Lethal and Sublethal Effects of Metals on the Physiology of Fish: An Experimental Approach with Monitoring Support

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Report to the Water Research Commission
by the
Department of Zoology
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EXECUTIVE SUMMARY

LETHAL AND SUBLETHAL EFFECTS OF METALS ON THE PHYSIOLOGY OF FISH: AN EXPERIMENTAL APPROACH WITH MONITORING SUPPORT

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3.1 Background and motivation

The Olifants River has been systematically impacted over the past few years because of increasing agriculture and mining activities, industrial development and urbanisation (Water *et al.*, 1992). This river system is often described as one of the most polluted systems in South Africa and is commonly known as "The Battered River". Agricultural pollution occurs mainly in the highveld region of the Olifants River catchment where various crops are produced. Any pollution contribution from agricultural practices results from substances dissolved in water or transported by water and sediment. The main threats to the aquatic environment resulting from agriculture include crop spraying (causing biocide pollution), leaching of fertilisers (causing organic pollution and eutrophication), erosion (causing siltation), damming (causing changes in aquatic habitats) and water abstraction (Engelbrecht, 1992). The middle region of the catchment, with its large number of informal settlements and extremely poor agricultural infrastructure, contributes largely to the increased silt loads and associated effects in this river system (Buermann *et al.*, 1995). Urban runoff from high population density areas in the catchment can be a major contributor to metal pollution and usually affects riverine biotas more negatively than well treated sewage effluent. Sewage treatment works are point sources of pollution in most river systems that usually contribute to high nutrient loads. Various sewage spills into the Olifants River have been reported over the past few years, e.g. at Namakgale into the Selati River in 1994. Along its length, it is being especially impacted by coal mining and industries in the Witbank-Middelburg and Phalaborwa areas. These mine effluents contain a complex of chemicals, many of which may have deleterious effects for aquatic ecosystems.

3.1.1 *Effects of mining on the aquatic environment.*

In the development and management of mining projects, it is important to ensure that waste materials, particularly tailing and waste rock containing potentially mobile heavy metals, are disposed of in a manner that will lead to a minimum of environmental contamination. Also, it is now an accepted practice to examine background metal levels in important terrestrial and aquatic organisms in areas where new mining developments will go into production, thus any significant changes in biological metal levels due to mining operations may be detected (Bohn & McElroy, 1976). Mining and processing activities have various impacts on the environment. These include:

- deterioration of the quality of the underground water;
- occurrence of sinkholes caused by water being pumped from the mines to prevent flooding, and
- deterioration of the water quality due to dissolved iron and manganese
- decrease in species diversity of macroinvertebrate populations.

The result of mine activities, especially coal mining, is the creation of effluent wat with a high acidity content and a corresponding low pH value. pH is an intensity factor, measuring the concentration of hydrogen ions. However, what is important in situations with acid-mine drainage is not the concentration alone but the availability of hydrogen ions to neutralise

bases; in other words, their excess over other ions. An understanding of the concept of total acidity is the key to understanding the differences between acid-mine drainage and other acid ecosystems such as peat drainage and acid rain-affected areas where low pH values are coupled with low acidity. Peat drainage differs from the others as the source of its acidity is weak carboxylic acid groups rather than strong mineral acids. However, while extreme acid-mine drainage characteristically has high concentrations of total acid and high conductivity, where this is diluted by other acid streams in the catchment the water quality can come to resemble the soft, low-acidity waters affected by acid precipitation (Kelly, 1988). One of the most important effects of low pH is the destruction of the bicarbonate buffer system, a feedback mechanism which controls the magnitude of shifts in pH. Below a pH of about 4.2 all carbonate and bicarbonate is converted to carbonic acid. This readily dissociates to water and free carbon dioxide that may be lost to the atmosphere. Water loses its capacity to buffer changes in pH. Many photosynthetic organisms use bicarbonate as their inorganic carbon source. All aquatic organisms which live below pH 4.2 will need to be adapted to the lack of bicarbonate buffering, but aquatic plants, in addition, will need to be able to utilize free carbon dioxide as their inorganic source for photosynthesis (Kelly, 1988).

At very low pH values the metal ions are soluble, but as the pH rises, some begin to precipitate out. The critical pH values are about 4.3 for iron (111) and 5.2 for aluminium. Flocs have been noticed at lower pH values but these probably formed when pH was higher. As the pH of acid-mine drainage is frequently below these values, the dissolved metal salts will not precipitate out until a certain amount of neutralisation has taken place. If conditions are favourable to oxidation of the pyrites, neutralisation of the drainage may be close to the outflow from the mine. In other cases it may not take place until much further downstream or where the acid stream joins a less acid river which can dilute the acidity and trigger the deposition of the floc. When the pH does rise the iron (111) salts come out of solution either to form colloids suspended in the water, fine suspended precipitates or heavier amorphous flocs. All of these can have severe effects on the biota. In suspension the classic orange-hued flocs of iron reduce light penetration and so interfere with photosynthesis and the vision of consumers. It can also cause some physical abrasion. When it settles out it can encrust rocks and stones, smothering all the benthic biota, filling gaps between stones and settling, especially in sludge pools, to give a deep layer of enveloping deposits. Even after the source of acid-mine drainage has been stopped, a severe spate can resuspend iron (111) deposits and, once again, affect the biota for some distance downstream. Metal ions are lost from solution by precipitation or adsorption, especially when iron (111) and aluminium flocs are present. The extent of adsorption is dependent upon pH. A decrease in pH is accompanied by a rise in the solubility of metals, making very high concentrations possible.

Two features of metal toxicity which should not be overlooked are their ability to form organometal complexes (the attachment of a trace element directly to carbon: Horowitz, 1991) and their potential for bioaccumulation There is some evidence that the presence of organic substances can reduce heavy metal toxicity considerably, or at least as measured in conventional toxicity tests. A number of organometal compounds are known to be particularly hazardous to aquatic life Tributyl tin, for example, a constituent of anti-fouling paints, is implicated in severe environmental damage in harbours, boatyards and inland waters, and appears in the *Black List* of substances compiled by international organisations, such as the P.P.C. and the United Nations Programme (Abel, 1989).

3.1.1.1 Effects of acid-mine drainage on the biota

Studies of the effects of pH in the field can conveniently be considered on the basis of the taxonomic categories that are principally involved, namely micro-organisms, macro-invertebrates and fish.

Macro-invertebrates

Acidification of waters can in principle influence metal-organism interactions in at least two ways: the decrease in pH may affect metal speciation in solution, or it may affect biological sensitivity at the level of the cell surface. Generally, pollution affects stream community structure predominantly by reducing species diversity. The elimination of non-tolerant species is often accompanied by (1) increases in stream productivity of benthic invertebrates due to lack of predation and competition, (2) changes and simplifications in food chains and (3) in the case of organic pollution a seemingly inexhaustible source of food for the remaining tolerant species (Koryak *et* al., 1972). Studies have shown that under conditions of constant acid mine drainage the Odonata, Ephemeroptera and Plecoptera were eliminated and Trichoptera, Megaloptera and Diptera were represented by fewer species. Species tolerant of these conditions included the caddis fly *Psilostomis siallis and Chironomus attenuatus.* Certain Hemiptera and Coieoptera were present in large numbers. In a stream affected by intermittent acid mine drainage the insect fauna differed little from similar unpolluted streams except for the absence of some sensitive Ephemeroptera and Diptera.

Fish

The addition of acid to a stream may release sufficient carbon dioxide from the bicarbonate in the water to kill fish even though the pH level itself would not be directly lethal. Lloyd & Jordan (1964) noted that one of the important conclusions to be reached from their investigations of factors which affected the resistance of fish to acid waters was the effect of sub-lethal concentrations of free carbon dioxide. They considered that this alone could account for the considerable variation in the lethal pH values which were quoted in the literature and observed that their own work covered most of the pH values said to be toxic by other authors.

From the discussion above, it is clear that anthropogenic activities in the Olifants River catchment and especially mining, industrial development and urbanisation can negatively impact on the aquatic environment. To assess impact on water quality, various issues have been identified, which require attention.

3.1.2 *Issues related to water quality*

3.1.2.1 The sources, nature and extent of the water pollution.

The sources, nature and extent of the water pollution will be site specific. Nevertheless, it can be expected that certain broad similarities will exist. Furthermore, it is anticipated that the major sources of water pollution are effluent from mining, industrial and agricultural activities.

3.1.2.2 The impact of the various sources of pollution on the aquatic environment that include the water, macro-invertebrates and fish.

An important consideration relates to the selection of sampling sites to evaluate the impact of the various sources of pollution on the aquatic environment. It must be sufficiently representive to allow reasonable extrapolation to the anthropogenic activities in the catchment. The sampling sites have to exhibit potential pollution characteristics which can be anticipated to be of relevance and significance in meeting the objectives of the research, and mining, industrial and agricultural operations should not be terminated or scaled down at the case study sites during the study period.

3.1.2.3 Steps to combat the possible deleterious effects of pollutants on the aquatic environment.

Based on the identification of the major sources of pollution and the significant environmental impacts, appropriate pollution control measures will be identified. The sources and mode of pollution will need to be taken into account when identifying the control options. According to the principles of integrated environmental management, the socio-economic benefits of any action must be weighed against the adverse environmental impacts. An important aspect to bear in mind is that whilst non-polluting industry is an unattainable dream, all environmental problems can be managed, and, if not solved, at least mitigated. Identified adverse impacts should be divided into those that can be avoided or mitigated and those that cannot. Those adverse impacts that cannot be avoided will need to be taken into account when determining waste load allocations in terms of the RWQ approach to water pollution control. The adverse impacts that can be avoided or mitigated will be assessed to identify appropriate pollution control measures. These measures will need to be evaluated with regard to their technical and economical feasibility.

3.1.2.4 Lethal and sublethal experimentation

Information on the lethal and sublethal effects of pollutants (e.g. metals) form an integrated part of ecosystem health assessment programmes and of procedures followed to develop water quality guidelines for the environment. During the workshop on the water quality of the Sabie River (March 1992), it was concluded that, at present, limited information is available on lethal and sublethal effects of pollutants and other water quality variables on aquatic species. There is an urgent need to evaluate the influence of these variables on aquatic species. This lack of data/information is presently affecting the establishment of water quality guidelines for the environment for Southern Africa by the Department of Water Affairs and Forestry, which are presently based on overseas data. It is envisaged that the same problems will be encountered when developing an ecosystem health assessment programme for South Africa. It is, therefore, very important for us, as freshwater biologists in South Africa, to study both the lethal and sublethal effects of water quality variables in order to assist in both ecosystem health assessments and water quality guidelines development.

The general objective with acute test with pollutants (eg. metals) is to determine the concentration that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled laboratory conditions. The acute lethality tests have been useful in providing estimates of the concentration of pollutants (eg. metals) that cause direct irreversible harm to organisms (such as fish). Furthermore, these tests provide practical means for (a) deriving estimates for upper limits of concentrations that produce toxic effects,

(b) evaluating the effects of other water quality variables (eg. pH, hardness, temperature, etc.) on the toxicity of pollutants (eg. metals) and (c) developing an understanding of the concentration-response relationships (Macek *et al.*, 1978). The acute toxicity test exposures are also used to develop chronic and other associated sublethal tests. Generally, concentrations that produce sublethal chronic effects are lower than those that produce more readily observable acute effects, such as death (Rand & Petrocelli 1985). Until recently, toxic effects of pollutants (e.g. metals) and therefore the health and well- being of organisms (such as fish) after exposure were mainly evaluated by the above mentioned acute test when the death of the organism was the only criterion (Larsson *et al.*, 1985). The possible sublethal effects of pollutants were generally neglected. However, biochemical/physiological processes would be effected or may even cease to function normally before the onset of death. The sublethal effects of pollutants are usually biochemical/physiological in nature since most of these pollutants affect the basic level of organisation that is, the sub-cellular level in an organism.

The toxic mode of action of a pollutant can be a reaction with enzymes or the metabolites, or a binding or interfering with membrane systems or other cellular constituents. These primary interactions between a pollutant and cellular processes or constituents result in functional and structural changes at a higher level of organisation. These changes would ultimately have a negative effect on essential processes such as osmoregulation, hormone regulation, muscle and nerve functions, immune reactions, respiration and circulation (Waldichuk, 1974; Larson *et. al.*, 1985).

It is clear, therefore, that both lethal and sublethal effects of pollutants on organisms such as fish must be studied to fully evaluate the potential impact of the pollutant on the organism. The information on both lethal and sublethal effects is essential to predict the possible impact of pollutants on the organisms in the natural environment. Furthermore, data on the sublethal effects of pollutants would also aid in the identification of pollution before dramatic changes (e.g. mass mortality) occur in the natural population. It may therefore be concluded that realistic water quality guideline development or ecosystem health assessment, cannot be achieved if information on lethal and sublethal effects of pollution is not available.

From the foregoing it is evident that the proposed research is important in that it will provide the information that is required to assist water quality managers in addressing environmental problems. At the same time, this research will make a positive contribution towards the development of equitable water management legislation. The water authorities and the mining industry share a firm commitment to the principle of integrated environmental management and responsible self-regulation. The practical implementation of these principles requires access to the type of information that will result from meeting the objectives of this research project. With this research project potential pollution problems were identified and procedures defined for assessing their environmental impacts.

3.2 Objectives

This project had the following main objectives:

3.2.1 To investigate the anthropogenic impacts on the water and sediment quality in the Olifants River, Mpumalanga. Research was therefore carried out at selected sites in the Upper Catchment of the Olifants River as well as in the Lower Catchment on the western boundary of the Kruger National Park, to:

- Provide spatial and to some extent, temporal changes in the values for the most important water quality variables (See sections 9.1 &9.2 of the report).

- Determine the extent of metal bioaccumulation in the tissues/organs of selected fish species (See section 9.3 of the report).

- To use the data obtained, together with other available information, to form a holistic picture of the present state of the water quality of the Olifants River. This will provide water quality managers with information to assess the levels of pollution in the river and set management priorities of constituents of concern.

3.2.2 To investigate, under controlled laboratory conditions, the sublethal effects of metals on fish (See section 10 of the report).

Laboratory experiments where fish are exposed under controlled conditions to different metals, according to toxicity, provided important information on lethal and sublethal levels of the pollutants. This information is essential for the improvement of water quality guidelines for metals. The "health" status of the fish can also be accessed. Fish were exposed to sublethal concentrations of the toxic metals. These sublethal concentrations were determined from already determined LC_{50} values for the selected metals. Standard techniques were employed in all experimentation. Results from these experiments are available to supplement the existing information obtained during the previous project on the Olifants River.

3.2.3 To provide information that could be used to improve water quality guidelines for metals. This would aid water quality managers, engineers as well as consultants in assessing the impact of these pollutants on the aquatic environment.

3.2.4 To further expand a water quality index (Water 2) which already exists but needs further refinement. The final product could be tested for suitability of use in other river systems in South Africa.

3.2.5 To train manpower in water quality management and the assessment of the detrimental effect of pollutants on aquatic life, especially fish, which may be of great value in pollution control. One Ph D and three M Sc - students worked on the project. Three completed their studies while the Ph D theses will be submitted by May 1998. Two of them registered for a Ph D while the other one took up a position at the Gauteng Department of Health.

3.3 General discussion and conclusions

3.3.1 *Water and Sediment*

Aquatic ecosystem contamination can be confirmed by examining the water, sediment and organisms occurring in such an environment. This is important to assess because the quality of the aquatic environment will determine the health and existence of aquatic organisms, as well as of the users reliant on the resource. This section of the study therefore investigated the extent of occurrence of various physical and chemical water quality variables, as well as

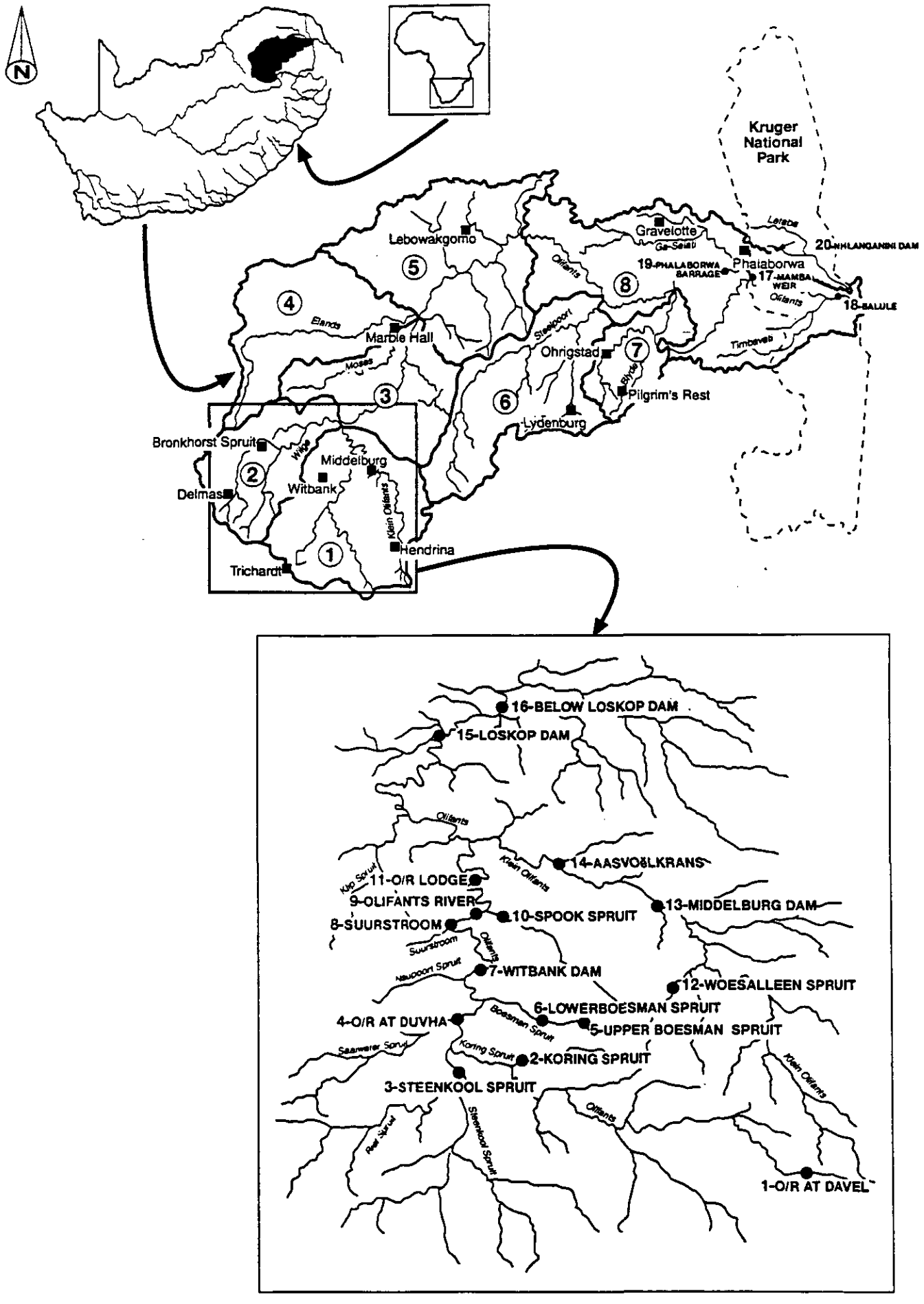

Figure 3.1: The study area with selected localities in the Upper and Lower Catchment of the Olifants River.

metal concentrations in the water and sediment of selected localities in the upper and lower catchments of the Olifants River.

Evaluation of the data for the macro- and trace elements (metals) in the water at selected sites (Fig. 3.1) indicated that many of the concentrations exceeded the water quality guidelines (Canadian, South African-DWAF) for aquatic ecosystems. This is alarming because many of these constituents have negative impacts on aquatic life, thereby posing a potential threat to ecosystem health. Evaluation of the physical and chemical water quality variables of selected sites (Table 3.1 and Fig. 3.2) showed that localities 2, 3, 6, 8, 9, 10, 12 and 17 were severely impacted. Elevated levels of certain variables (eg. total dissolved salts & sulphates) suggest that runoff originating in the catchments of these localities is being impacted by mining. This is further confirmed by low pH-values at localities 3, 5, 8, 9, 10 and 12, which indicates acid mine drainage from the many coal mines in the upper catchment of the Olifants River.

Nutrient enrichment (elevated phosphates, nitrates and nitrites) occurred at many sites in the catchment, but in particular at localities 3, 4, 6, 10, 11, 14, 15 and 17. Point source pollution from sewage treatment works and non-point sources from agricultural runoff and informal settlements are the main contributors to these elevated levels of nutrients. This is clearly evident at Localities 11 and 14 where the high phosphate also caused excessive growth of algae and aquatic weeds in the river at the two localities. The 1 mg/l phosphate standard for effluent water is therefore not adhere to and should be revised to also take into account drought and low flow periods when there is practically no dilution.The elevated phosphate effluents in the Selati River are the main contributor to the high nutrient levels detected in the lower Olifants River catchment at Locality 17.

Table 3.1 Evaluation of the physico-chemical water quality variables at selected sites in the Olifants River, to indicate problematic areas that need to be addressed.

Variable	Locality																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0_2-*sat.*	✓	✓	x	✓	✓	⊗	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓
Turbidity	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	☺	✓	✓	✓	x	x
PH	✓	✓	✓	✓	✓	✓	⊗	x	⊗	✓	x	✓	✓	✓	✓	✓	✓	✓
TAL	☺	✓	✓	✓	x	✓	⊗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
TDS&EC	✓	x	✓	✓	✓	⊗	✓	⊗	✓	⊗	✓	✓	✓	✓	✓	✓	⊗	⊗
$S0_4$	☺	x	✓	✓	✓	⊗	✓	⊗	✓	⊗	✓	⊗	X	✓	✓	☺	⊗	⊗
Cl^-	✓	x	x	✓	✓	x	x	✓	x	✓	x	x	✓	x	x	✓	⊗	x
Na^+	✓	x	x	✓	✓	x	✓	x	x	✓	x	x	✓	x	x	☺	⊗	⊗
K^+	✓	x	x	✓	✓	x	✓	x	x	x	x	x	✓	x	x	☺	⊗	⊗
Mg^{2+}	✓	x	✓	✓	✓	⊗	✓	⊗	✓	x	✓	⊗	✓	✓	✓	☺	x	x
Ca^{2+}	☺	x	✓	✓	x	⊗	✓	⊗	✓	⊗	✓	⊗	✓	✓	✓	☺	x	x
F^-	☺	✓	⊗	✓	✓	x	x	⊗	x	x	x	x	X	x	x	x	⊙	⊗
$P0_4$-P	✓	✓	⊗	✓	✓	✓	✓	✓	⊗	x	⊗	✓	✓	⊗	x	✓	☺	✓
NO_3-NO_2	⊗	✓	⊗	x	✓	x	☺	x	⊗	x	x	✓	☺	⊗	x	☺	x	✓
NH_4-N	✓	☺	x	x	x	x	✓	⊗	x	x	x	✓	X	x	✓	✓	✓	x

☺ - Levels of variable generally well within guideline limits. Locality seems to be unimpacted by pollutants containing/influencing this variable. Levels occurring at this site seem to be of no threat to the health of the aquatic ecosystem. There is a possibility that this site can be used as a reference site

for this specific variable in future studies in this area.

✓ - Levels were generally within guideline limits. Levels of variable detected seem to bear no direct threat to aquatic life occurring at this site.

x - Values exceeded the guideline limits and/or seemed to be impacted by some source of pollutant containing/influencing this variable. Concentrations detected could have a negative effect on the aquatic ecosystem and will have to be investigated and addressed.

⊗ - Levels of variable detected exceeded guideline limits and/or other sites investigated by large margin. Seems to be heavily polluted by a source containing/influencing variable. Major possibility of negatively impacting ecosystem at present (especially sensitive organisms). Urgent need for improvement !

It is evident from the evaluation of metal concentrations in the water and sediment (Table 3.2 and Fig. 3.2), that most of the sites along the Olifants River are being affected by metal pollution. Acid mine drainage at localities 3, 5, 8, 10 and 12 is most likely responsible for the release of metals from the sediment, resulting in the high metal loads detected in the water at these sites. The negative impact of the Selati River on the Olifants River is also stressed by its contribution to the elevated metal levels detected in the water at Locality 17.

However, efficient evaluation of the metal pollution in the catchment is difficult because the data is based on single seasonal samples. Furthermore, most guideline values set by the Department of Water Affairs and Forestry are primarily based on dissolved metals, while this study focussed on total metal concentrations. The contribution of natural processes such as geological weathering to metal levels in the Olifants River catchment is also unknown, thereby complicating the overall evaluation. Despite these difficulties, the present study clearly indicates that the Olifants River is subjected to metal pollution. Specific impacts of observed metal concentrations on aquatic communities of this river system should be investigated, if possible by the use of on-site toxicity testing.

3.3.2 *Bioaccumulation of selected metals in the organs and tissues of fish.*

Fish are mentioned in the literature as good bioaccumulative indicators of metal pollution because they are known to readily accumulate metals from their environment. This can be detrimental to the health of both the organism itself, as well as to consumers, be they animals or humans. The investigation of metal bioaccumulation in fish is important because it supports the monitoring of the chemical and physical quality of water and sediment in aquatic ecosystems. It is also important for the assessment of the spatial and temporal extent of accumulation as well as organism health. Fish are an important food source to humans and it is therefore necessary to investigate the potential consumption of contaminated fish.

Metals were bioaccumulated mainly by the gills, but copper and iron concentrations were the highest in the liver tissue. It is therefore suggested that that these organs be used in a general biomonitoring programme for the assessment of the extent of bioaccumulation of metals in fish tissues/organs. The lowest concentrations of the selected metals were found in the muscle and skin tissue. However, this should also be included in the biomonitoring programme, as it is the edible part of the fish. Since the skin only forms a small

percentage of the edible part of the fish, its tissue may for the purposes of analysis be replaced by vertebrae which, in general, appear to accumulate higher levels of metals. Nevertheless, it is suggested that skin, muscle, gills, liver and vertebrae are included in a metal bioaccumulation monitoring programme.

Although different sampling techniques were used, it was not possible to capture fish of a specific size throughout the sampling period. For the present data set both positive and negative correlations between size and metal levels were detected, but in many cases these relationships were not significant. Evaluation of the present data and the literature suggests that, for each data set, size of the fish must be considered, especially if it is not possible to select a specific size range of fish. The data from young, fast-growing fish should be considered as a separate data set. The present study indicates that there were generally no significant difference or specific trends in metal bioaccumulation between different sexes of fish. This indicates that the organs and tissues sampled during this study do not give a true reflection of the preferences of bioaccumulation between sexes. Gonads will be the most obvious tissues to sample for differences in accumulation between sexes, but according to the literature levels of metals usually vary between different species as well as seasons. Gonads will therefore be of limited value in bioaccumulation studies aimed at investigating the extent of metal pollution in aquatic ecosystems.

Table 3.2 Evaluation of the metal concentrations detected in the water and sediment at selected localities in the Olifants River, in an attempt to identify problematic areas.

Variables	Locality																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Water																		
Cu	X	x	⊗	x	x	x	x	⊗	✓	x	x	x	X	⊗	x	x	x	x
Zn	X	x	⊗	x	x	x	⊗	⊗	✓	⊗	x	x	⊗	x	x	x	x	x
Al	x	x	x	x	x	x	✓	⊗	✓	✓	x	✓	✓	x	x	x	x	⊗
Fe	x	x	x	x	x	x	x	x	✓	✓	x	✓	X	x	x	x	x	x
Ni	✓	✓	⊗	x	✓	x	⊗	⊗	✓	⊗	x	x	⊗	⊗	x	x	x	x
Mn	✓	✓	✓	⊗	✓	⊗	✓	⊗	✓	⊗	✓	x	✓	✓	x	⊗	✓	x
Pb	x	x	⊗	x	✓	⊗	⊗	x	✓	x	⊗	x	⊗	⊗	x	x	x	x
Cr	✓	✓	⊗	x	x	✓	⊗	x	x	x	⊗	✓	X	x	x	x	x	x
Sediment																		
Cu	x	✓	✓	x	x	x	x	x	x	⊗	x	x	X	x	⊗	x	x	✓
Zn	✓	✓	✓	✓	✓	x	⊗	⊗	x	⊗	⊗	⊗	⊗	✓	x	x	x	✓
Al	✓	✓	✓	x	⊗	⊗	x	⊗	x	⊗	✓	⊗	⊗	✓	⊗	x	⊗	✓
Fe	x	✓	✓	x	x	⊗	✓	⊗	x	⊗	x	⊗	✓	✓	⊗	x	⊗	x
Ni	x	✓	✓	x	x	⊗	✓	⊗	x	⊗	x	x	✓	✓	x	x	⊗	x
Mn	⊗	x	✓	✓	✓	⊗	✓	✓	x	⊗	⊗	⊗	X	x	x	x	x	x
Pb	✓	✓	✓	x	x	⊗	✓	⊗	x	⊗	✓	⊗	X	✓	x	x	x	✓
Cr	x	x	✓	x	x	x	x	⊗	x	⊗	x	x	✓	✓	x	x	⊗	⊗

✓- Concentrations of metal are low and/or slight seasonal variation occurs. This locality therefore seems to be unimpacted or only slightly impacted by sources containing this metal.

x- The concentrations detected were generally above guideline limits and the levels detected at other sites and/or seasonal fluctuations in the metal concentrations were evident. Levers occurring at this site could affect the aquatic ecosystem negatively and an attempt should be made to reduce

concentration of metal at this site. Further investigation and monitoring definitely recommended.

⊗- The concentrations of the metal detected at this site were generally far higher than the guideline limits and/or the levels detected at the other localities and/or major seasonal fluctuations occurred in the level of metal. It is obvious that these levels could be detrimental to the health of aquatic organisms (especially sensitive species) occurring at this site. Urgent need for reduction in the concentration of the metal and further monitoring is proposed.

Metal concentrations varied mostly between *O. mossambicus* and *L. umbratus* as well as *C. gariepinus,* and *L. umbratus* which can possibly be attributed to the differences in general feeding behaviour of the species. It is, however, not only the levels in food source that can be an important pathway for uptake but also the intake of sediment particles associated with the food. Metals adsorbed onto sediment particles play an important role in their availability to aquatic organisms and usually become more available as pH levels decrease. The low pH in the stomachs of fish could therefore cause increased levels of bioavailable metals which could in turn be taken up and accumulated by fish. This route of metal uptake should be investigated, especially at contaminated sites.

Temporal variation in metal accumulation by fish occurred due to variation in metal concentrations in the water and sediment at a locality. This is the result of seasonal variation in climatic conditions (eg. rainfall, temperature) as well as fluctuations in pollutant inputs into the river system over a period of time. Seasonal patterns of accumulation varied between different localities because of variations within their subcatchments. In some cases, decreased accumulation occurred during high flow periods; this is ascribed to the diluting effect of more water on pollutant concentrations. In other cases, increased flow caused fish to be exposed to higher levels of metals, due to their increased contact with metal-polluted sediment in the more turbid waters of the high flows or flushing of dams, such as the case with the Phalaborwa Barrage. Various factors such as water quality and variation in behaviour of fish during different seasons (eg. decreased metabolism during winter) could also have contributed to the seasonal trends observed for bioaccumulation.

Geological differences could result in different levels of metals in different reaches of a river. However, as discussed, anthropogenic activities are responsible for point and diffuse sources of pollutants causing measurable differences in metal concentrations between localities. This caused a variation in the extent of bioaccumultion that occurred at different sites. In general, fish at Locality 11 (Olifants River at Olifants River Lodge) accumulated more Aluminium, iron, nickel, manganese and chromium than from Locality 14 (Klein Olifants River at Aasvoëlkrans). Zinc and manganese were accumulated in higher levels at Locality 15 while fish at Locality 17 accumulated higher levels of copper, aluminium, iron and nickel. The impact of the highly polluted Selati River in the lower catchment was evident in the difference between metal concentrations detected in the fish at locality 17, and locality 19 (Phalaborwa Barrage) upstream of the Olifants-Selati confluence. From this data, literature data on the metal levels in fish as well as water and sediment quality information, it can be concluded that the aquatic biota at the selected sites in the Olifants River system are to some extent subjected to metal pollution.

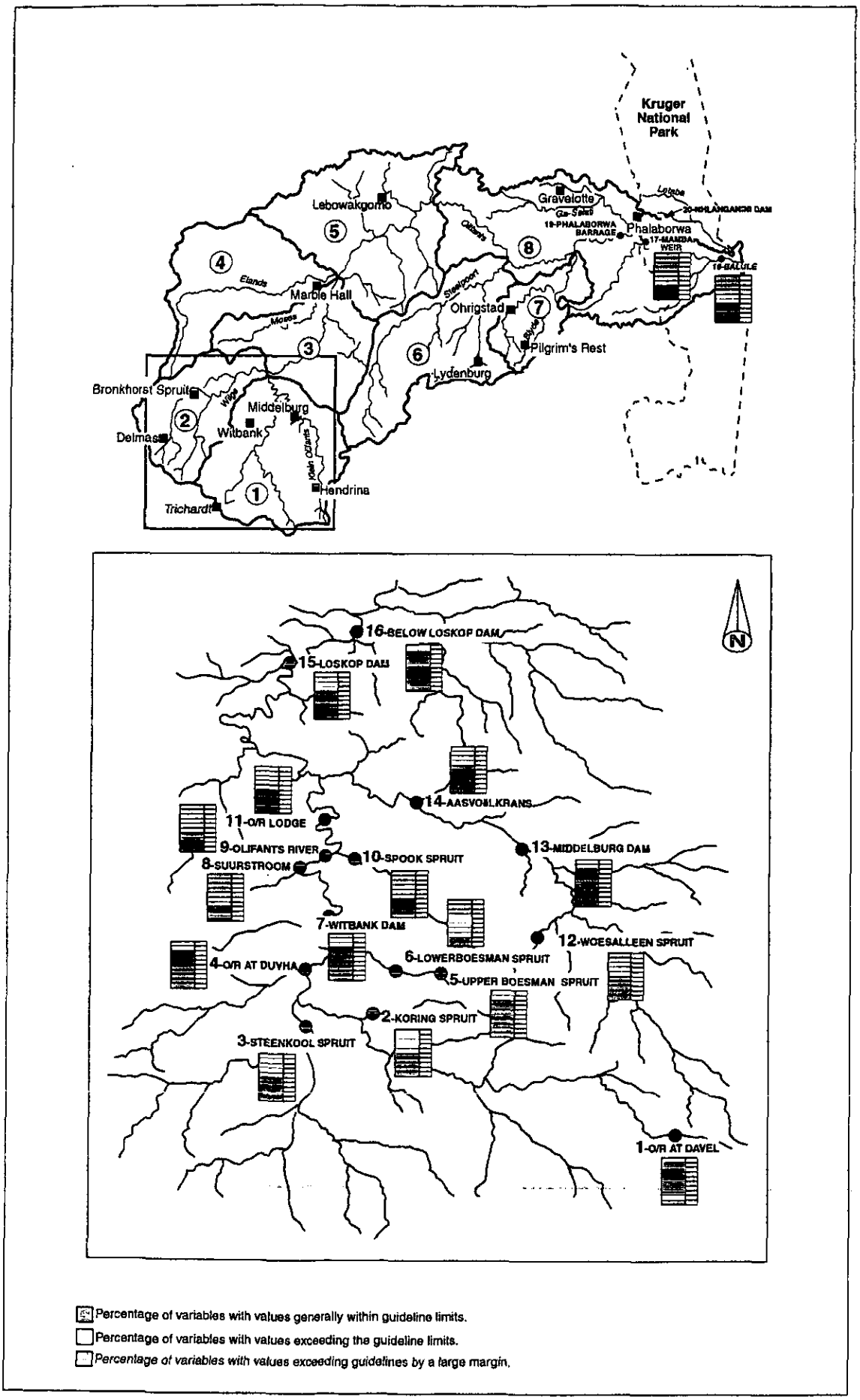

Figure 3.2: Evaluation of the physico chemical water quality variables at selected sites in the Olifants River. Increments of 10% are given. See legend for explanation of colours.

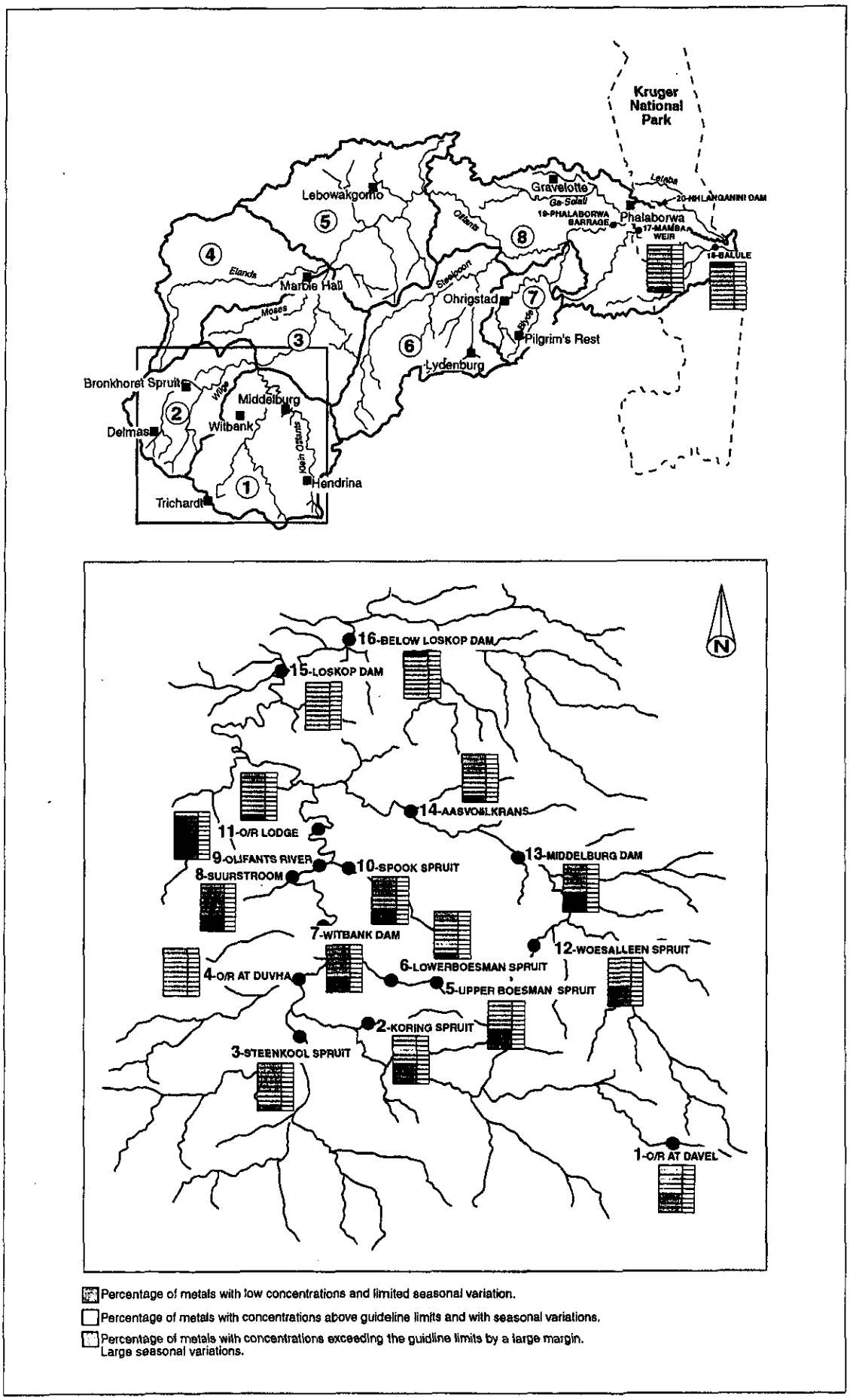

Figure 3.3: Evaluation of metal concentrations detected in the water at selected sites in the Olifants River. Increments of 10% given. See legend for explanation of colour

3.3.3. *Experimental work*

Firstly, it was clear that the haematology and osmoregulation of *O. mossambicus* were altered after the exposure to copper or zinc at a neutral and acidic pH, respectively. In most cases the acidic pH caused the opposite result to the neutral pH e.g., [Na]: was decreased at the neutral pH, and increased at the acidic pH.

Secondly, it was clear that low pH and sublethal aluminium concentrations had a physiological effect on *O. mossambicus*. In some cases however, for example an aluminium concentration of 0.06 $mg.l^{-1}$ aluminium and pH 5.2, the toxicity of the acid was reduced rather than increased by the aluminium as was clearly seen in the blood glucose concentration. At pH 5.2 it seems the most toxic concentration of aluminium was in the range of 1.00 $mg.l^{-1}$ and of 1.50 $mg.l^{-1}$ aluminium. Higher concentrations, in this case 2.00 $mg.l^{-1}$ aluminium at pH 5.2, had statistically little effect on the haematology of *O. mossambicus*. The metabolism of *O. mossambicus* was severely affected after exposure to low pH as well as the combination of aluminium and pH 5.2.

Thirdly, the sublethal manganese exposures resulted in clinical diagnostic haematological changes.

All the changes after exposures (Cu, Zn, Al and Mn) indicate that haematological variables can be used as indicators in detecting the effects of sublethal metal exposure on fish (Tables 3.3 and 3.5). These toxicity tests, performed under controlled laboratory conditions, provide essential information concerning the sublethal effects of copper, zinc, aluminium and manganese on the haematology of fish (*O. mossambicus*). Some of the values obtained during this study fluctuated widely which indicated that the influence of the "stress" condition was handled differently by each individual. Although individual variations are a very important phenomena when conclusions are drawn, this does not mean that diagnostic tools of this nature cannot be used in the process of water quality guidelines. The more information available the better the prediction of effects of metals on fish survival. Water quality guidelines could be a source in the identification of pollution, before it causes changes in the natural population or environment. Figure 3.4 shows the levels of acceptability of copper, zinc and aluminium at the sampling localities investigated during this project. These metals are already present at concentrations were it could affect the survival of fish negatively.

3.3.4 *Macroinvertebrates*

A great diversity and number of aquatic macroinvertebrates occurred at the different localities. Metal concentrations for the various organisms give an indication of the metal levels these organisms were exposed to. Aluminum and iron concentrations averaged high levels in the organisms analysed. High aluminum and iron concentrations were also observed for the water and sediment analysed (Table 11.10). Thus aluminum and iron from both the water column and sediment compartment contributed to elevated levels in the macroinvertebrate. Aquatic macroinvertebrates occurring in the upper Olifants River catchment have to some degree adapted to high metal concentrations, whether from the water column or sediment compartment. These organisms such as the Chironomidae, Tubificidae and Crustacea thrived and built high populations (Table 11.10). These organisms' exposure and their consequent survival in large numbers might also be caused by the developmental stage when exposed to metal

Table 3.3 Pathological conditions of haematology, caused in *O. mossambicus* after 96 hour exposures to copper, zinc, aluminium and manganese.

Metal ($mg.l^{-1}$)	Condition											
	Leucocytes		Erythrocytes		Haemoglobin (hypoxia)		Haematocrit		Mean corpuscular volume		pH	
	Leucocytosis	Leucopaenia	Erythrocytosis	Erytropaenia	Increase	Decreas	Haemoconcentration	Haemodilution	Increase	Decrease	Alkalosis	Acidosis
[Cu]: 0.0191		⊗		⊗	⊗			⊗		⊗		●
0.0124	⊗		⇔		⊗			⊗		⊗		⊗
0.0439	⊗		⊗		⊗		⊗			⊗		●
0.0264	⊗		⊗		⊗			⊗		●		●
0.0050	⊗		⊗		●		⊗		⊗			●
0.2000	⊗		●		●		⊗		⊗			●
pH5.2 + 0.0191		●		⊗		⊗		⊗	⊗		⊗	
pH5.2 + 0.0124		●		●		●		⊗		⊗		⊗
pH5.2 + 0.0439		⊗	⊗			⊗	⊗			⊗		⊗
pH5.2 + 0.0264		⊗		⊗		⊗		⊗		⊗		●
[Zn] : 0.2099		⊗		⊗		⊗		⊗		⊗		●
0.2535	⊗		⊗		⊗		⊗			⊗		●
0.3674		⊗	⊗		⊗			⊗		⊗		●
0.8391	⊗		⊗		⊗			⊗		⊗		●
0.0300		⊗		●	⊗			●		⊗		●
0.1000	⊗			⊗	⊗			⊗		●		⊗
pH5.2 + 0.2099		●		⊗		●	⊗		●		⊗	
pH5.2 + 0.2535		●	⊗			⊗	⊗			⊗	●	
pH5.2 + 0.3674		●		⊗		●		⊗	⊗		●	
pH5.2 + 0.8391		●		⊗		⊗		●		⊗	●	
[Al]:												
pH5.2 + 0.06	●		⊗		⊗		⊗			⊗	N/A	
pH5.2 + 1.00	●		⊗		⊗		⊗			●	N/A	
pH5.2 + 1.50	●		⊗			⊗	●		●		N/A	
pH5.2 + 2.00	⊗			⊗		⊗		⊗		⊗	N/A	
[Mn]:												
172.30	⊗			⊗		⊗	⊗		⊗		N/A	
259.00	⊗		⊗		⊗		⊗			⊗	N/A	
345.00		●		●		●		●		⊗	N/A	

N/A = Not available
● = Significant increases or decrease
⊗ = Insignificant increases or decrease
⇔ = No detected change (same value)

Table 3.4 Pathological conditions of osmotic and ion regulation, caused in *O. mossambicus* after 96 hour exposures to copper, zinc, aluminium and manganese.

[Metal] ($mg.l^{-1}$)	Condition: Total osmolality		Plasma sodium concentration		Plasma potassium concentration		Plasma calcium concentration		Plasma chloride concentration	
	Increase	Decrease	Hypernatraemia	Hyponatraemia	Hyperkaliaemia	Hyporkaliaemia	Hypercalcaemia	Hypocalcaemia	Hyperchloraemia	Hypochloraemia
[Cu]: 0.0191	●			●	⊗		⊗		⊗	
0.0124	●			●	⊗			⊗	⊗	
0.0439		●		●	●			⊗		⊗
0.0264		●		●	●			⊗		●
0.0050	●			●		⊗		⊗	⊗	
0.2000	⊗			●	●			⊗		⊗
pH5.2 + 0.0191	●		●			⊗	⊗		●	
pH5.2 + 0.0124	⇔		⊗		⊗			⊗	⊗	
pH5.2 + 0.0439	⊗		⊗		●		⊗		⊗	
pH5.2 + 0.0264	⇔			⊗	●			⊗	⊗	
[Zn] : 0.2099	⊗			●		⊗	⊗		⊗	
0.2535		⊗		●	⊗			⊗		⊗
0.3674		⊗		●		⊗		⊗	⊗	
0.8391	⇔			●		⊗		⊗	●	
0.0300	⇔			●	⊗			⊗	⊗	
0.1000	⇔			●		⊗		⊗	⊗	
pH5.2 + 0.2099	●		●		⊗		⇔		●	
pH5.2 + 0.2535	●		⊗		●			●	●	
pH5.2 + 0.3674	●		●		⊗			⊗	●	
pH5.2 + 0.8391	●		●		⊗			⊗	●	
[Al]:										
pH5.2 + 0.06		⊗		⊗		⊗	●			⊗
pH5.2 + 1.00	●			⊗	●		●			●
pH5.2 + 1.50	●			●		⊗		⊗		⊗
pH5.2 + 2.00	●			●	⊗			●		⊗
[Mn]:										
172.30	⊗			●	●		●		●	
259.00	⊗			⊗		●		●	⊗	
345.00	⊗			⊗		●		●	⊗	

N/A = Not available
● = Significant increases or decrease
⊗ = Insignificant increases or decrease
⇔ = No detected change (same value)

Table 3.5 Pathological conditions of metabolism, caused in *O. mossambicus* after 96 hour exposures to copper, zinc, aluminium and manganese.

[Metal] ($mg.l^{-1}$)	Condition									
	Choline Esterase activity		Pyruvate Kinase activity		Glucose-6-Phosphate dehydrogenase activity		Lactate concentration (Hypoxia)		Blood glucose concentration	
[Al]:	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease	Hyperglycaemia	Hyporglycaemia
PH5.2 + 0.06		⊗		⊗	🔥		🔥		⊗	
PH5.2 + 1.00		⊗		🔥	🔥		⊗		🔥	
PH5.2 + 1.50		🔥		🔥	🔥		🔥		🔥	
PH5.2 + 2.00		🔥		🔥	🔥		🔥		⊗	
[Mn]:										
172.30		🔥	⊗		N/A			🔥	🔥	
259.00	⊗		⊗			⊗		🔥	🔥	
345.00	⊗			🔥		⊗	⊗		🔥	

N/A = Not available
🔥 = Significant increases or decrease
⊗ = Insignificant increases or decrease

Table 3.6: Description of sampling localities in the study area in the Upper and Lower Olifants River as given in Figure 3.4.

1: Davel	A locality in the most upper reaches close to the origin of the Olifants River near a town called Davel.
2. Koring Spruit	A locality in the Koring Spruit South of Van Dyksdrift
3. Steenkool Spruit	A locality in the Steenkool Spruit before its confluence with the Riet Spruit
4. Duvha	A locality in the Olifants River upstream of the Witbank Dam
5. Upper Boesman Spruit	A locality in the upper reaches of the Boesmankrans Spruit before it flows through a coal mining area.
6.Lower Boesman Spruit	A locality in the lower reaches of the Boesmankrans Spruit before its confluence with the Olifants River (after passing through a mining area).
7. Witbank Dam	Witbank Dam - this impoundment on the Olifants River is the biggest municipal dam in the country, with a storage capacity of 10 402 million m^3. It provides water for urban and industrial use in the Witbank area. Compensation releases for Loskop Dam are made monthly which influences the flow of the river between these two dams.
8. Suurstroom	Suurstroom - A locality in a small stream arising from mine drainage flowing into the Olifants River between Witbank and Middelburg.
9. Olifants River	A locality in the Olifants River at the bridge on the old road to Middelburg after it passes the urban and industrial areas of Witbank.
10. Spook Spruit	A locality in the Spook Spruit before the confluence with the Olifants River.
11. Olifants River Lodge	A locality in the Olifants River between Witbank and Middelburg at Olifants River Lodge.
12. Woesalleen Spruit	A locality in the Woesalleen Spruit before its confluence with the Klein Olifants River.
13. Middelburg Dam	Middelburg Dam - an impoundment on the Klein Olifants River close to Middelburg. It has a storage capacity of 47,9 million m^3 and mainly supplies the town of Middelburg with domestic water.
14.Aasvoëlkrans	A locality in the Klein Olifants River in the vicinity of Aasvoëlkrans, after it passes through Middelburg.
15. Loskopdam	Loskop Dam - this is the largest dam in the Olifants River basin, with a storage capacity of 348,1 million m^3. The major land use sectors are irrigation, domestic and industrial.
16. Below Loskop Dam	A locality in the Olifants River just below the Loskop Dam wall.
17. Mamba Wier,	Mamba weir Kruger National park (KNP) - A locality in the Olifants River, after it crosses the western boundary of the KNP. It is ± 15 km downstream of the Phalaborwa Barrage and ± 10 km downstream of the Selati-Olifants River confluence.
18.Balule	Balule weir (KNP). This is a locality in the Olifants River ± 40 km downstream of locality 17.
19. Phalaborwa Barrage	Phalaborwa Barrage. This dam has a storage capacity of 4,5 million m^3 and provides water to the towns, mines and industries in the area.
20. Nhlanganini Dam	Nhlanganini Dam. A dam built for water provision to game in a tributary of the Letaba River, a major tributary of the Olifants River. This site was sampled as a control because there are no known anthropogenic activities affecting its water quality.

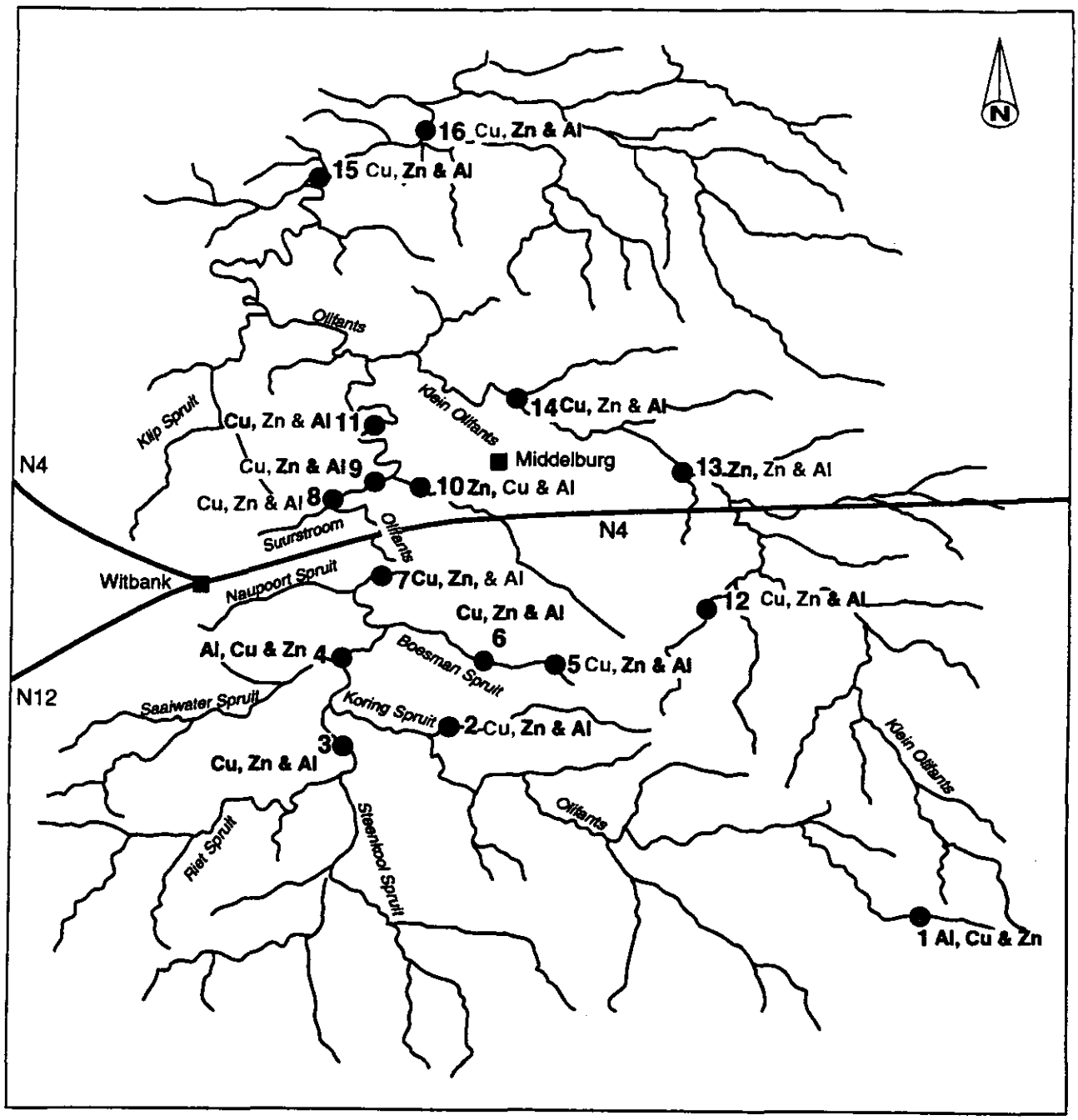

Figure 3.4: Acceptability of copper, zinc and aluminium levels to fish in the Upper Olifants River determined through sublethal experimental

- Acceptable
- Low Risk (Low Flow only)
- High Risk (High and Low Flow)
- Hazardous to aquatic life

concentrations, feeding habits, the organism's ability to exclude or regulate metals by their physiological abilities, the availability of food and the presence/absence of predators.

The lower numbers of organisms (water insect larvae, *Coelenterata, Gastropoda and Pelecypoda*) may be indicative of their sensitivity towards very high metal concentrations from their surrounding water column and sediment compartment. Thus, the populations of organisms found in this system are significant in offering clues to the intensity of pollution and the consequent degree of recovery concentrations, feeding habits, the organism's ability to exclude or regulate metals by their physiological abilities, the availability of food and the presence/absence of predators.

The lower numbers of organisms (water insect larvae, Coelenterata, Gastropoda and Pelecypoda) may be indicative of their sensitivity towards very high metal concentrations from their surrounding water column and sediment compartment. Thus, the populations of organisms found in this system are significant in offering clues to the intensity of pollution and the consequent degree of recovery

3.4 **Recommendations**

3.4.1 *Monitoring*

The general objective of this section of the study was to investigate the water and sediment quality and bioaccumulation of metals in fish at selected sites along the Olifants River. This was achieved, showing that these catchments are impacted by present and past anthropogenic activities. The data gained from this study will not only be available for use in future management strategies aimed at reducing impacts on the Olifants River, but can also serve as a foundation for further investigations and monitoring. It is recommended that future research should at least address the following aspects:

* To identify specific sources/bodies/organisations directly responsible for point or diffuse sources of pollution affecting water quality in severely impacted areas. Tables 3.1 & 3.2 and Figures 3.2 & 3.3 could be used to identify specific areas of concern that need to be investigated and addressed. Special attention should be given to to the areas *with low pH water, while the sulphate and phosphate loads of the system should be* investigated and modelled. The party responsible for pollution must be informed about its impact on the river. It should then become their responsibility to develop and implement a management plan to reduce their impacts. Measures should be taken by the Department of Water Affairs and Forestry and other interested or affected parties, to ensure and enhance rehabilitation, as well as improvemnet of the water quality. The Olifants River Forum can play a prominent role in this regard.

* Due to the metal loads detected in the water and sediment of the Olifants River, it is recommended that DWAF should also implement a more frequent sampling programme to investigate metal loads, maybe similar to the procedures followed for macro-constituents. As mentioned previously, special attention should be given to dissolved metal concentrations as many of the proposed guidelines are set on this criterion. The speciation of metals in the water and sediment of the Olifants River should also be investigated to improve our understanding of the possible impacts of such metals on aquatic organisms.

* Bioaccumulation studies should in future be included in monitoring programmes because they not only identify possible metal polluted sites, but also result in the assessment of fish quality for consumption by humans. Bioaccumulation studies on fish should include at least 20 specimens of each species under investigation, to ensure more reliable results by reducing variation in the data. More than one sample of a specific tissue from a specimen (eg. muscle tissue being abundantly available) should be sampled and investigated to ensure more reliable data. These investigations should include species with different behavioural patterns and food requirements, for example one predatory and one omnivorous species. A few surveys should also be done to investigate the extent of accumulation of a metal in different age groups of the species under investigation. The combination of organs and tissues used in this study should be sufficient to give a reliable evaluation of the metal pollution status.

* A detailed monitoring programme must be implemented on the Olifants River. This programme should in future not only focus on the physical and chemical qualities of the river, but also include a well-designed biomonitoring component. As an initial phase the protocols of the national biomonitoring programme, currently being tested in the Crocodile River Catchment, can be adopted. These should then be expanded to incorporate bioaccumulation assessment for metals and biocides. Furthermore, on-site and laboratory toxicity testing should form the major bioassays aimed at the investigation of the impacts of pollutants on resident biota of the Olifants River. It is thus evident that a multi-disciplinary approach should be followed to ensure the future existence of a sustainable freshwater system.

3.4.2 *Experimental work*

From this study it was evident that a physiological effect was exerted on *O. mossambicus* causing changes in the haematology, osmotic and ionic regulation, and metabolism of these fish. Thus copper, zinc, aluminium and manganese concentrations recorded in the water of the Upper Olifants River Catchment, even at levels which are considered non-lethal, can have a detrimental effect on aquatic organisms. It is important to note that in freshwater ecosystems where chronic stress, resulting from metal pollution, is operative for a long time, the organism's ability to adjust behaviourally and/or physiologically may be reduced. If an organism does not adapt to these changes, the population's survival will be in danger in that specific ecosystem.

It is important to remember that in general metals rarely occur singly. Thus, for the purpose of environmental protection, it is necessary to know the lethal and sublethal toxicity of mixtures under various environmental conditions to various aquatic species (e.g., algae, macro invertebrates and fish). Therefore, future field monitoring studies should include experimental studies on the lethal and sublethal effects of metals, both singly and in mixtures, on biochemical and physiological processes under various environmental conditions, because this is of utmost importance to gain more knowledge of their interactions and toxic effects.

Laboratory experiments when fish (or other aquatic organisms) are exposed to different metals, according to toxicity, under controlled conditions, provide important information on lethal and sublethal levels of the metals and should be continued. This information is essential for the improvement of water quality guidelines for metals and the "health" status of fish can also be assessed. These experiments are also important in developing improved water quality guidelines for metals which could aid water quality managers, engineers as well

as consultants in assessing the impact of metals on the aquatic environment. Furthermore, the results also contribute to the development and expansion of an existing water quality index (WATER 2), which needs further refinement. The refined product of (WATER 2) for the Olifants River is in the final stages of preparation and is renamed to RAUWATER. This index could be tested for suitability of use in other river systems in South Africa.

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"LETHAL AND SUBLETHAL EFFECTS OF METALS ON THE PHYSIOLOGY OF FISH: AN EXPERIMENTAL APPROACH WITH MONITORING SUPPORT"

The Steering Committee responsible for this project consisted of the following persons:

Dr. PCM Reid	:	Water Research Commission (Chairman 1994)
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Mr. D Huyser	:	Water Research Commission (Committee Secretary)
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7. Glossary

acute - involving a stimulus severe enough to rapidly induce a response; in toxicity tests, a response observed in 96 hours or less is typically considered acute. An acute effect is not necessarily measured in terms of lethality, but can make use of a variety of effects. (Note that acute means short and not lethal).

anaemia - more red blood cells are lost, through haemolysis, than produced.

anthropogenic chemicals - chemicals with a synthetic origin (e.g. herbicides and insecticides).

bioaccumulation - refers to the combined uptake from the surrounding water food ingested as well as from the non-food particles ingested.

bioassays - a method for determining the relative biological activity (potency) of a chemical by comparing its effect on a living organism with the effect of a standard preparation on the same type of organism, under controlled conditions. A toxicity test is a type of bioassay that measures the toxic effects of a chemical.

bioavailability - the property of a toxicant that governs its effects on exposed organisms. A reduced bioavailability would have a reduced toxic effect.

bioconcentration - of a pollutant is generally taken to refer to uptake from the surrounding water.

biomagnification - refers to the increases in concentration of a pollutant in successive members of a food chain (for example: crabs -> fish -> birds/man) and can be seen as accumulation from the food consumed.

blood acidosis - the pH of blood becomes more acidic.

erythrocytes - red blood cells

erythrocytosis - increase in the number of erythrocytes

food chain - the scheme of feeding relationships by trophic levels that unites the member species of a biological community.

haematocrit - measurement of packed erythrocytes.

haematology - the study of blood and blood forming tissue.

haemoconcentration - inspissation of blood

haemodilution - dilution of blood.

haemolysis - the lysis of a suspension of red blood corpuscles.

haemopoietic tissue - blood-forming tissue.

homeostasis - the maintenance of the constancy of the internal environment of the body.

hypokalemia - reducing the potassium level of blood.

hypercalcemy - elevating the calcium level of blood.

hypoxia - a low PO_2 in the blood, or in specific tissues.

an **impact** - a change in the chemical, physical and biological quality or condition of a waterbody caused by external sources.

LC50 - lethal threshold concentration is an estimate of concentration of a test material which will produce a specific effect (immobilise or kill) 50% of the exposed test organisms in a specific period.

lethal - causing death.

leucocytes - decrease in the number of leucocytes.

leucopaenia - increase in the number of leucocytes.

mean corposcular volume (MCV) - size or status of red blood cells.

permeability - functional integrity of the membrane.

pollutant - any substance which when present renders water less fit for use.

sublethal - involving stimulus below the level that causes immediate death.

toxicology - the science treating of poisons and their effects.

toxic pollutant (toxicant) - any of thousands of natural or synthetic chemical substances which can cause adverse effects, even when present at low concentrations. These substances strongly sorb to suspended and bedded sediments and consequently are associated with long-term contamination. Toxic pollutants are often transformed to chemicals with lower, the same or higher toxicity, while others are resistant to degradation and bioconcentrate.

trophic level - one of the successive levels of nourishment in a pyramid of numbers, food web, or food chain; plant producers constitute the first (lowest) trophic level, and dominant carnivores constitute the last (highest) trophic level.

8. General Introduction

8.1 Overview and scope of study

Water quality deteriorates mostly due to man's activities. Activities that may lead to water pollution include the following: industries, urbanisation, mining, powerstations, agriculture and transport (Department of Water Affairs and Forestry, 1991). The use of metals for industrial, mining and agricultural purposes and the subsequent occurrence as trace contaminants have resulted in increasing loads thereof in the aquatic environment. Metal and organic pollution, acidification, mineralisation, and salinisation are consequences of diffuse and point source effluents from these activities. Contaminants such as metals find their way into surface waters where it poses a threat to the natural habitats of a variety of aquatic organisms.

Metal pollution is one of the major toxic pollutants in the world (Mason, 1991). The fact that heavy metals firstly can not be destroyed through biological degradation and secondly, have the ability to accumulate in the environment, makes these toxicants deleterious to the aquatic environment (Förstner & Prosi, 1979). Animals need many of these metals in trace concentrations for normal physiological function. When these concentrations are exceeded it may lead to altered physiological functions within an organism (Heath, 1991). All metals are potentially harmful to most organisms at some level of exposure and absorption. Some of these metals are toxic to most organisms even at the lowest concentration although they are also beneficial to living systems (Enk & Mathis, 1977). However, the susceptibility of aquatic organisms to metals is influenced by various physical, chemical and biological factors.

Information on the potential pollution of the Olifants River was supplemented by the results obtained in a project conducted by the Rand Afrikaans University in the Kruger National Park (Van Vuren *et al.*, 1994). Much work still has to be done to identify the presence and source of metal pollutants in the Olifants River between the Upper catchment and the western boundary of the Kruger National Park. Little is known about the potential pollution by mining, industrial and agricultural activities along the river before it reaches the Kruger National Park. It is important to determine the contribution of the above mentioned activities on the water quality of the river. Furthermore, the impact on the aquatic biota is very important in the conservation of the aquatic environment and needs urgent attention.

It is well documented that pollutants such as trace metals and organic compounds can be accumulated by aquatic biota (Förstner & Wittmann 1979; Ellis 1989; EPA 1991). Bioaccumulation - that is, the uptake and retention of chemicals in the body of an organism (Roux 1994) - may take place via absorption through the gills (e.g. fish and crabs) and/or through ingestion of contaminated food (EPA, 1985). The extent of bioaccumulation of a specific chemical can, however, be influenced by factors related to the organism itself (e.g. species, physiological condition, growth, age, sex, pollutant, interactions and physical/chemical features of the environment.

Monitoring of the bioaccumulation of metals in a river system is essential because it has the following advances:

⇨ Ascertaining the extent of accumulation, both temporal and spacial.

- ⇨ Assessing organism health.
- ⇨ Assessing possible effects on human health, if consumed.

Temporal changes in bioaccumulation will provide information regarding the trend of bioaccumulation, that will be used to identify stability, improvement or deterioration (Mance, 1987). Spatial monitoring may generate information to assist in the identification of potentially unknown areas with high concentrations, whilst at known discharges it will provide information regarding the area being affected. The monitoring of concentration levels in fish and any other organism, which are used as food is essential because it aids in the protection against the consumption of contaminated food which is a risk on human health (Van Vuren *et al.*, 1994).

The accumulation of pollutants in aquatic biota can ultimately affect factors such as survival and reproduction (Timmermans, 1993). For instance, in invertebrates it can affect life cycles or predator-prey interactions. The accumulated levels in aquatic organisms may also pose a risk to animals such as birds and mammals that feed on them, resulting in a decline in population numbers (Lloyd, 1992).

According to Hellawell (1986) fish are suitable organisms to use in biological biomonitoring for the following reasons: (1) Metals accumulate in their organs and tissues, (2) they are readily identified and present in large numbers and (3) their distribution are cosmopolitan. The monitoring of concentration levels in fish and other organisms that are used as food is essential, because it helps to protect people from the consumption of contaminated food. Furthermore, the detected levels can be judged against standards set for food in general (Mance, 1987). In addition, with water and sediment data, this can provide valuable information on metal concentrations in the aquatic environment. The present project focuses on the concentrations of selected water quality variables and metals in water, sediment and fish. Samples were collected from selected localities in the Olifants River, Klein Olifants River, from the source to Loskop Dam, after their confluence at Aasvoëlkrans. Middelburg and Witbank dams were also included in the surveys.

Manganese, copper and aluminium have been identified in completed projects as pollutants according to its general toxicity. Concentrations of these metals detected in the abiotic and biotic components of the Olifants River, Mpumalanga, as well as preliminary surveys in the study area in the upper section of the Olifants River which include Loskop Dam, lead to this selection (Seymore, 1994). With this information available, it was decided to expose fish to sublethal concentrations of these metals to establish physiological effects of these potentially harmful metals.

The general objective of acute tests with pollutants (e.g. metals) is to determine the concentration that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled laboratory conditions. The acute lethality tests have been useful in providing estimates of the concentration of pollutants (e.g. metals) that cause direct irreversible harm to organisms (such as fish). Furthermore, these tests provide practical means for (a) deriving estimates for upper limits of concentrations that produce toxic effects, (b) evaluating the effects of other water quality variables, such as pH, hardness and temperature on the toxicity of metal pollutants and (c) developing an

understanding of the concentration response relationships (Macek, *et al.*, 1978). The acute toxicity test exposures are also used to develop chronic and other associated sublethal tests.

Information concerning the sublethal effects of metals form an integrated part of the ecosystem health assessment programmes and of procedures followed to develop water quality guidelines for the environment (Roux *et al.*, 1994). Generally, concentrations that produce sublethal chronic effects are lower that those that produce more readily observable acute effects, such as death. Toxic effects of pollutants (e.g. metals) and therefore the health and well being of organisms (such as fish) after exposure were mainly evaluated by the above mentioned acute test when the death of the organism was the only criteria (Larsson *et al.*, 1985). The possible sublethal effects of pollutants were generally neglected. However, biochemical/physiological processes would be effected or even cease to function, generally before the onset of death. The sublethal effects of pollutants are usually biochemical/physiological in nature, since most of these pollutants effect the basic level of organisation that is the sub-cellular level in an organism. Data on the sublethal effects of pollutants would aid in the identification of pollution before drastic changes occur in the natural population. Information of sublethal effects of fish populations is therefore needed to develop realistic water quality guidelines and ecosystem health assessment.

To determine the subtle, non-lethal effects induced by sublethal concentrations on the physiology of fish, it is necessary to monitor certain haematological variables:

Red blood cell counts, white blood cell counts, haematocrit percentages, haemoglobin concentrations, mean corpuscle volumes, blood protein levels and the enzyme acetyl choline esterase activity (Wedemeyer & Yasutake, 1977).

- ⇨ The regulation of osmotic ionic concentration by aquatic organisms is essential for the maintenance of water balance and ion homeostasis. In order to evaluate the effects of metals on the osmoregulation variables such as sodium, potassium, chloride and calcium ions could be measured (Heath, 1987).
- ⇨ An increase in glucose and lactate concentration in the blood of fish are typical indicators of stress (Hattingh, 1976). The hormones glucocorticoid and catecholamine causes a classic change in the blood sugar levels induced by pollutants such as metals (Heath, 1987).

Enzymes involved in carbohydrate metabolism are sensitive to chemical stress which may result in a decrease in key enzymes of glycolysis (e.g. pyruvate kinase, glucose-6phosphatase) and of the Krebs cycle (Heath, 1987).

Standard techniques were employed to obtain values for the different variables measured after exposure to selected metal concentrations.

8.2 Metals and the Environment

Pollutants, especially metals, act as stressors in the aquatic environment and may affect the survival of the organisms involved. Metals are non-biodegradable and once they enter the environment, bioaccumulation may occur. Thus, the bioaccumulation of metal ions is ecologically very significant. Even though metals occur in nature, the trace concentrations needed for normal physiological function sometimes become excess concentrations. These excess concentrations of metals (metal pollution) are already dissolved in and mobilised by water. The organism absorbs it and the effects might be toxic. In nature, metals are common in the atmosphere, in natural waters and soil/rock. In the atmosphere the natural resources are surface waters, soils and vegetation as well as volcanic activity. Metals derive from rock weathering, land run-off and from atmospheric deposition to the surface waters. A major contribution to metal sources is man-made. These contributions are the combustion of fossil fuels, mining and smelting operations, processing and manufacturing industries, as well as waste disposal such as dumping, release of domestic sewage and scrap metal handling. The metal load also increases due to erosion caused by farming and forestry.

The circulation of metals occurs naturally in the biosphere, lithosphere, hydrosphere and atmosphere. The most common types of metal transport are:

- ⇨ Atmospheric transport, where metals are carried over distances by the wind and are then deposited, or are washed out of the air onto land or onto the surface waters.

- ⇨ Man (e.g. industrial effluent, mining activities and sewage) does aquatic transports, where the major metal transport system of metals emits to the environment. Rivers are an important transport medium. In lakes, it will settle in areas with active sedimentation. These sediments can release the metals again with the help of microbial activity and through change in various physical and chemical variables.

- ⇨ Biological transports where transport is carried out by living organisms. In nature, the metal ions occur as free aquo ions, simple complexes with inorganic ligands, chelates with multidentate organic ligands and absorbed onto particle surfaces.

The chemical form, in which the metals occur, influence the bioavailability and toxicity of metal ions to aquatic organisms. The chemical form of the metal is dependent on the water characteristics in which it is present. The water characteristics is controlled by the following variables:

- ⇨ pH
- ⇨ Redox potential.
- ⇨ Dissolved oxygen.
- ⇨ Ionic strength.
- ⇨ Salinity.
- ⇨ Alkalinity.
- ⇨ Hardness.
- ⇨ The presence of organic and particulate matter.

⇨ The biological activity of the solution.

Thus, the transformation between the different chemical forms is induced by changes in these variables.

Due to the rapid growth in mining, industrial and agricultural activities, as well as the growing human population, metal pollution is increasing at an alarming rate. The following metals are found in the upper reaches of the Olifants River at increased concentrations and reviewed in this chapter: zinc, copper, iron, chromium, nickel, manganese, lead and aluminium. Furthermore, these metals are also important according to toxicity and more information on the distribution and fluctuation in concentration would be valuable in assessing the pollution status of the upper reaches and tributaries of the Olifants River, Mpumalanga. The metals concerned were reviewed with regards to biological function, toxicity and occurrence.

8.3 Aims

Information on the potential pollution of the Olifants River was supplemented by the results obtained in a project currently conducted by us in the Kruger National Park. Much work still has to be done to identify the presence and source of metal pollutants in the Olifants River between the upper catchment and the western boundary of the Kruger National Park. Little is known about the potential pollution by mining, industrial and agricultural activities along the river before it reaches the Kruger National Park. It is important to determine the contribution of the abovementioned activities on the water quality of the river. Furthermore, the impact on the aquatic biota is very important in the conservation of the aquatic environment and needs urgent attention. More research on the impact of pollutants, and specifically metals, should be done to fully understand the effect on the survival and "health" of aquatic organisms.

Water and sediment quality is important and samples were taken at selected sites along the Olifants River and the tributaries in the coal mining areas near Witbank and Middelburg. These selected sampling sites included the major impoundments in the river system (Figure 8.1). Through these surveys it would probably be possible to establish the quality of river water, the changes in water quality and the concentration of metals in the river from the upper catchment until the entrance to the Kruger National Park. Surveys at sampling sites in the Kruger National Park were included.

Zoobenthos samples were taken to determine whether stream community structures experience any reduction in species diversity as a result of deteriorating water quality. Tissue and organ samples of selected fish species were also collected to determine the extend of bioaccumulation of metals in fish. The results will be compared to the values already obtained in the Kruger National Park and other river systems investigated.

Samples were taken seasonally and an attempt made to establish increases and decreases in metal concentrations in the fish sampled. Metal concentrations were compared to water quality and flow regimes of the river, since metal concentrations and water quality variables can fluctuate during seasons, floods and droughts. The surveys were conducted over 3 years to enabled the project team to monitor the situation for more than one seasonal cycle.

After the potential metal pollutants were identified according to toxicity and concentration, fish were exposed to these metals in an experimental flow through system under controlled laboratory conditions. The results obtained supplement the results already available in the research completed on metals with a potential detrimental effect on the physiology of riverine fish. At the same time the toxicity of other metals, which have not been identified or the effect on fish physiology not yet established, could be included in the experimental programme. Critical levels of metal concentrations will be further investigated which will be useful in the determination of water quality guidelines. The synergistic effects of metals in a specific water body also need much more research and should be dealt with in a specific project. Changes in haematological variable values as well as metabolic processes were used to assess the effect of the pollutants on the "health" and survival of freshwater fish.

Fish were exposed to sublethal concentrations of the toxic metals. These sublethal concentrations were selected from available LC_{50} values for the different metal pollutants in the Olifants River previously determined were by employing standard techniques. Metals present at high concentrations were also considered without performing LC_{50} determinations. Results from these experiments will supplement the existing information obtained the last three years.

The results obtained on the tolerance of fish to pollutants in the Olifants River and data from literature, made it possible to compile a water quality index for the Olifants River (Van Vuren *et al.*, 1994). Information from the present study is useful in refining the existing index that could be a valuable tool in the hands of water quality managers. This information will become available through dedicated as well as continuous monitoring and laboratory experimentation.

8.4 The Olifants river basin and locality description

8.4.1 *General description*

The Olifants River originates in the Bethal/Trichardt area and flows in an easterly direction before crossing the Kruger National Park into Mozambique (Fig 8.1). The Olifants River basin in the Transvaal drains a large area of over 54 575 km^2 (Theron *et al.*, 1991). The main tributaries of the Olifants River in the upper catchment of the system, from the source near Bethal, to its confluence with the Wilge River, north of Witbank, are the Klein Olifants River (with its tributaries being the Keori Spurt and Rietkuil Spruit), the Steenkool Spruit, Trichardt Spruit and Riet Spruit (which flow into the Olifants River before it joins with the Klein Olifants River) and the Klip Spruit, which flows into the Olifants River before its confluence with the Wilge River.

Topographically, the catchment falls mainly on the undulating highveld, between 1 200m to 1 800m above mean sea level. The climate is warm and the average temperatures show moderate fluctuation, with average summer temperatures varying between 18 °C and 26 °C, while the winter averages are between 0 °C and 13 °C. Frost may occur from May to September with seasonal rainfall occurring predominantly in summer from October to March (Theron *et al.*, 1991). The mean rainfall for the area is in the range of 650 - 800

mm. Evaporation is in the range of 75 - 190 mm per month with June the month with the lowest and December the highest evaporation.

The vegetation can be described as a grassland biome, which can be seen as an ecological unit, which represents large, natural and homogeneous areas of biotic and abiotic features. The biotic component is closely related to physical factors, particularly soil type and climate (Steffen, Robertson & Kirsten, 1991). Two veld types, namely Turf Highveld type, which is a pure grassveld that occurs in the upper reaches of the Olifants River tributaries, Steenkool Spruit and Trichardt Spruit and the false grassveld type, Bankenveld, which dominates the rest of the area (from the perspective of Acock's veldtypes). The vegetation has a uniform physical appearance and is dominated by hemicryptophytes of Poaceae and the number of threatened plant species is less than ten (Theron *et al.*, 1991). Soil erosion is also limited because of the high vegetation cover.

The main geological outcrops are the bushveld Complex, the Waterberg Group, the Karoo Supergroup and the major Dyke/Sill intrusives. Structurally the most significant feature within the catchment is the intrusion of the Bushveld Complex into older Transvaal sediments. Dolerite, mostly in the form of sills, has intruded the Karoo rocks in the catchment. The chemical make-up and geometry of these sill intrusives are not conducive to groundwater development, except along the contact zones where sedimentary strata are often fractured (Theron *et al.*, 1991).

8.4.2 *Sources of water*

8.4.2.1 Groundwater

Two hydrological regions can be found namely the Karoo Sequence Region and the Granitic Region. The Karoo Sequence Region in the Middelburg and Witbank area, is made up of interbedded shale, sandstone and coal strata, with intrusive dolerite and ultramafic dykes, which will provide good borehole sites, and sills. The Granitic Region is confined to all areas where granite, gneiss and granitic type rocks are found in the area. Boreholes are at present an important source of water supply to rural, domestic and stock watering and are expected to yield in the region of 1.5 l/s to 5 l/s. Although roughly 70 % of the Upper Olifants River Catchment has high to moderate groundwater potential, dry boreholes are becoming more plentiful. The chemistry of groundwater is greatly influenced by the media with which it has been in contact, as well as the duration of the contact. The dissolution of minerals from a rock produces water that contains the same minerals. Two or more types of water from different geological origins can also combine to produce a composite groundwater assemblage. Possible groundwater types associated with various geological sequences are thus subject to migration influences (Theron *et al.*, 1991). Sodium, calcium, silica, iron and magnesium concentrations will dominate groundwater originating from the granitic type rocks and the degree of mineralisation is expected to be moderately high. The rocks of the Bushveld complex are made up of ferro-magnesian silicates and the resulting groundwater will be mineralised with iron, magnesium, calcium, sodium and phosphate dominating (Theron *et al.*, 1991)

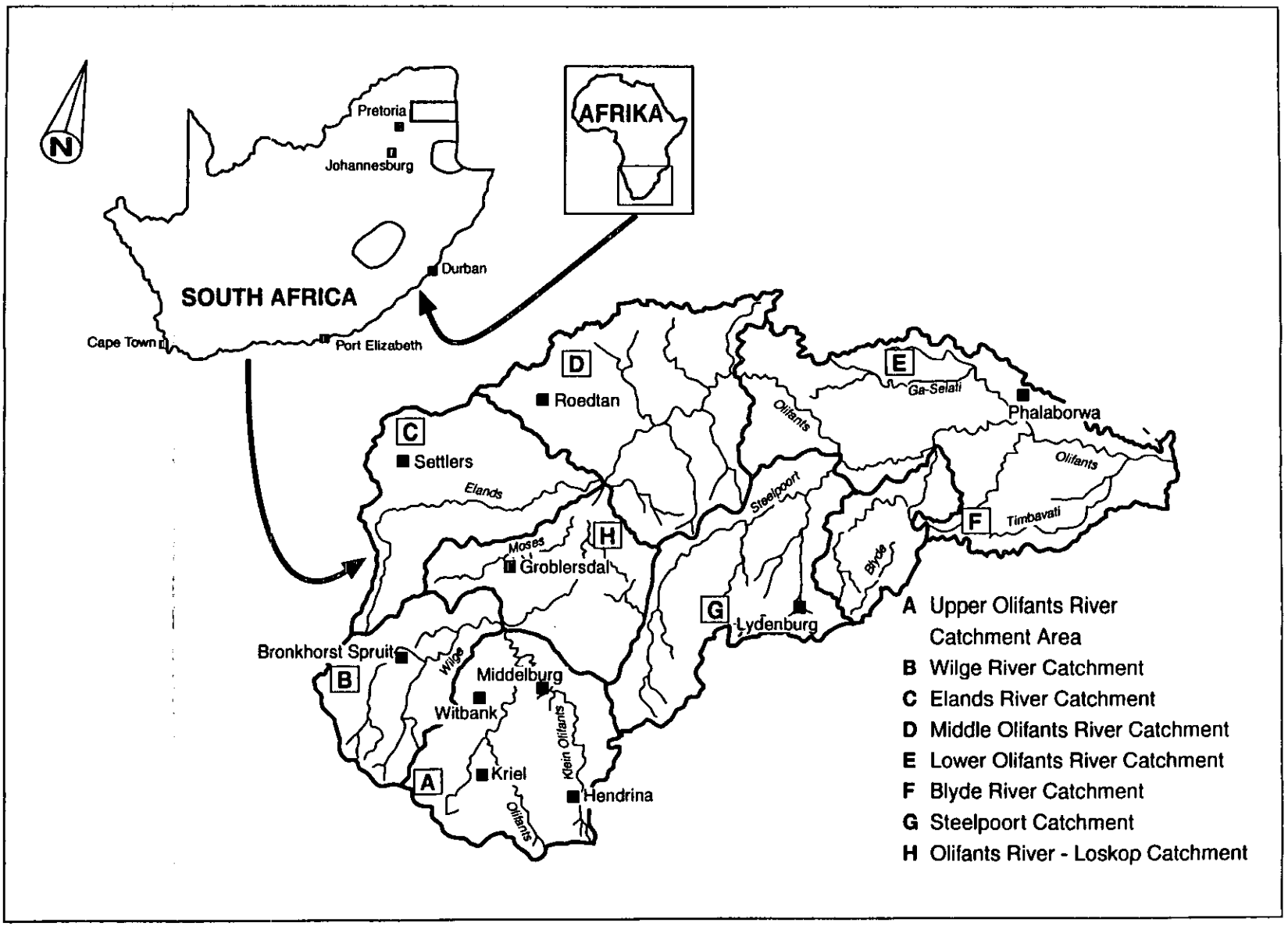

Figure 8.1: The Olifants River Catchment, indicating the different sub-catchments, in particular the Upper Olifants River Catchment

8.4.2.2 Surface water

Dams play an important role in supplying water at a high level of assurance. In the Olifants River catchment in South Africa, thirty major dams, with a total percent storage capacity of 1 064.87 million m^3 are on record. Over 2 000 dams can be found with a surface area of less than 1 ha and a volume of less than 20 000 m^3 (Theron *et al.*, 1991). The dams in the Upper Olifants River catchment can be divided into three categories (Fig 8.2):

- ⇨ Major dams which have a great impact on the runoff regime in a river and can be defined as those that perform a major function in the community and have a capacity greater than 2 million m^3. In the upper catchment, the Witbank, Middelburg, Doornspoort, Riet Spruit and Trichardsfontein Dams fall into this category.

- ⇨ Small dams, which are defined as those individually insignificant with regards to the hydrology of a basin. Small dams have a capacity of less than 100 000 m^3. Small dams in the upper catchment are mainly used for stock-watering and soil conservation (Theron *et al.*, 1991).
- ⇨ Minor dams that are defined as those dams which are significant only on a local level. There are over 200 minor dams in the Olifants River basin, each with a capacity of between 100 000 m^3 and 2 million m^3 and generally supply only one institution with water. In the Upper Olifants River Catchment, minor dams are used for irrigation, mining, stock watering, recreation and power generation (Theron *et al.*, 1991).

Rainfall is the most important determinant of runoff and occurs predominantly in summer in the form of showers and thunderstorms. Runoff at the major impoundments, Witbank Dam and Middelburg Dam decreased from 125 million m^3/annum at Witbank Dam and almost 44 million m^3/annum at Middelburg Dam to 107.4 and 37 million m^3/annum respectively. This is due to the effect of afforestation, irrigation and evaporation from minor and small dams on the natural runoff at the dams. It is expected that the runoff will decrease further to 104 and 36 million m^3/annum respectively by the year 2010, due to possible increase in irrigation and minor and small dams.

8.4.2.3 Re-use of effluent

The re-use of effluent as a source of water is important, particularly in areas of urban and industrial growth. Effluent should therefore be considered as a supplementary source that can be purified to different standards for various categories of use, for example industrial consumers that are presently supplied with raw water from Witbank or Middelburg Dams. Re-use of effluent would however involve the cost of additional purification and also the installation of pumping systems, therefore there is at present no significant merit in adopting the reuse of effluent (Theron *et al.*, 1991).

8.4.3 *Water user sectors*

Most of the water in the Upper Olifants River catchment is used for afforestation, mining and power generation, irrigation, domestic and industrial purposes, as well as for

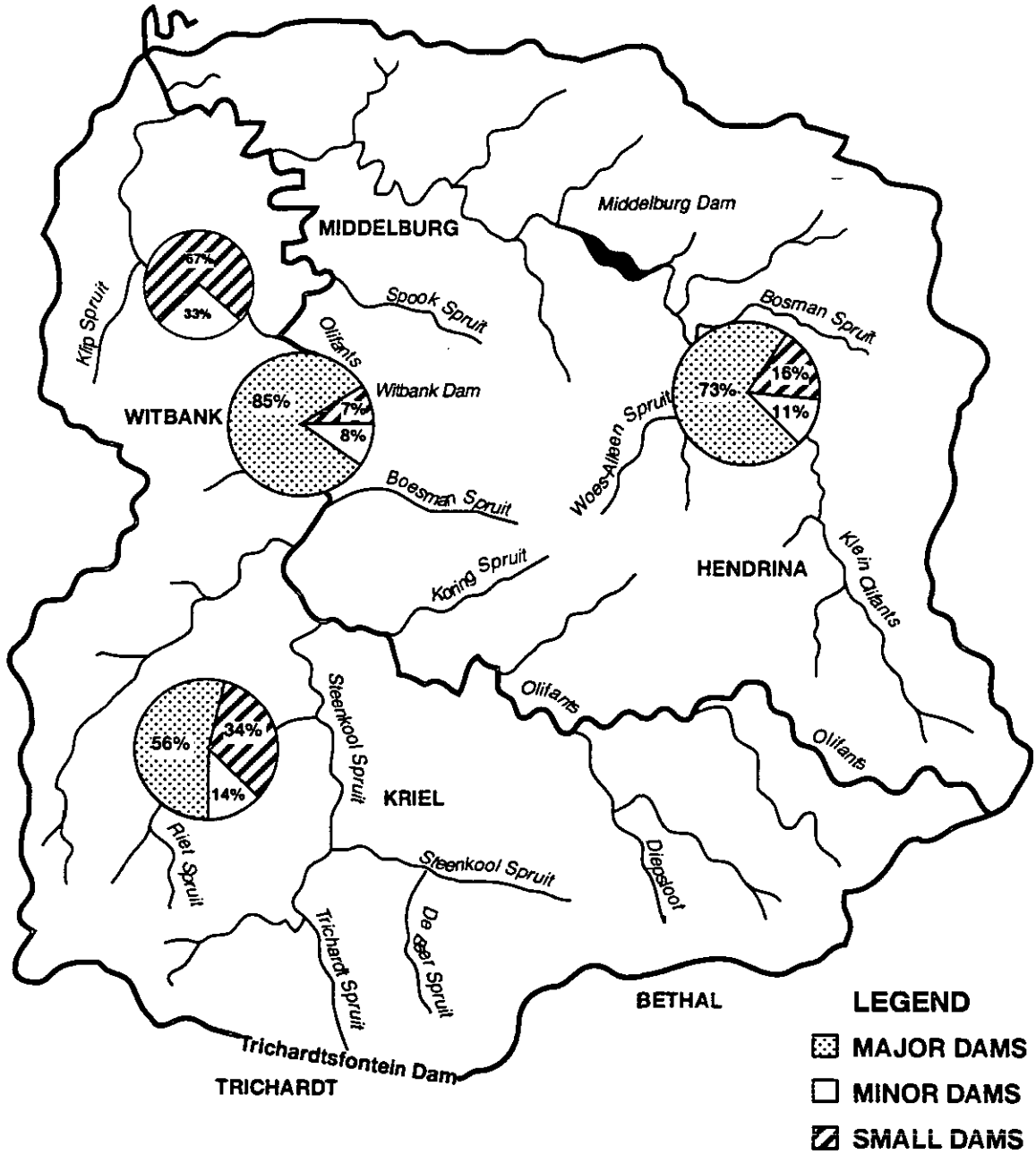

Figure 8.2: Percentage of water stored in dams in the Upper Olifants River Catchment

maintaining the ecological systems. The locations of all the major abstractions are showed in Figure 8.3 (See Table 8.1).

8.4.3.1 Afforestation

Although conditions are not ideal for afforestation due to low rainfall, approximately 17 680 ha of exotic afforestation occurs in the Upper Olifants River Catchment, which is concentrated around Witbank and Middelburg in the catchment of the Olifants and Klein Olifants Rivers respectively. No significant indigenous forests occur and only 7 300 ha are managed as commercial forests. The balance comprises stands of unattended wattles, pines and gums. The timber produced is used for mining poles, pulping and charcoal. No new afforestation is expected, as natural climatic conditions limit the suitability of the area for afforestation. Present exotic plantations decrease the mean annual runoff by ± 5% (Theron *et al.*, 1991)

8.4.3.2 Power generation

The upper catchment of the Olifants River system, with its tributaries, drains a portion of the Mpumalanga highveld, where most of the thermal power stations in the country are located, as six of the eight power stations are situated in the upper catchment. These power stations receive imported water, with the Komati- , Hendrina- , Arnot- and Duhva power stations receiving water from the Komati system and Kriel- and Matla power stations from the Usutu system. Each power station is designed to use water of a specific quality, with the TDS concentrations being one of the most important variables in determining the necessity for pre-treatment of the water, used for purposes like the cooling of the circuits, for steam generating circuits, utilising demineralised water and for the transportation of ash from boilers to ash dams. Power stations are one of the most important sources of heat pollution, which can have serious effects on the aquatic environment and its organisms, since it may change the natural temperature range of the water, while a decrease in dissolved oxygen is also experienced with increasing temperatures.

8.4.3.3 Mining

The operating mines in this region consist of 37 coal, 6 brick, 17 sand, 4 felsite and 7 clay mines. These mines used both surface and underground water, but now mainly consume imported water from neighbouring catchment. Mining consumption presently totals about 22 million m^3 water per year of which 10.7 million m^3 are imported and 7.2 million m^3 are from surface water sources (Theron *et al.*, 1991). With the development of new power stations in the area, the coal-mining sector also expanded, with the subsequent increased demand for water.

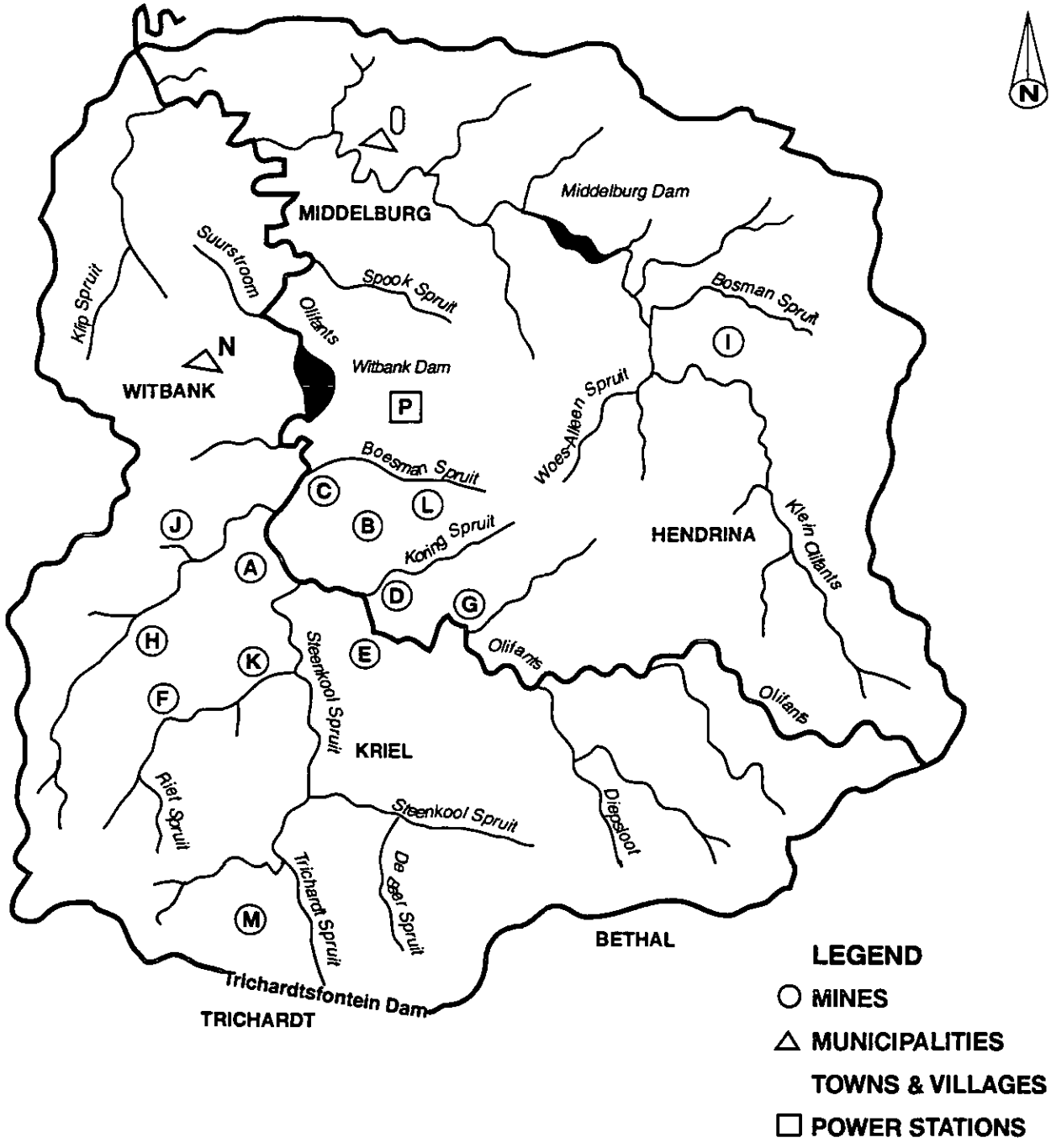

Figure 8.3: The major abstaction points in the upper Olifants River Catchment, with the mines indicated as A-M, the towns as N & O and the power station as P. For a description see Table 8.1.

Table 8.1: Major abstractions in the study area

No. (See Fig. 8.3	Abstraction	Source of abstraction
A	Albion colliery	Steenkool Spruit
B	Douglas colliery (Douglas section)	Olifants River (Douglas Dam) Imported
C	Douglas colliery (Wolwekrans section)	Olifants River
D	Douglas colliery (Van Dyksdrift section)	Olifants River
E	New Clydesdale	Olifants River
F	Tavistock colliery	Olifants River/Phoenix Dam
G	Transvaal Navigation colliery	Olifants River/Underground
H	Witbank Consolidated colliery	Zaaiwater Spruit
I	Arnot colliery	Klein Olifants River/Imported
J	Klienkopje colliery	Olifants River
K	Riet Spruit Opencast Services	Riet Spruit Dam
L	Duhva Opencast Services	Witbank Dam/Imported
M	Zwakfontein Sand	Trichardt Spruit/Underground
N	Witbank	Witbank Dam
O	Middelburg	Middelburg Dam
P	Duhva Power Station	Witbank Dam

8.4.3.4 Irrigation

Irrigation is a major water use sector in the Upper Olifants River catchment and at present 4 760 ha of land is irrigated. Huge areas under irrigation carry fodder crops and posture to support the extensive livestock farming activities. Maize and potatoes, followed by vegetables, lucerne, groundnuts and dried beans are irrigated in this area. Water used for irrigation comes from the Olifants River and its tributaries. Development for irrigation is estimated to be about 1 800 ha which will increase the water demands by ± 9.9 million m^3 per year, making the total water required for irrigation almost 35 million m^3 per year. Water availability is, however, the main restriction on irrigation use, but if not, ± 25 million m^3 per year would be abstracted in the sub-catchment (Theron *et al.*, 1991).

Above the Witbank Dam however, difficulty is experienced with the water quality, for example runoff in the Koring Spruit is unsuitable for irrigation, while in a number of other areas low flows, in particular, can be of such poor quality that crops and soils cannot be irrigated. Runoff in the Klein Olifants River, the Wilge River and Kranspoort Spruit is, however, of good quality for irrigation purposes.

8.4.3.5 Stock watering

The highveld is traditionally a cattle-farming district, with the large stock population in the Upper Olifants River catchment ± 457 000 large stock units (LSU) which use an estimated amount of 8.7 million m^3 per year. Stock watering relies on surface water, springs and boreholes for water supply.

8.4.3.6 Domestic and industrial use

Seven towns are situated in the Upper Olifants River Catchment. The Witbank/ Middelburg complex is a major industrial area with smaller towns Kinross, Trichardt, Hendrina and Ogies. Several magisterial districts fall partly inside the catchment boundaries, namely Bethal, Belfast, Ermelo, Highveld Ridge, Middelburg and Witbank. The total domestic and industrial water demand is expected to be 65.8 million m^3 for the year 2010, based on the probable population size (Theron *et al.*, 1991). Of this projected demand, almost 67% will be by Witbank and the township, Kwa Guqu, from the Witbank Dam and 26% by Middelburg and the township, Mhluzi, from the Middelburg Dam. A total of 36.6-million m^3 water per year is presently used for domestic purposes in the Upper Olifants River Catchment, which can be divided into:

- ⇨ 33.6 million m^3 surface water from the Bronkhorst Spruit Dam, the Middelburg Dam and the Witbank Dam, which serves Ogies, Middelburg and Witbank respectively.
- ⇨ 2.3 million m^3 from boreholes, used by the majority of the rural population.
- ⇨ 0.9 million m^3 is imported water supplied by Rand Water to Kinross and Trichardt, the Usutu-Vaal Scheme to Davel and by the Nooitgedacht-Komati pipeline to Hendrina (Theron *et al.*, 1991).

The industrial towns of Middelburg and Witbank are expected to grow rapidly and due to the enormous population growth, accelerated water demand is therefore also expected.

8.4.4 *Water quality*

It is important to take into account the different sources of pollution, which can be divided into two categories:

8.4.4.1 Point sources of pollution

Mines: Much of the pollution in this catchment is due to the extensive mining activities. Generally, coal, brick, sand, felsite and clay mines are found in the Olifants River catchment, with coalmines most commonly found in the upper catchment. Coalmines generally have an influence on the water pH, turbidity and total dissolved salt concentrations (Fig 8.4; Table 8.2).

Industries: Different industries have different effluent discharges, for example the vanadium industry contributes to the sulphate load in the water. These industries do not have permits to discharge effluents into any river and all their effluent is disposed off through the municipal sewage systems.

Sewage treatment works: There are a number of sewage treatment works in the area:

- ⇨ The Hendrina sewage treatment works, which is at present overloaded and it is necessary to double the size of the works to cope with the expansion of the Jonkerville township.
- ⇨ Two sewage works at Kinross, which discharge, into tributaries of the Vaalbank Spruit.

- ⇨ Newer works that are situated on the farm Zondagskraal and serve the western part of Kinross and the rehabilitation school.
- ⇨ Middelburg or Boschkraans sewage works that is an activated sludge system.
- ⇨ The Trichardt sewage works where the effluent is discharged into the Trichardt Spruit.
- ⇨ The Riverview works that discharges into the Olifants River.
- ⇨ The Ferrobank works which discharge into the Brug Spruit, a tributary of the Klip Spruit.
- ⇨ The Naauwpoort sewage works where the effluent is discharged into a spruit that flows into Witbank Dam.
- ⇨ The Davel sewage effluent is processed in an oxidation ponds system, where the effluent is evaporated, thus there is no discharge into the river system.
- ⇨ The origin, volume and concentration of the effluent of these point sources can be quantified (Theron *et al.*, 1991).

8.4.4.2 Non-point sources of pollution

- ⇨ **Agriculture and forestry** where irrigated areas are a diffuse source of nutrients like nitrates and phosphates, as well as biocides. Surface runoff and high loads of suspended solids may be a result of the physical disturbance of soil and vegetation (Dallas & Day, 1993). Irrigation and subsequent evaporation of water from the land can result in concentration of dissolved solids and therefore high TDS (Theron *et al.*, 1991). Algal growth can be stimulated by nutrients, from disturbance of land and application of fertilisers and livestock excreta that enter receiving water bodies.

- ⇨ **Surface runoff** from urban areas where pollutants such as soil, garden- chemicals and pet wastes are carried to the river system via storm water drains. Potential sources also include road pavement materials, motor vehicles, atmospheric fallout, litter, domestic spraying and unauthorised dumping and washing. Contaminated groundwater can also seep to surface streams.

The volumes and concentrations of effluent from non-point sources of pollution cannot be quantified and the origin is diffuse (Theron *et al.*, 1991).

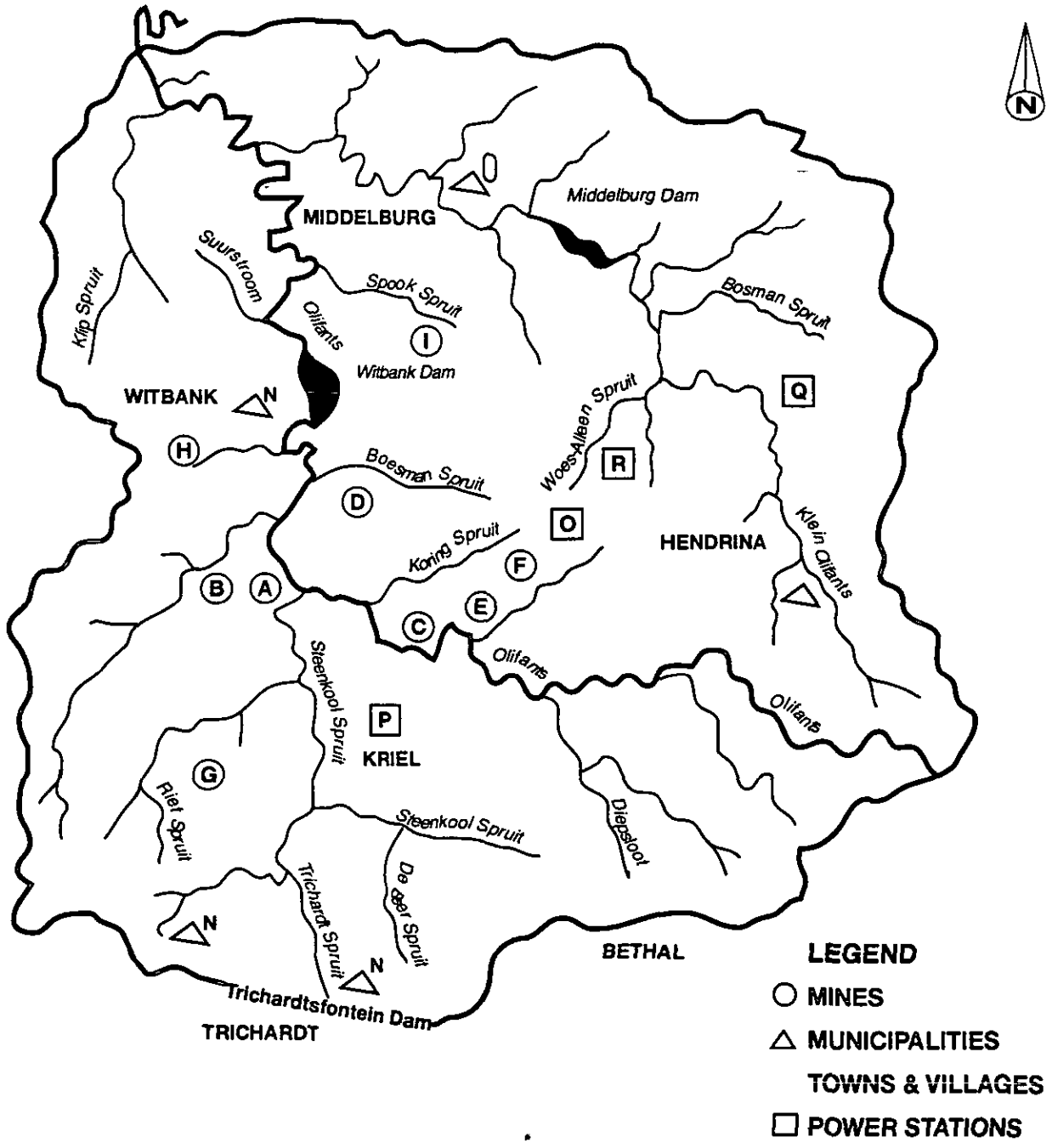

Figure 8.4: Major affluent discharge points in the Upper Olifants River, mines indicates as A - I, municipalities as J - N and the power stations as O - R. For description, see Table 8.2

Table 8.2: Major effluent discharge points

No. (See Fig. 8.3)	Effluent	Point of return
A	Albion colliery	Steenkool Spruit
B	Tavistock collieries	Zaaiwater Spruit
C	Transvaal Navigation	Olifants River
D	Douglas colliery (Douglas section)	Douglas Dam
E	Goedehoop colliery	Olifants River
F	Blinkpan colliery	Koring Spruit
G	Matla colliery	Riet Spruit
H	Greenside colliery	Witbank Dam
I	Middelburg Mine Services	Niekerk Spruit
J	Witbank	Olifants River/Brug Spruit
K	Middelburg	Klein Olifants River
L	Hendrina	Klein Olifants River
M	Trichardt	Trichardt Spruit
N	Kinross	Vaalbank Spruit
O	Komati Power Station	Koring Spruit
P	Kriel Power Station	Steenkool Spruit
Q	Arnot Power Station	Bosman Spruit
R	Hendrina Power station	Woesalleen Spruit

8.4.5 *The study area*

Due to the vastness of the total Olifants River catchment, the study was focused on the upper catchment (localities 1 to 16). This area covers the Upper Olifants River and its many tributaries from their origin down to just below the Loskop Dam wall. Two localities were also selected in the Lower Olifants River inside the Kruger National Park namely, Mamba weir (locality 17) on the western border where the Olifants River enters the park, and Balule weir (locality 18) approximately 30 km downstream (Fig. 8.5). The final survey included two new localities: the Phalaborwa Barrage (locality 19) which was selected to evaluate the quality of the Olifants River before its confluence with the Selati River and the Nhlanganini Dam (locality 20) in the Nhlanganini River, a tributary of the Letaba River which is part of the Olifants River system which was selected as a control. This dam ought to be "less polluted" as the majority of its catchment is situated inside the KNP.

8.4.5.1 Upper Catchment:

⇨ **Locality 1**: A locality in the most upper reaches close to the origin of the Olifants River near a town called Davel.

⇨ **Locality 2**: A locality in the Koring Spruit South of Van Dyksdrift.

⇨ **Locality 3**: A locality in the Steenkool Spruit before its confluence with the Riet Spruit.

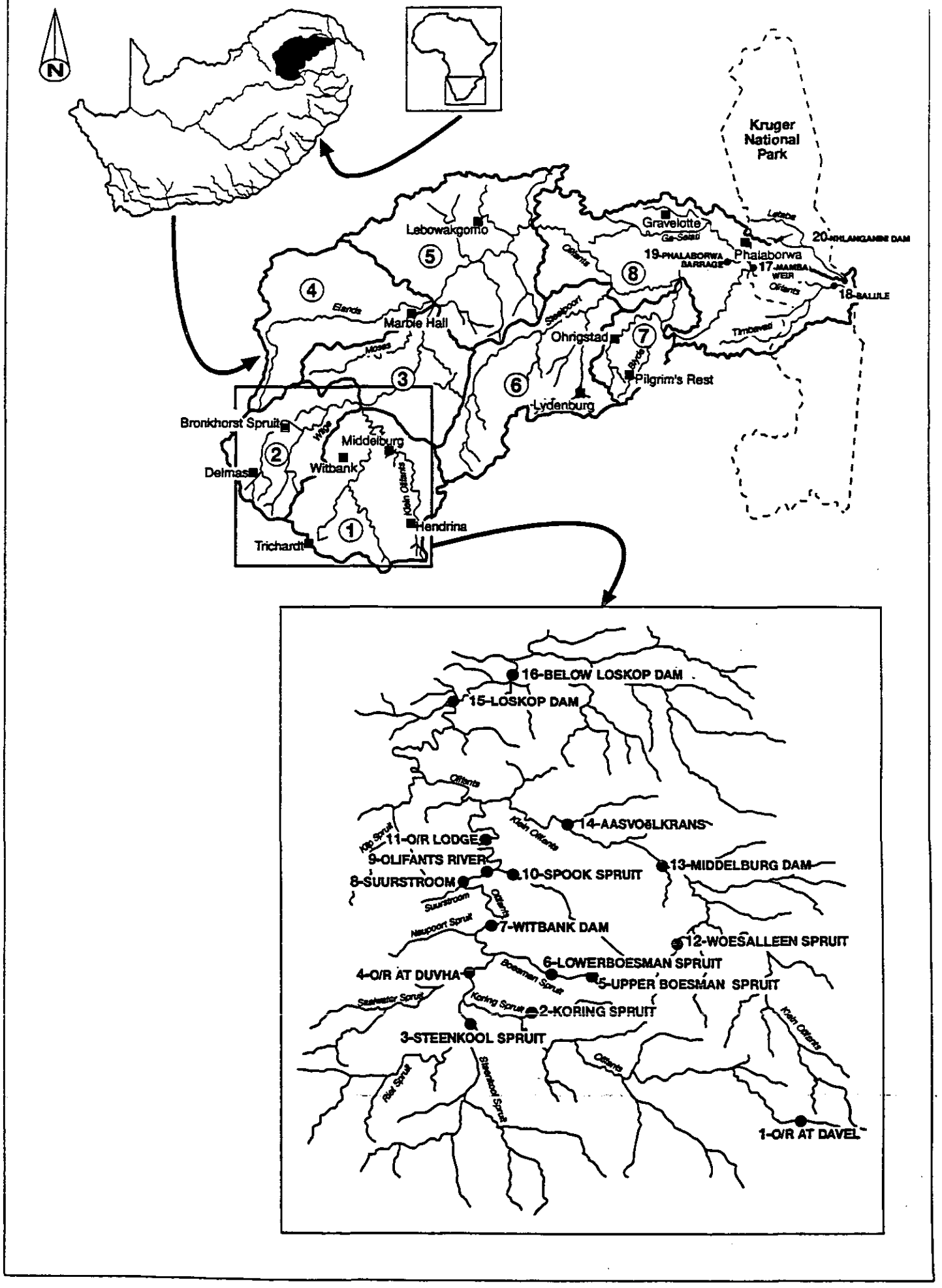

Figure 8-5: Sampling sites in the study area

⇨ **Locality 4**: A locality in the Olifants River upstream of the Witbank Dam.

⇨ **Locality 5**: A locality in the upper reaches of the Boesmankrans Spruit before it flows through a coal mining area.

⇨ **Locality 6**: A locality in the lower reaches of the Boesmankrans Spruit before its confluence with the Olifants River (after passing through a mining area).

⇨ **Locality 7**: Witbank Dam - this impoundment on the Olifants River is the biggest municipal dam in the country, with a storage capacity of 10 402 million m^3. It provides water for urban and industrial use in the Witbank area. Compensation releases for Loskop Dam are made monthly which influences the flow of the river between these two dams.

⇨ **Locality 8**: Suurstroom - A locality in a small stream arising from mine drainage flowing into the Olifants River between Witbank and Middelburg.

⇨ **Locality 9**: A locality in the Olifants River after it passes the urban and industrial areas of Witbank.

⇨ **Locality10**: A locality in the Spook Spruit before the confluence with the Olifants River.

⇨ **Locality11**: A locality in the Olifants River between Witbank and Middelburg at Olifants River Lodge.

⇨ **Locality 12**: A locality in the Woesalleen Spruit before its confluence with the Klein Olifants River.

⇨ **Locality 13**: Middelburg Dam - an impoundment on the Klein Olifants River close to Middelburg. It has a storage capacity of 47,9 million m^3 and mainly supplies the town of Middelburg with domestic water.

⇨ **Locality 14**: A locality in the Klein Olifants River in the vicinity of Aasvoëlkrans, after it passes through Middelburg.

⇨ **Locality 15**: Loskop Dam - this is the largest dam in the Olifants River basin, with a storage capacity of 348,1 million m^3. The major land use sectors are irrigation, domestic and industrial.

⇨ **Locality 16**: A locality in the Olifants River just below the Loskop Dam wall.

8.4.5.2 Lower Catchment:

⇨ **Locality 17**: Mamba weir (KNP) - A locality in the Olifants River, after it crosses the western boundary of the Kruger National Park. It is ± 15 km downstream of the Phalaborwa Barrage and ± 10 km downstream of the Selati-Olifants River confluence.

⇨ **Locality 18**: Balule weir (KNP). This is a locality in the Olifants River ± 40 km downstream of locality 17 (Mamba weir).

⇨ **Locality 19**: Phalaborwa Barrage. This dam has a storage capacity of 4,5 million m^3 and provides water to the towns, mines and industries in the area.

⇨ **Locality 20**: Nhlanganini Dam. A dam built for water provision to game in the Letaba River, a major tributary of the Olifants River. This site was sampled as a control because there are no known anthropogenic activities affecting its water quality.

The localities were visited seasonally (summer/autumn/winter/spring) from February 1994 to May 1995 (Locality 19 & 20 only once during a final survey) during which time selected water quality properties were measured on site, and water samples were collected for macronutrient analyses. Sediment and water samples were also collected for metal content analysis. Bioaccumulation of selected metals in the organs/tissues of fish was investigated in three fish species *Labeo umbratus*, *Oreochromis mossambicus* and *Clarias gariepinus*. These were sampled seasonally at localities 3, 7, 11, 13, 15 and 17 and once during autumn 1995 at localities 19 and 20.

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- *Water Resources planning of the Olifants River Basin.* Demography of primary water users. Annexure 6. 5 pp.
- *Water Resources planning of the Olifants River Basin.* Water use for domestic, industrial, mining and power generating purposes. Annexure 8. 67 pp.
- *Water Resources planning of the Olifants River Basin.* Irrigation. Annexure 9 Part 1. Sub-catchments B100, B200 and B320 upstream of Loskop Dam. 7 pp. *Water Resources planning of the Olifants River Basin.* Water availability from major dams. Annexure 16 Part 1. Basin upstream of Loskop Dam. 52 pp.
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9. Monitoring

9.1 Physical and chemical water quality variables at selected sites in the Olifants River.

9.1.1 *Introduction*

Water quality as defined by DWAF (1995), refers to the physical, chemical, biological and aesthetic properties of water which determine its fitness for use and for the protection of the health and integrity of aquatic ecosystems. The aquatic biota living in this substance are directly dependent on the state of this medium. As they are developed to survive in relative narrow water quality ranges in the natural state, any dramatic change in the quality of their environment could lead to a shift in equilibrium either positive or negative. This could be detrimental to the aquatic organisms occurring in such an ecosystem and it is therefore important to monitor the changes in water quality. The quality of the water is controlled or influenced by various constituents or variables (eg. temperature, pH, dissolved or suspended substances) which form a complex aqueous solution that has a specific quality. When a chemical compound enters an aqueous solution it will dissociate into its different ions which can again form compounds with other ions in the water. The species of a chemical constituent, reacting additively, synergistic or antagonistic with the other constituents, will determine the final quality of the water (Mason, 1991; Dallas & Day, 1993). Water quality could therefore be seen as a complex integrative system with constituents which are interdependent of each other.

Due to various anthropogenic activities in the catchment of the Olifants River, the water quality of this river system have been degraded over the past few decades (Seymore *et al.*, 1994). The rich coal mines in the upper catchment as well as the mining of other mineral deposits in the upper, middle and lower catchment contributed largely to this deterioration. Industrial development and urbanisation have furthermore increased pollutant runoff into the river (see Chapter 8). This is of concern since freshwater systems in South Africa are scarce resources that are deemed essential for the future development of the country. In this sense, the Olifants River plays a cardinal role in the Mpumalanga and Northern Province of South Africa, for the provision of drinking water, water for mining, industrial and agricultural activities as well as for the functioning of the aquatic and riparian ecosystems. Furthermore, from a conservational point of view, the Kruger National Park is the most downstream user of this system in South Africa. Deterioration in the quality of the water has already impacted on the aquatic biota of this stretch of the Olifants River causing the loss of several fish species (Dr. Deacon, personal communication, 1994). This is in direct contrast with the policy of the National Parks Board which stands for the protection of species diversity. Keeping all of these important aspects in mind, further deterioration of the water quality of this river can not be afforded.

This section of the study investigated the occurance of various physical and chemical water quality constituents in the upper and lower Olifants River system.

9.1.2 *Materials and methods*

Seasonal surveys were conducted from summer 1994 to autumn 1995 at 18 selected localities in the Olifants River catchment, Mpumalanga. Sixteen of these sites were situated in the upper catchment and two in the lower catchment (Chapter 8 - Fig. 8.5). During the autumn 1995 survey samples were also collected from the Phalaborwa Barrage (locality 19) to evaluate its present state and to asses the impact of the Selati River on the Olifants River. Nhlanganini Dam (locality 20) was also sampled during this survey as a control site since relatively natural conditions prevail here (Chapter 8 - Fig. 8.5).

The following surface water quality variables were measured on site: pH (ORION, Model SA250), water temperature (WTW microprocessor, Model OXT 96), dissolved and percentage oxygen saturation (WTW microprocessor, Model OXT 96), turbidity (Nephelometer ANALITE 152) and conductivity (Jenway, Model 4070). One surface water sample was also collected at ±10 cm beneath the surface, preserved with mercuric chloride ($HgCl_2$) and refrigerated until analysed by the Institute for Water Quality Studies (IWQS) for ammonia (NH_4-N), nitrate and nitrite (NO_3+NO_2-N), fluoride (F^-), total alkalinity (as $CaCO_3$), sodium (Na^+), magnesium (Mg^{2+}), silica (Si), phosphates (PO_4-P), sulphates (SO_4), chloride (Cl^-), potassium (K^+), calcium (Ca^{2+}), total dissolved solids (TDS) and electrical conductivity at 25°C. The *Statgraphic 7* computer package was used for the statistical analysis.

9.1.3 *Results*

The physical and chemical water quality variables of the surface water, measured during every survey at the selected localities in the study area are presented in Figures 9.1 to 9.19. In the results, reference is made to the upper catchment including localities 1 to 16, and the lower catchment including localities 17 to 20 (Chapter 8-Fig. 8.5). Note that values given in brackets refer to the median level ± the standard deviation of a variable.

Water temperature (Fig. 9.1) in the upper catchment ranged from 7.2°C (locality 8-winter 1994) to 30.7°C (locality 16-summer 1994) and for the lower catchment from 6.9°C (locality 18-winter 1994) to 29.4°C (locality 17-summer 1995). As can be expected temperatures were the lowest during winter months increasing towards the summer months. With declination in altitude going from the highveld towards the lowveld, temperatures increased. The most prominent seasonal temperature fluctuation was detected at locality 5 (11.8 to 28.7°C) and at localities 1, 2, 11 and 16. Oxygen saturation (Fig. 9.2) in the upper catchment varied between 34% (locality 8-summer 1995) and 204% (locality 9-winter 1994) and in the lower catchment between 77% (locality 20-autumn 1995) and 152% (locality 18-summer 1995). Locality 6 had the lowest oxygen saturation (56±10%) and dissolved oxygen (4±1.3 mg/l) while relatively low levels of oxygen saturation were also measured at locality 3 (76±24%) and locality 20 (70%). Locality 9 displayed a major temporal oxygen saturation fluctuation, ranging from 47% (summer 1995) to 204% (winter 1994), with localities 1, 8, and 18 also having relatively high variation. The largest fluctuation in dissolved oxygen (Fig. 9.3) was observed at localities 18 (8.5 to 27.3 mg/l), 9 (3.4 to 19.5 mg/l) and 8 (2.6-13.2 mg/l).

Turbidity levels (Fig. 9.4) in the upper catchment ranged from 1 NTU (localities 13 & 16) to 100 NTU (locality 5) and in the lower catchment from 4 NTU (locality 17) to 60 NTU

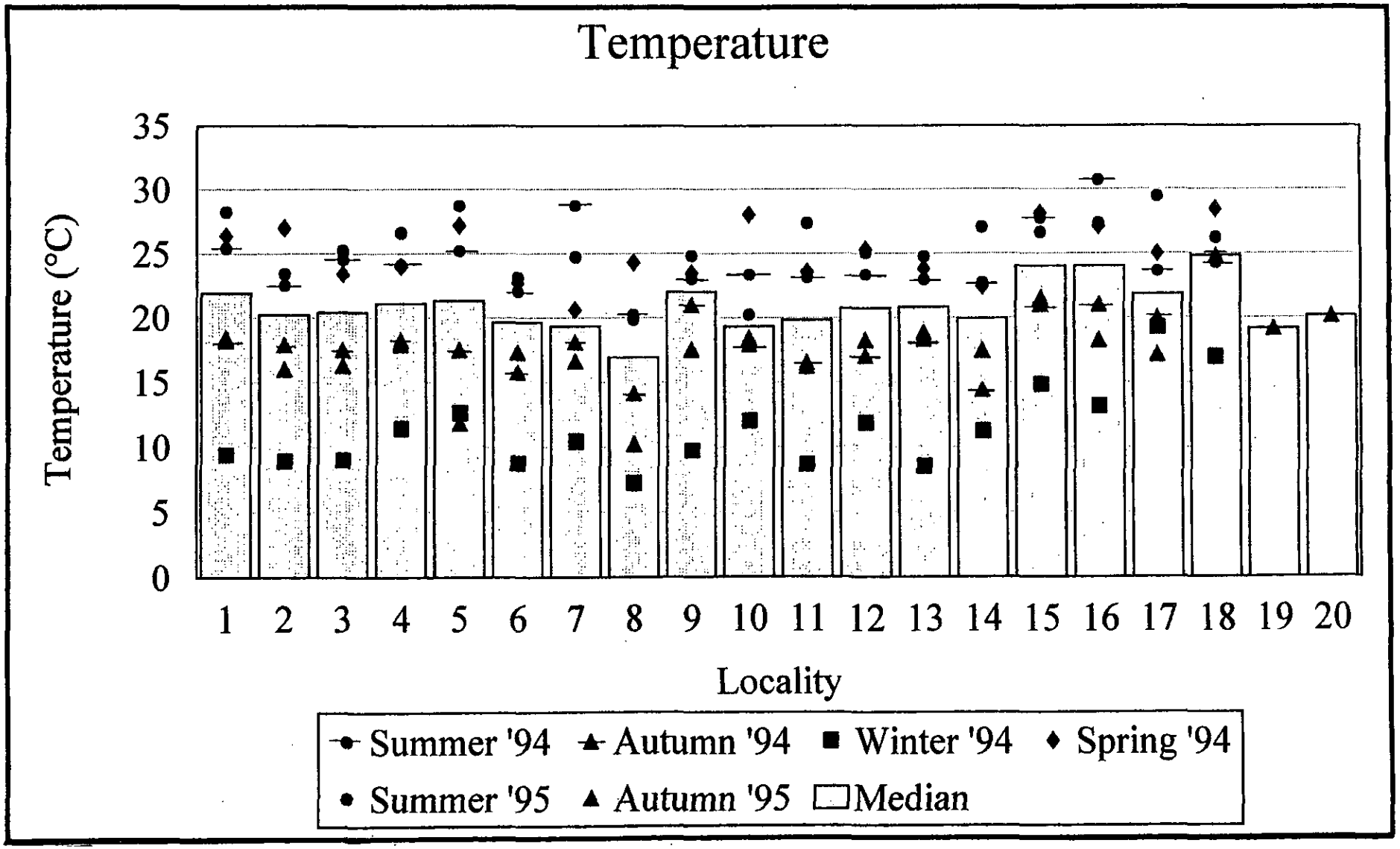

Figure 9.1: Spatial and temporal temperature variation of the surface water at the selected localities in the Olifants River.

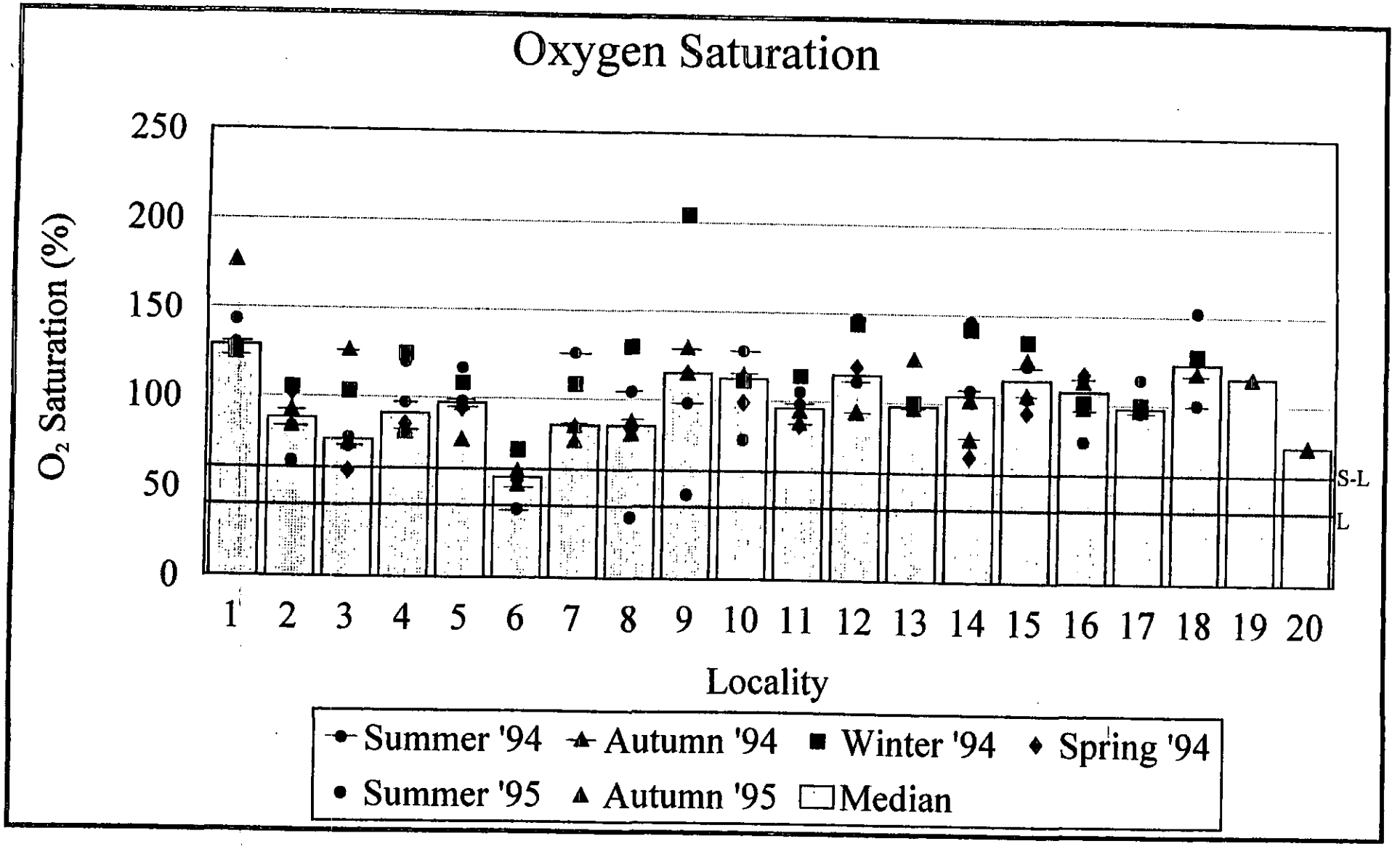

Figure 9.2 Spatial and temporal oxygen saturation variation of the surface water at the selected localities in the Olifants River. S-L = Sub-lethal and L = Lethal values for South African aquatic ecosystems (DWAF., 1995).

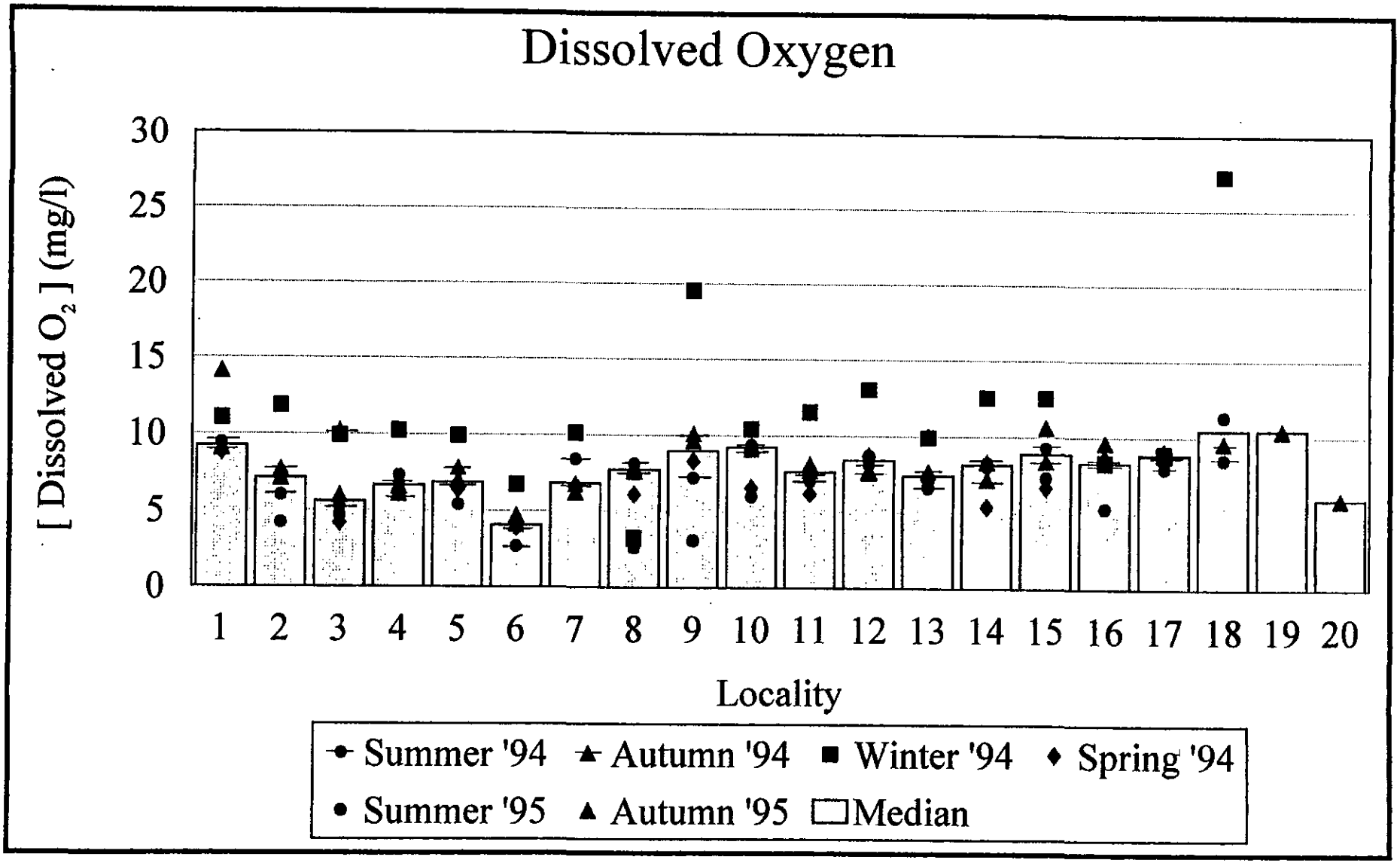

Figure 9.3: Spatial and temporal dissolved oxygen variation of the surface water at the selected localities in the Olifants River.

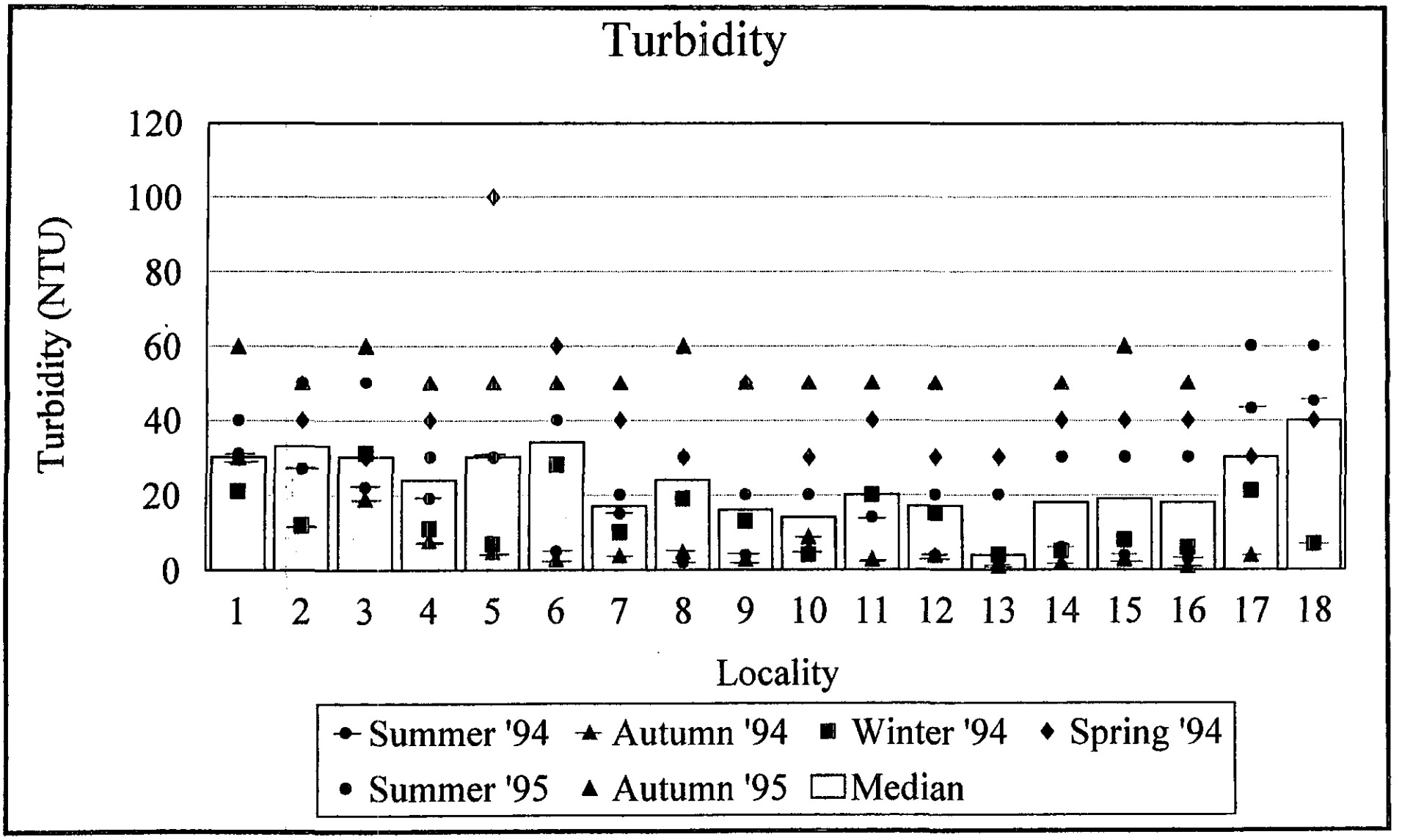

Figure 9.4: Spatial and temporal turbidity variation of the surface water at the selected localities in the Olifants River.

(localities 17 and 18). Locality 13 (4±13 NTU) had the lowest turbidity and locality 18 (40±23 NTU) the highest. In both catchments investigated, the turbidity levels were generally the lowest during the winter survey of 1994. Most of the localities in the upper catchment indicated a similar seasonal variation in turbidity, while in the lower catchment localities 17 (Mamba weir) and 18 (Balule) had similar trends.

The surface water pH (Fig. 9.5) of the upper catchment localities varied from 3.32 (locality 8) to 9.40 (Locality 9) and for the lower catchment from 6,86 to 9,01 (both at locality 18). Locality 8 (Suurstroom) was the most acidic of all localities sampled with a pH ranging from 3.32 (summer 1994) and 5.53 (autumn 1995). The most alkaline localities were 1 (9.40 during summer 1994) and 18 (9.01 during winter 1994). Besides a few exceptions (mainly tributaries) the Olifants River water is generally more alkaline than acidic. Temporal fluctuation of pH were usually low at most localities with locality 10 (Olifants River) indicating the highest variation i.e. pH values ranging from 4.90 (winter 1994) to 8.49 (autumn 1995).

Total alkalinity (TAL) measured as calcium carbonate (Fig. 9.6) ranged from 4 mg/l (locality 8) to 283mg/l (locality 1) in the upper catchment, and from 11 mg/l (locality 17) to 309 mg/l (locality 18) in the lower catchment. The lowest TAL was detected at localities 5 (42±15 mg/l), 8 (4±11 mg/l) and 15 (38±26 mg/l). Locality 1 (241±50 mg/l) had the highest TAL, followed by localities 17 (167±77 mg/l) and 18 (165±75 mg/l). Large temporal variation in TAL occurred at localities 1, 2 and 10 in the upper catchment, and at both localities 17 and 18 in the lower catchment.

As expected, total dissolved salts (Fig. 9.7) and electrical conductivity (Fig. 9.8) indicated similar variation between localities and surveys. In the upper catchment the total dissolved salts (TDS) ranged from 4 mg/l (locality 7-summer 1995) to 3177 mg/l (locality 6-spring 1994) and the electrical conductivity (EC) from 20 mS/m (locality 2-autumn 1994) to 328 mS/m (locality 6-spring 1994). In the lower catchment, the TDS values ranged from 174 mg/l (locality 19-autumn 1994) to 1736 mg/l (locality 18-autumn 1994) and the EC ranged from 24.3 mS/m (locality 20) to 236 mS/m (locality 18-winter 1994). The highest TDS and EC values were usually observed at localities 6, 8, 10, 12, 17 and 18).

Sodium concentrations (Fig. 9.9) in the upper catchment ranged from 4 mg/l (locality 2-autumn 1994) to 90 mg/l (locality 2-spring 1994 & locality 12-winter 1994) and in the lower catchment from 17 mg/l (locality 20-autumn 1995) to 175 mg/l (locality 18- summer 1994). Sites with high sodium levels were localities 6 (53±10 mg/l), 12 (81±21 mg/l), 17 (68±42 mg/l) and 18 (57±57 mg/l). The lowest levels of sodium were detected at localities 15 (18.5±14 mg/l), 16 (18.5 mg/l) and 20 (17 mg/l-autumn 1995). Potassium concentrations (Fig. 9.10) varied from 3.2 mg/l (locality 1) to 23.4 mg/l (locality 6) for the upper catchment and ranged between 2.1 mg/l (locality 19) and 58 mg/l (locality 18) for the lower catchment. Locality 17 (21.9±21 mg/l) had the highest median potassium levels while localities 6 (13±5 mg/l), 8 (17±3 mg/l) and 12 (17±3 mg/l) also had relatively high potassium levels. The lowest potassium levels were detected at localities 1, 15, 16 and 19. Levels of potassium detected during winter and spring 1994 at localities 17 and 18 were extremely high.

Localities 5 (165±59 mg/l), 8 (197±47 mg/l) and 12 (168±65 mg/l) had high levels of

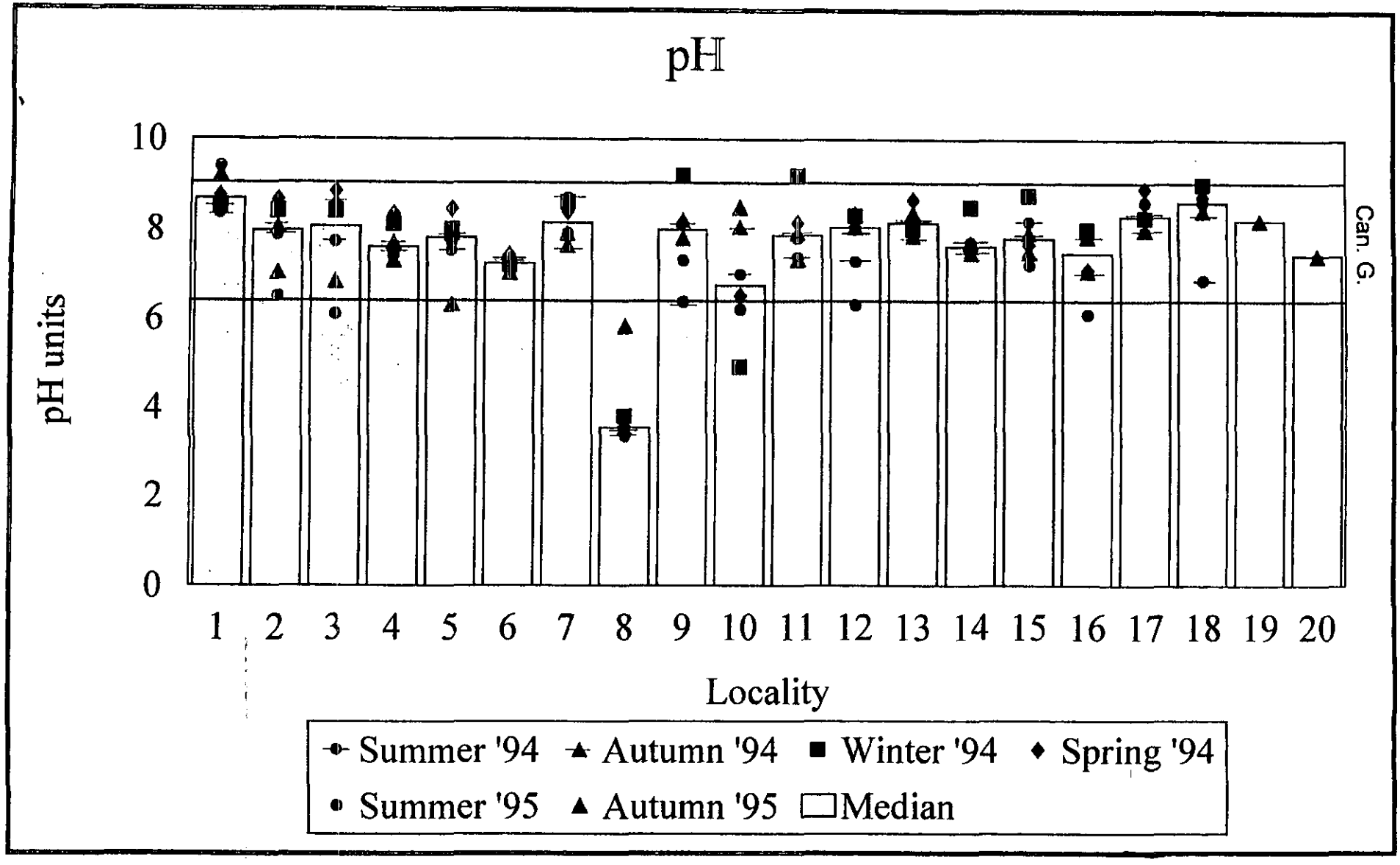

Figure 9.5: Spatial and temporal pH variation of the surface water at the selected localities in the Olifants river. Can. G = Canadian guideline values (upper and lower limit).

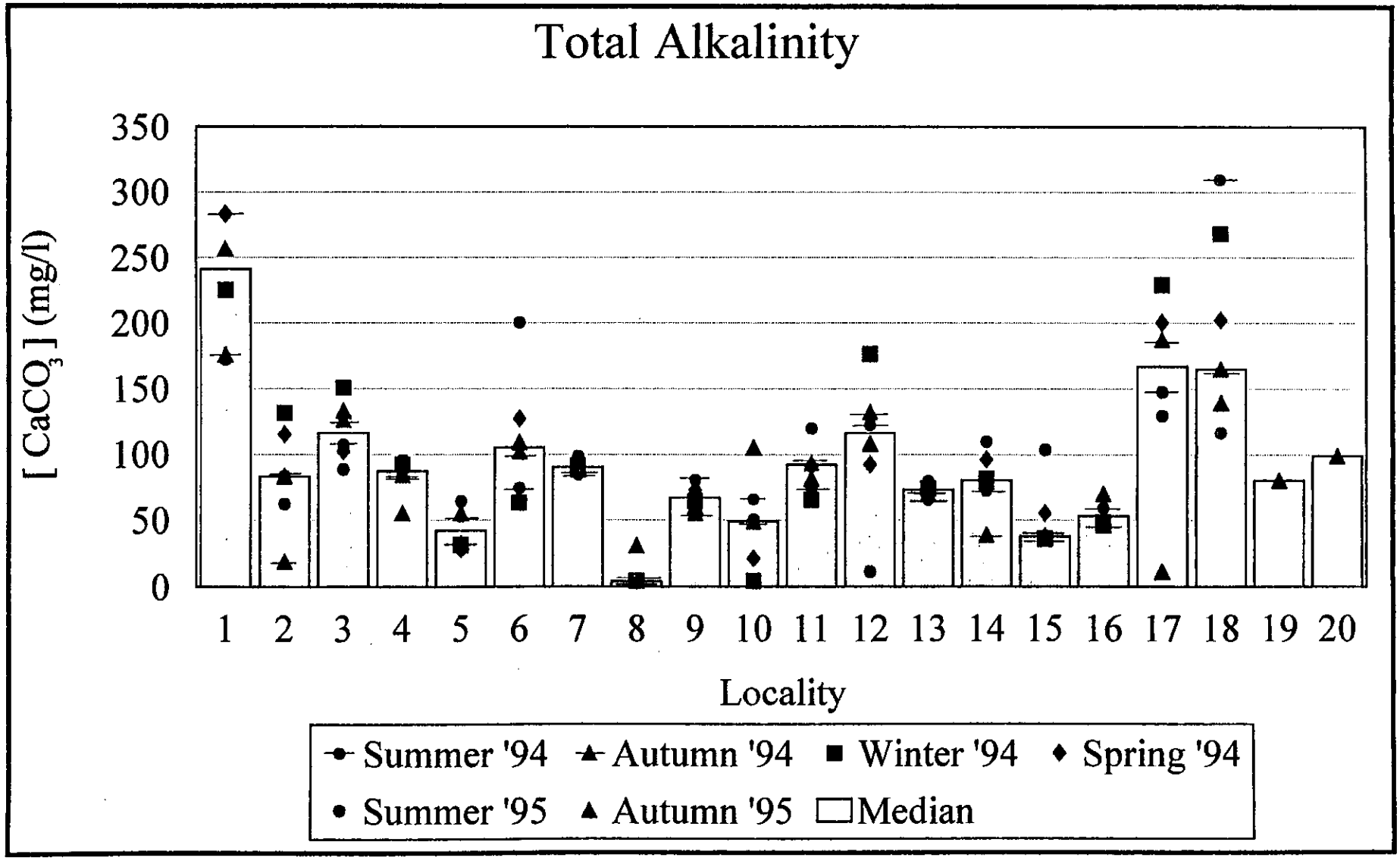

Figure 9.6: Spatial and temporal total alkalinity variation of the surface water at the selected localities in the Olifants River.

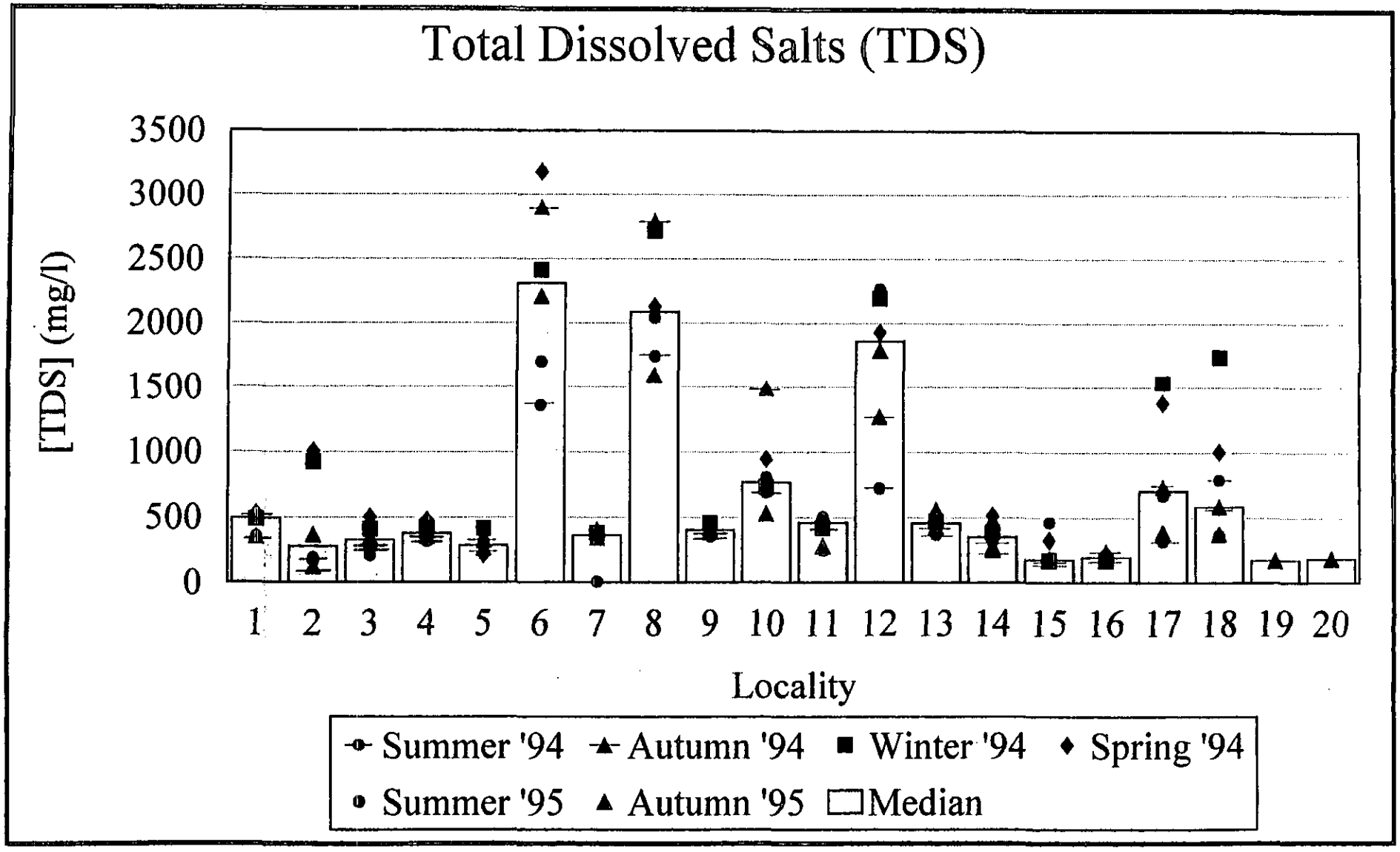

Figure 9.7: Spatial and temporal dissolved salts (TDS) of the surface water at the selected localities in the Olifants River.

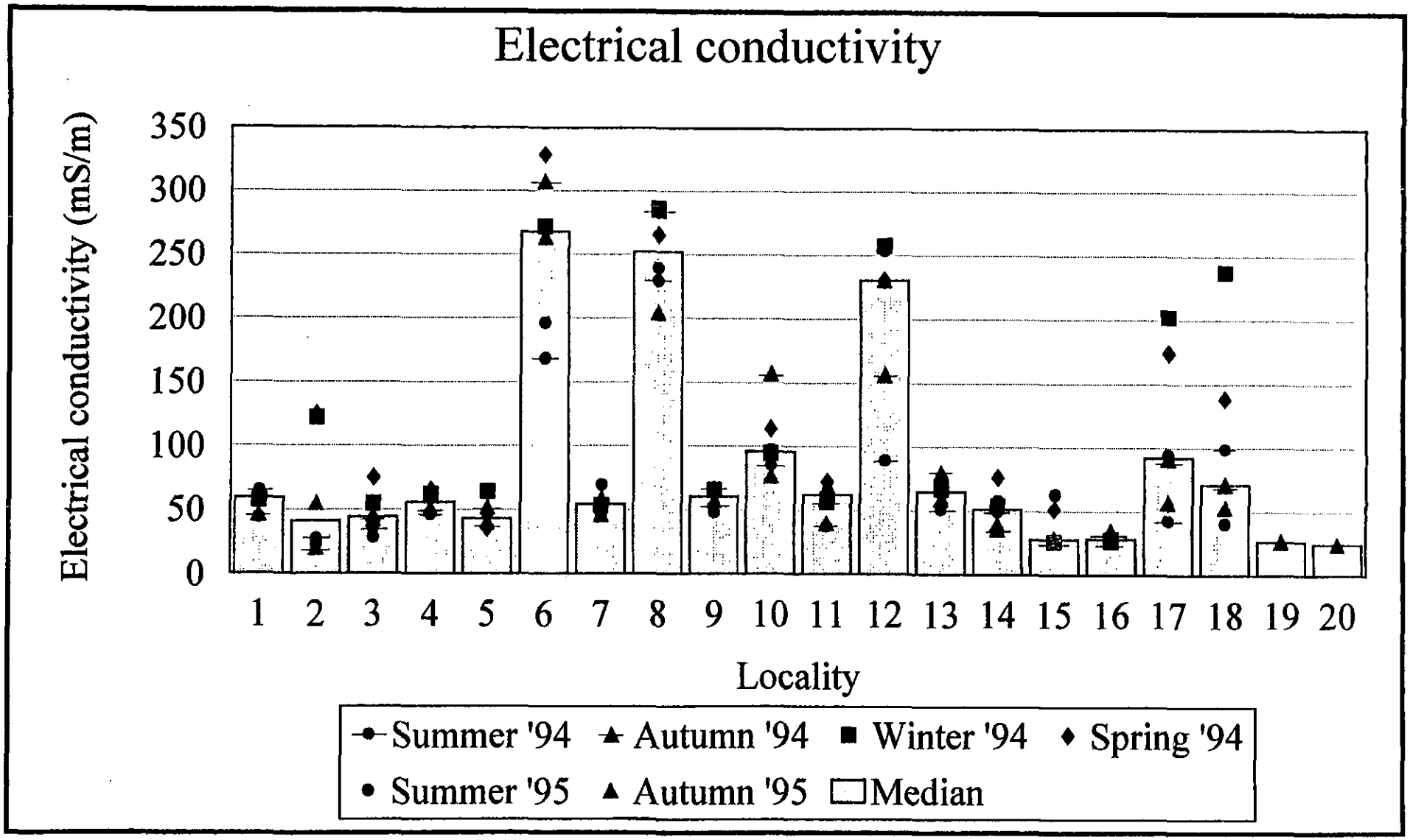

Figure 9.8: Spatial and temporal electrical conductivity variation of the surface water at the selected localities in the Olifants River.

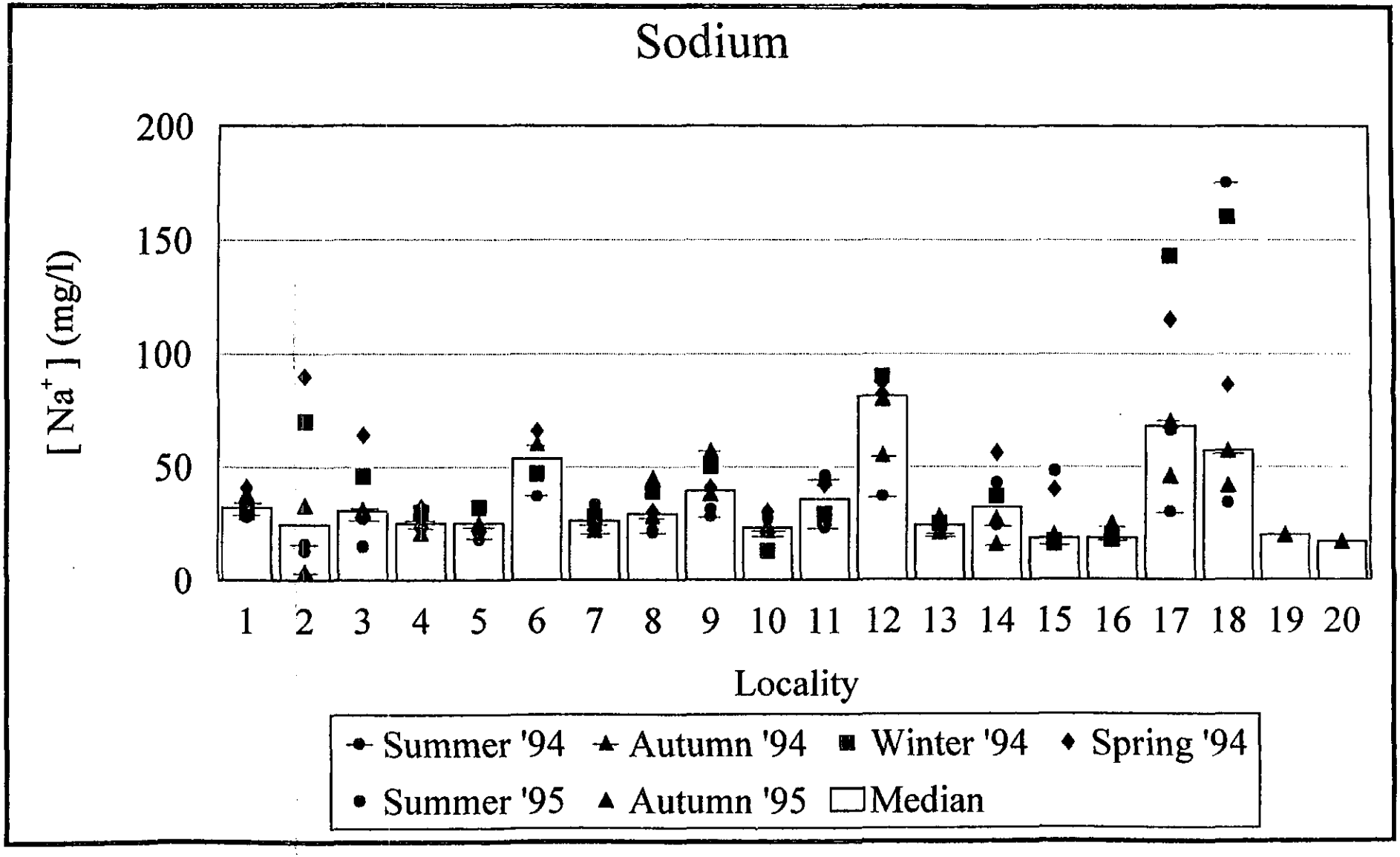

Figure 9.9: Spatial and temporal sodium variation of the surface water at the selected localities in the Olifants River.

magnesium (Figure 9.11). Other sites with relatively high levels of magnesium were localities 1 (43±8 mg/l), 10 (55±22 mg/l) and 17 (56±51 mg/l). The lowest magnesium levels were detected at localities 15 (9.0±5 mg/l), 16 (9.5±08 mg/l), 19 (10 mg/l) and 20 (5 mg/l). Calcium concentrations (Fig. 9.12) in the upper catchment ranged from 14 mg/l (locality 2) to 457 mg/l (locality 6), and from 10 mg/l (locality 20) to 92 mg/l (locality 18) in the lower catchment. Sites with very high calcium levels were localities 6 (369±109), 8 (296±60), 10 (114±61) and 12 (213±71). Low calcium levels were detected at localities 1, 2, 3, 5, 15, 16, 19 and 20. Major temporal calcium fluctuations occurred at localities 6, 8, 10 and 12 while the levels of calcium stayed relatively constant across surveys at localities 1, 7, 9 and 16. Fluoride concentrations (Fig. 9.13) ranged from 0.3 mg/l (locality 9) to 9.8 mg/l (locality 8) in the upper catchment, and from 0.1 mg/l (locality 18) to 3.0 mg/l (locality 17) in the lower catchment. Locality 8 (5.9±2.2 mg/l) contained extremely high levels of fluoride. Other sites with high fluoride levels were localities 9 (1±0.3 mg/l), 10 (0.95±0.2 mg/l), 17 (1.05±1.1 mg/l) and 18 (0.8±0.5 mg/l). Fluoride concentrations at both localities 17 and 18 were particularly high during winter and spring 1994. Locality 20 (control) had the lowest median fluoride level (0.3 mg/l) of all localities sampled.

Chloride concentrations (Fig. 9.14) detected in the upper catchment, varied from 1 mg/l (localities 4, 7 & 15) to 52 mg/l (locality 2). In the lower catchment chloride concentrations were generally much higher than those in the upper catchment, ranging from 9 mg/l (locality 20) to 175 mg/l (locality 18). Localities 17 (75±52 mg/l) and 18 (42±30 mg/l) contained extremely high levels of chloride with localities 9 (29.5±11 mg/l), 11 (25±11 mg/l) and 12 (35.5±7 mg/l) also having relatively high levels compared to other sites sampled. Sites with extremely high nitrite-nitrate levels (Fig. 9.15) were localities 3 (1.56±1.6 mg/l), 9 (5.60±4.2 mg/l) and 14 (1.63±1.8 mg/l). Locality 1 (11.60 mg/l), 11 (3.84 & 3.94 mg/l) and 15 (2.93 mg/l) had high levels during specific surveys (Fig. 9.15). Median levels detected at localities 1 and 4 (0.37±0.2 mg/l), 8 (0.39±0.2 mg/l), 11 and 20 (0.55 mg/l) were also relatively high. Lower nitrite-nitrate levels with little temporal variation were detected at localities 2, 7, 13, 15, 16 and 18. Locality 8 (0.6±3.7) had extremely high ammonium levels (Fig. 9.16), especially during autumn 1995 (9.76 mg/l) and winter 1994 (1.80 mg/l). Other sites with relatively high ammonium levels were localities 3 (0.15±0.08 mg/l), 6 (0.12±0.3 mg/l) and 20 (0.20 mg/l). Great temporal fluctuations in ammonium were detected at localities 5, 6, 8, 10 and 13, while ammonium levels at localities 2, 15 and 16 stayed relatively stable over the study period.

Phosphate concentrations (Fig. 9.17) in the upper catchment ranged from 0.019 mg/l (locality 16) to 3.641 (locality 9), and in the lower catchment from 0.011 (locality 19) to 0.103 (locality 17). Locality 9 (2.229 ± 1,49 mg/l) had extremely high phosphate levels in comparison to the other sites sampled. Other sites with relatively high levels were localities 11 (0.131 ± 0.66 mg/l) and 14 (0.427 ± 1.19 mg/l), as well as localities 3 (0.104 ± 0.18 mg/l) and 17 (0.064 ± 0.03 mg/l). Sulphate concentrations (Fig. 9.18) ranged from 2 mg/l (locality 7) to 2182 mg/l (locality 6) in the upper catchment and from 4 mg/l (locality 20) to 750 mg/l (locality 18) in the lower catchment. Compared to other sites sampled, localities 6 (1570 ± 519 mg/l), 8 (1536 ± 393 mg/l) and 12 (1218 ± 435 mg/l) had extremely high levels of sulphate. Other sites with relatively high levels of sulphate were localities 10 (529 ± 263 mg/l), 13 (228 ± 46 mg/l) and 17 (240 ± 267 mg/l).

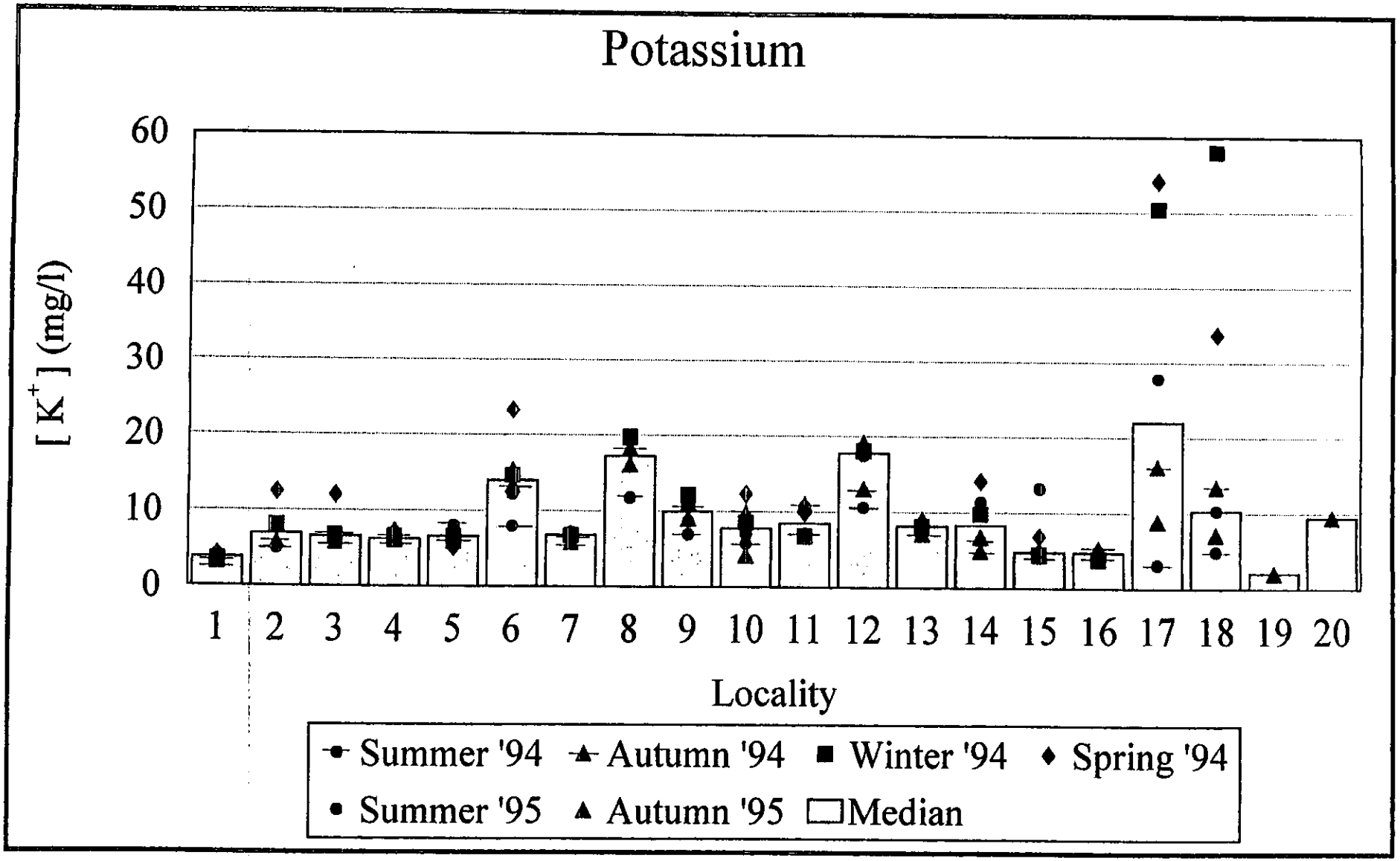

Figure 9.10: Spatial and temporal potassium variation of the surface water at the selected localities in the Olifants River.

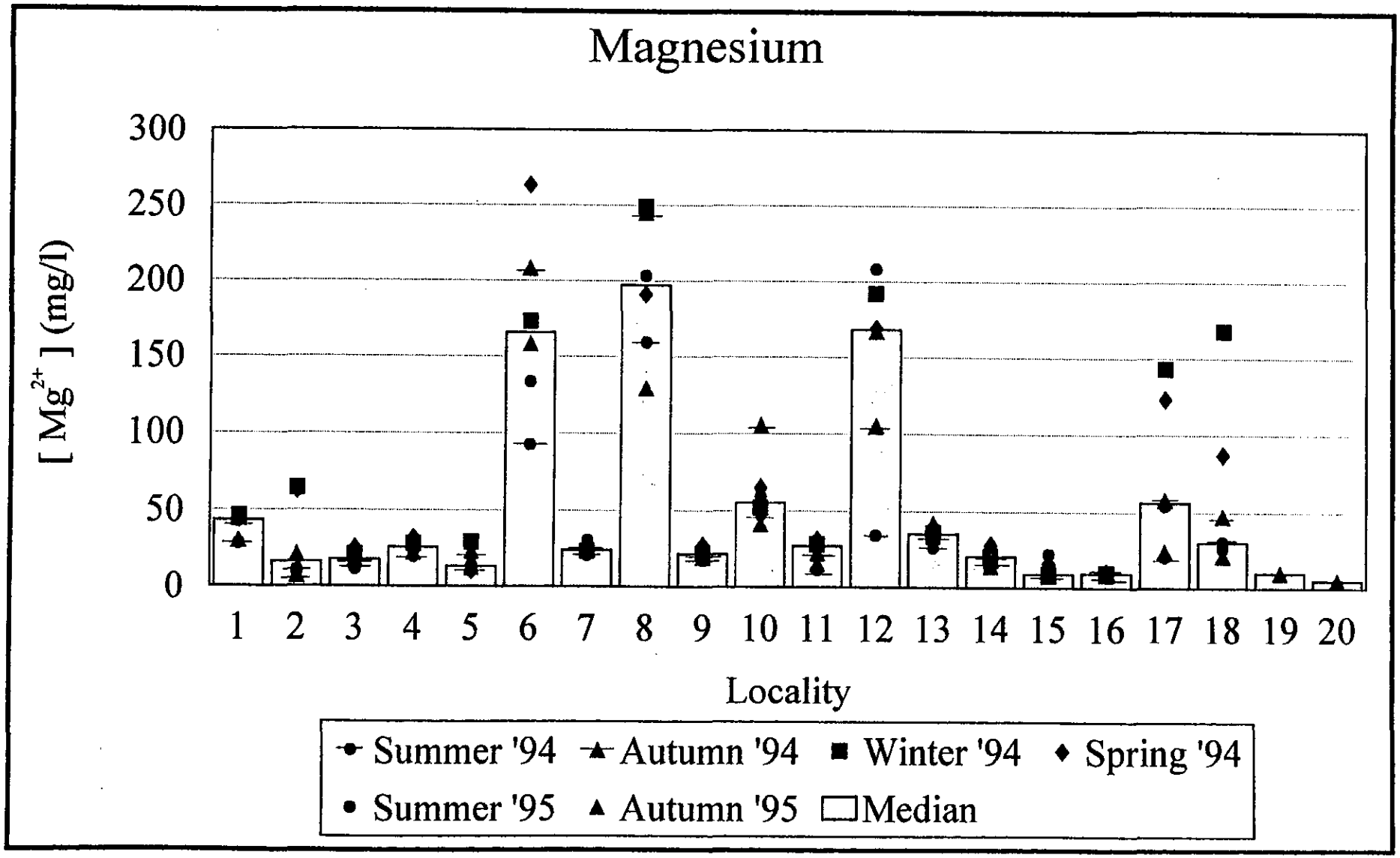

Figure 9.11: Spatial and temporal magnesium variation of the surface water at the selected localities in the Olifants River.

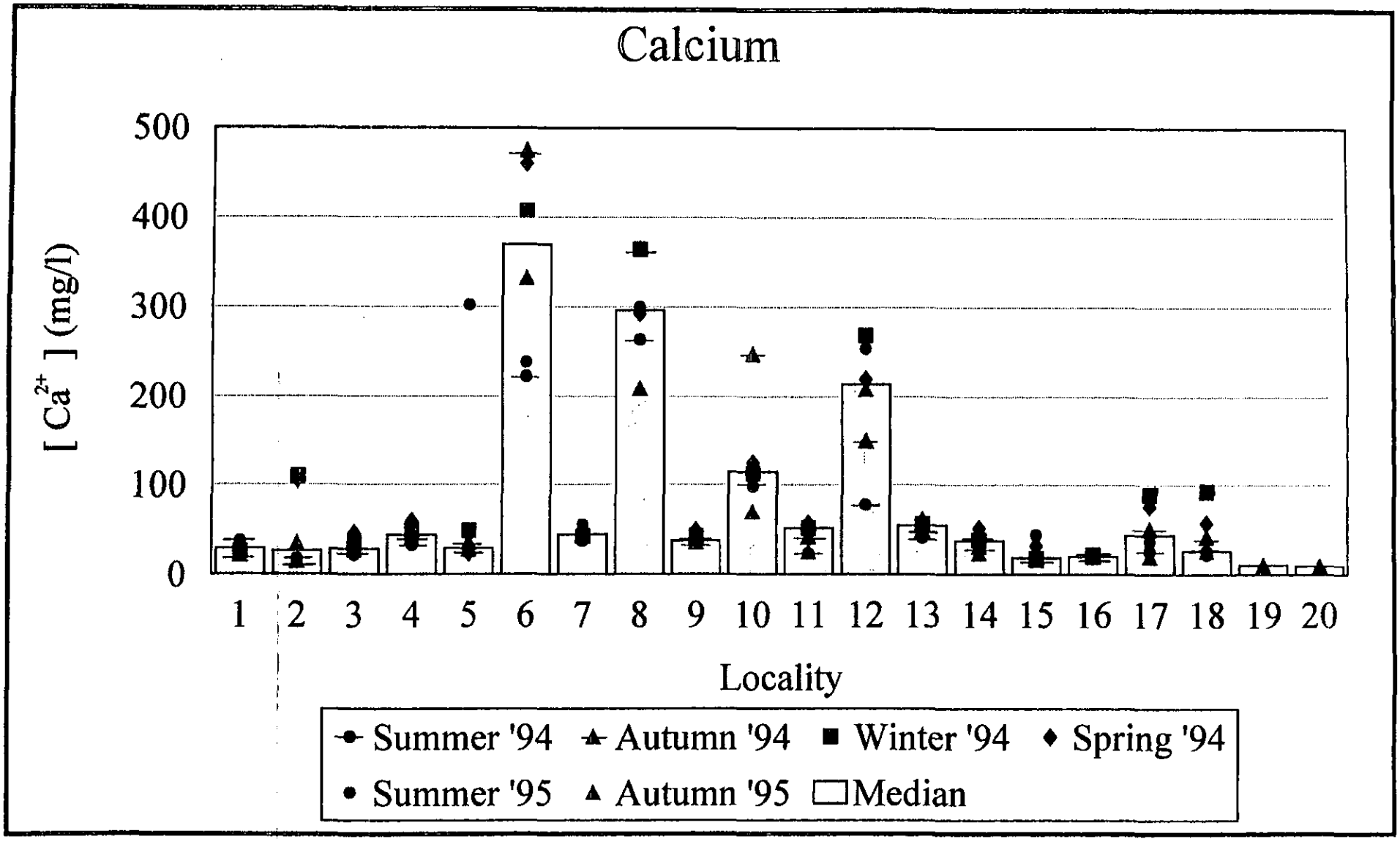

Figure 9.12: Spatial and temporal calcium variation of the surface water at the selected localities in the Olifants River.

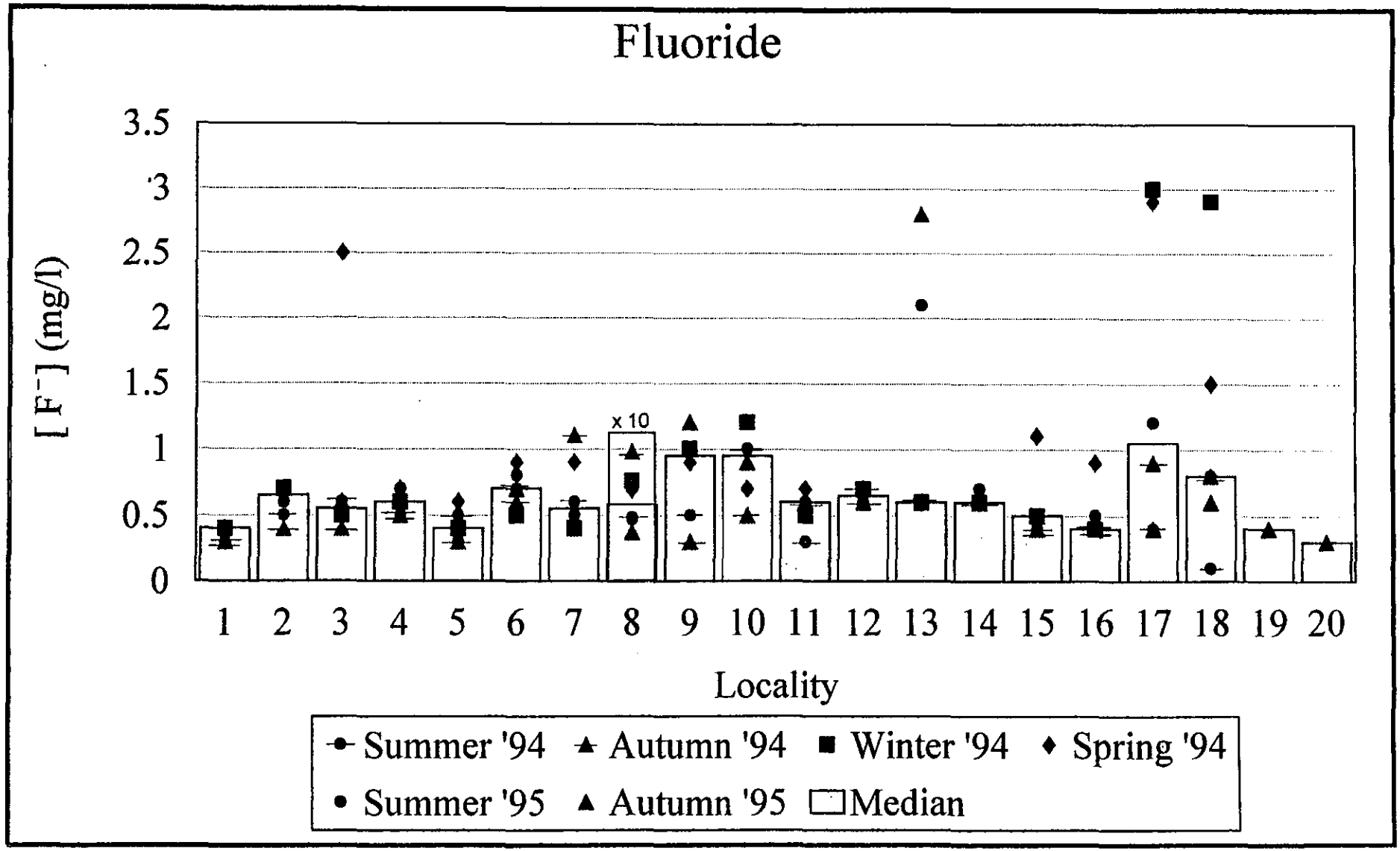

Figure 9.13: Spatial and temporal fluoride variation of the surface water at the selected localities in the Olifants River.

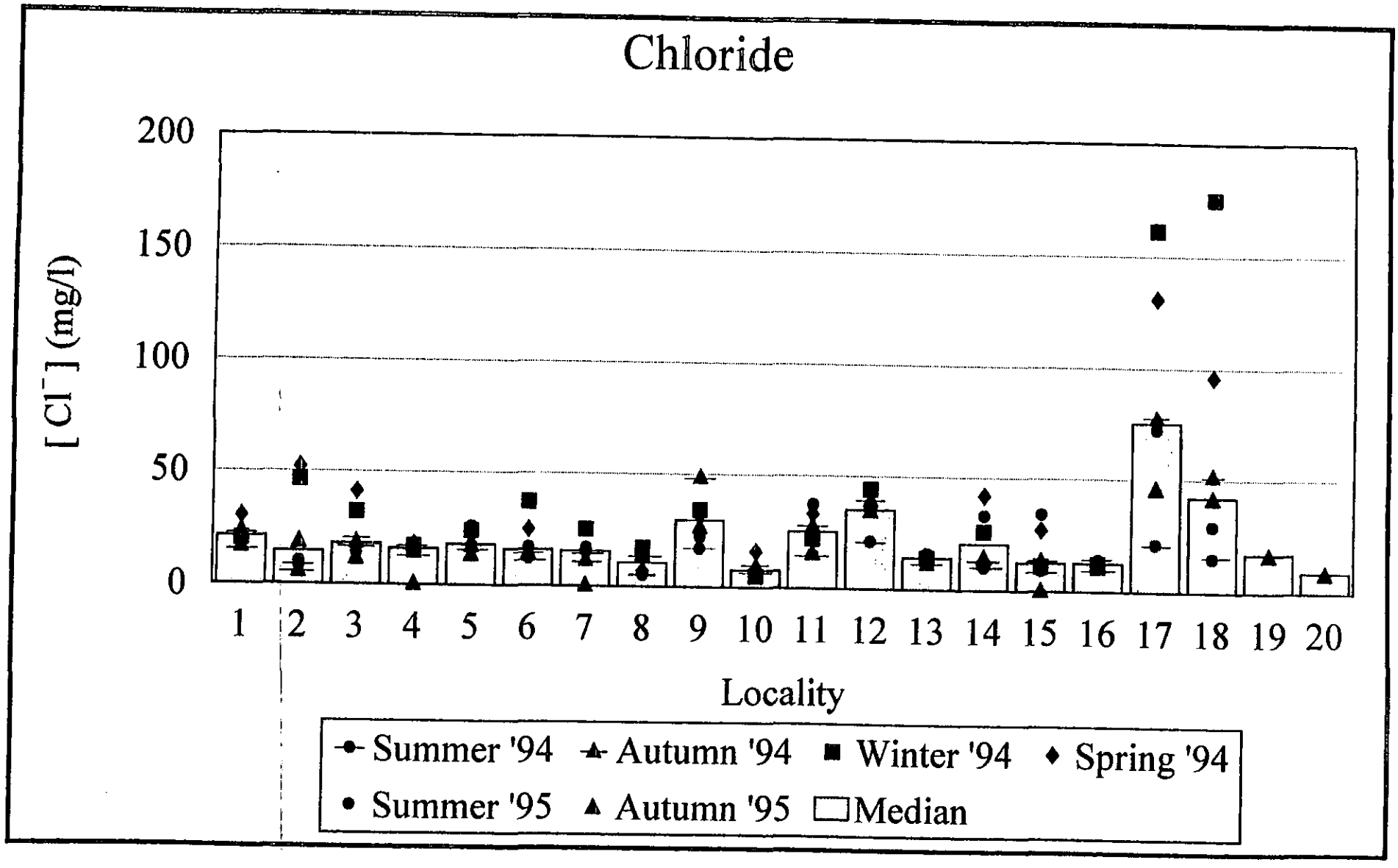

Figure 9.14: Spatial and temporal chloride variation of the surface water at the selected localities in the Olifants River.

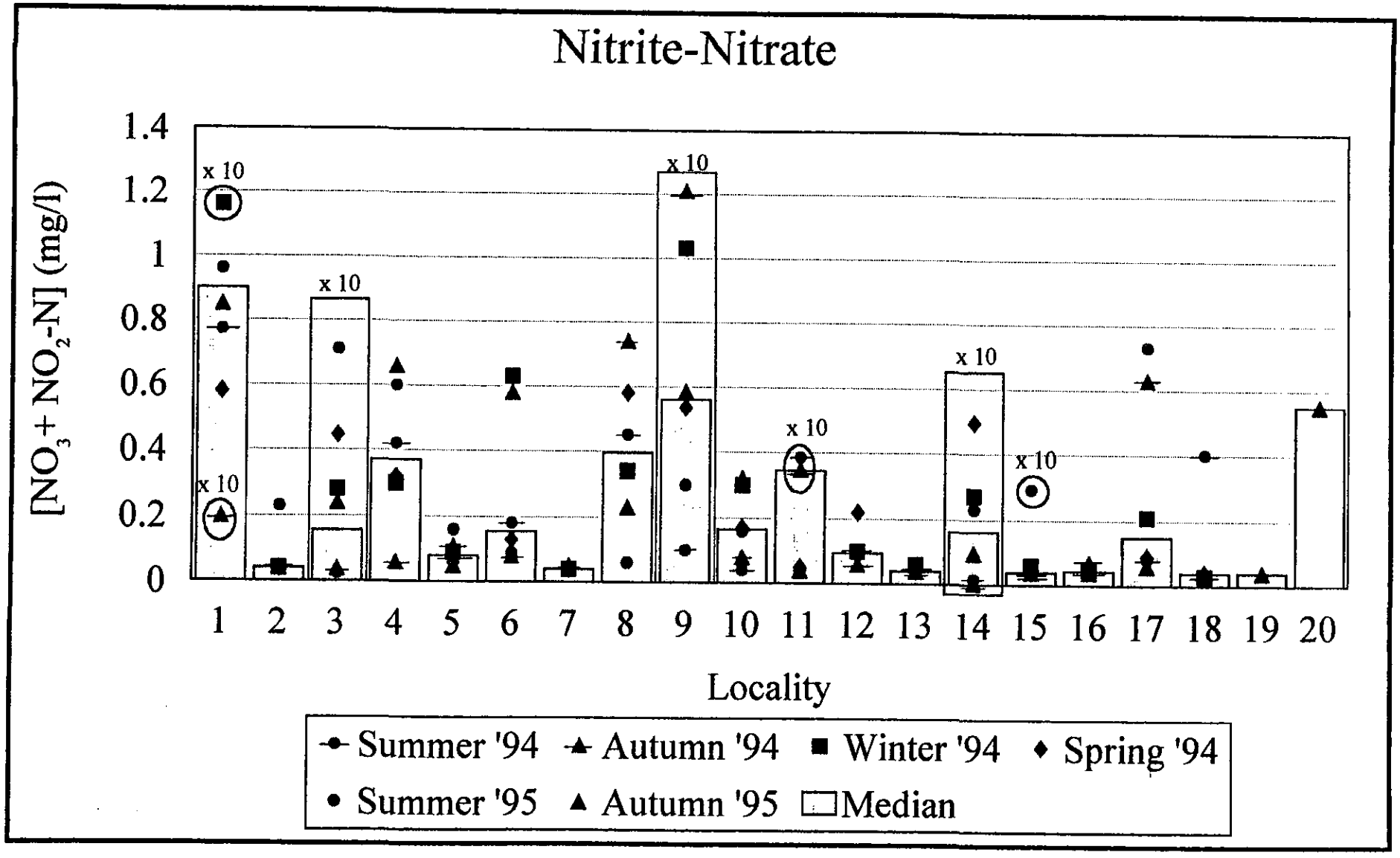

Figure 9.15: Spatial and temporal nitrite-nitrate variation of the surface water at the selected localities in the Olifants River. All values within blue circles and rectangles should be multiplied with 10 (as indicated).

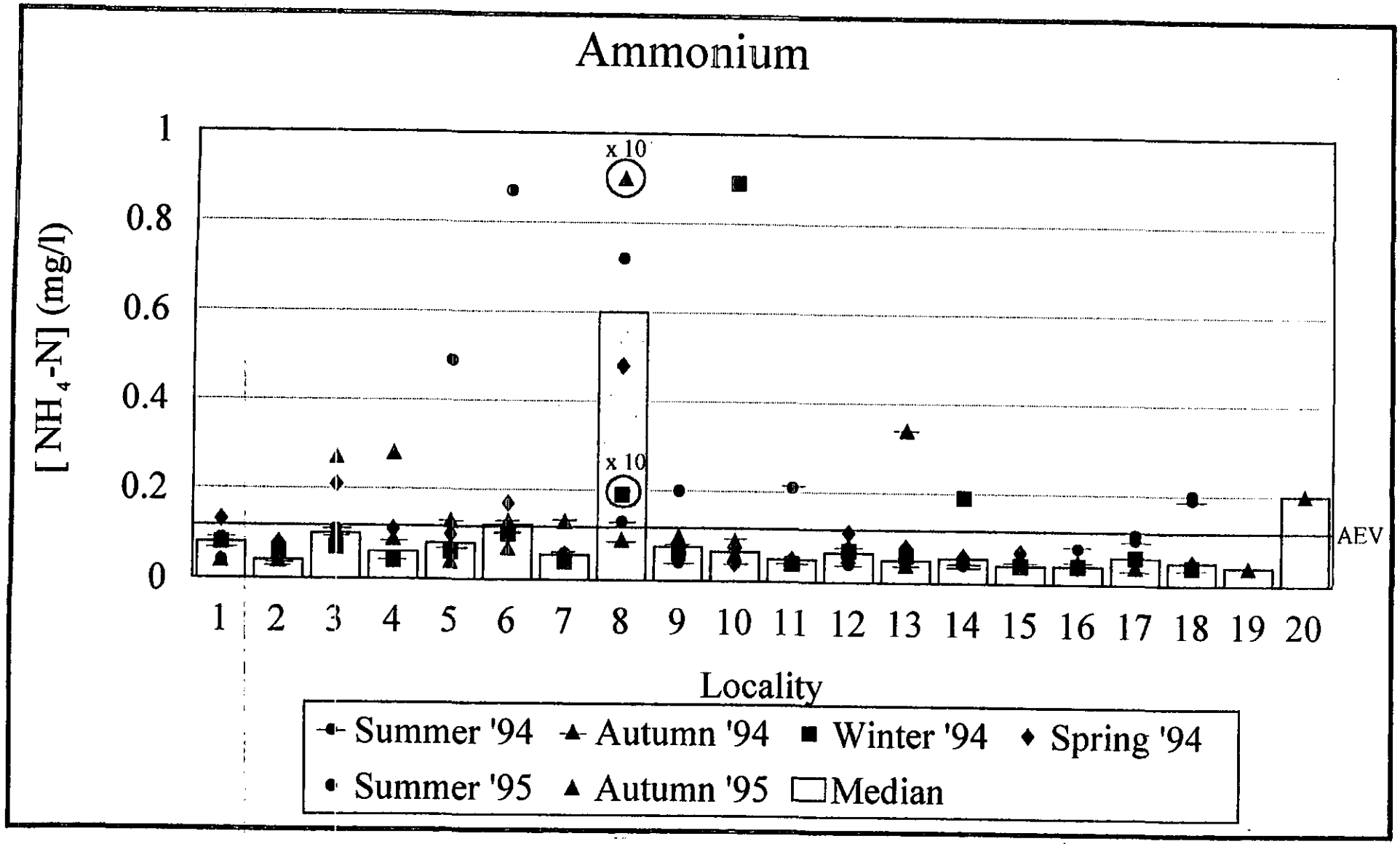

Figure 9.16: Spatial and temporal ammonium variation of the surface water at the selected localities in the Olifants River. AEV = Acute effect value for total ammonia proposed for South African aquatic ecosystems (DWAF., 1995).

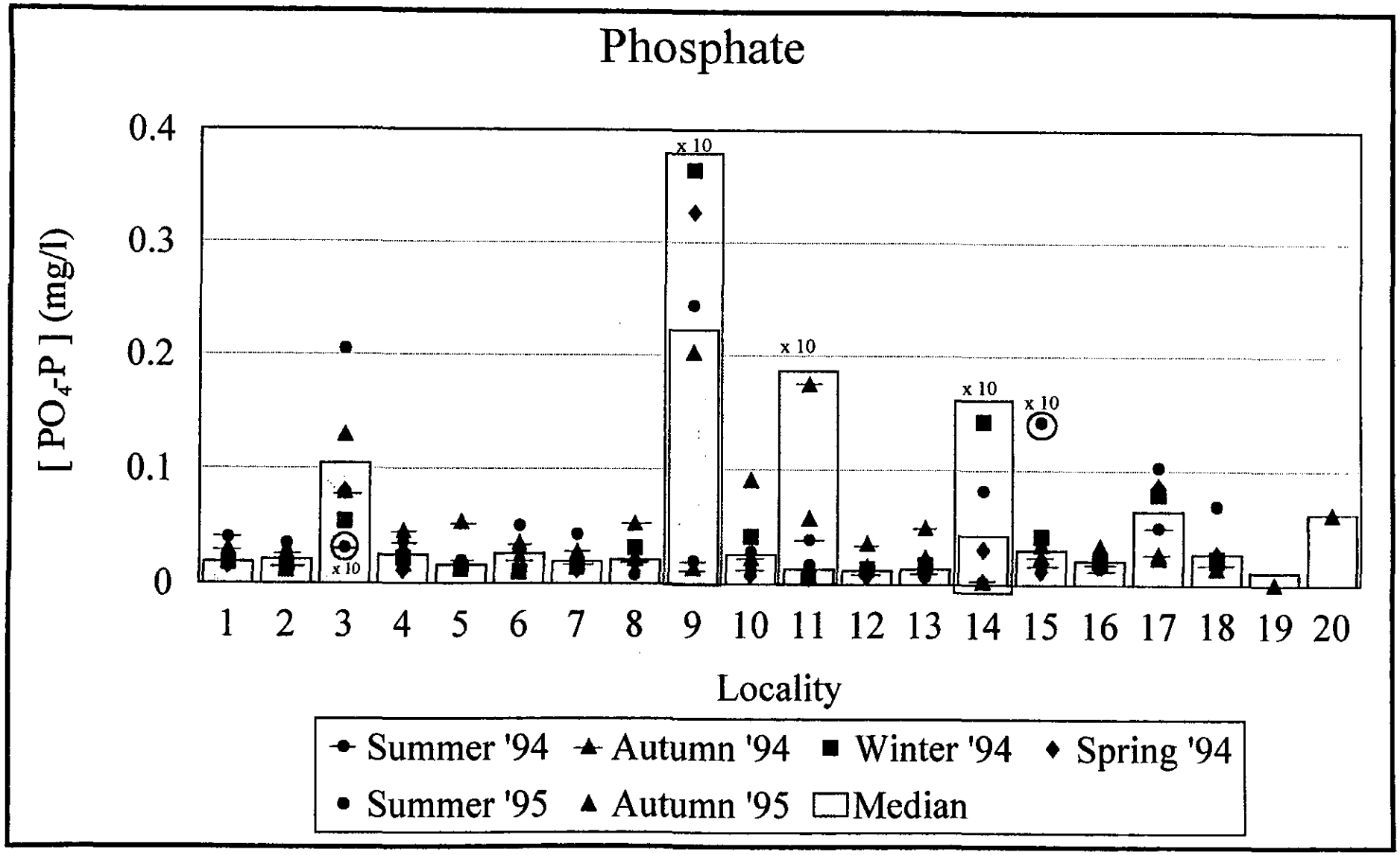

Figure 9.17: Spatial and temporal phosphate variation of the surface water at the selected localities in the Olifants River.

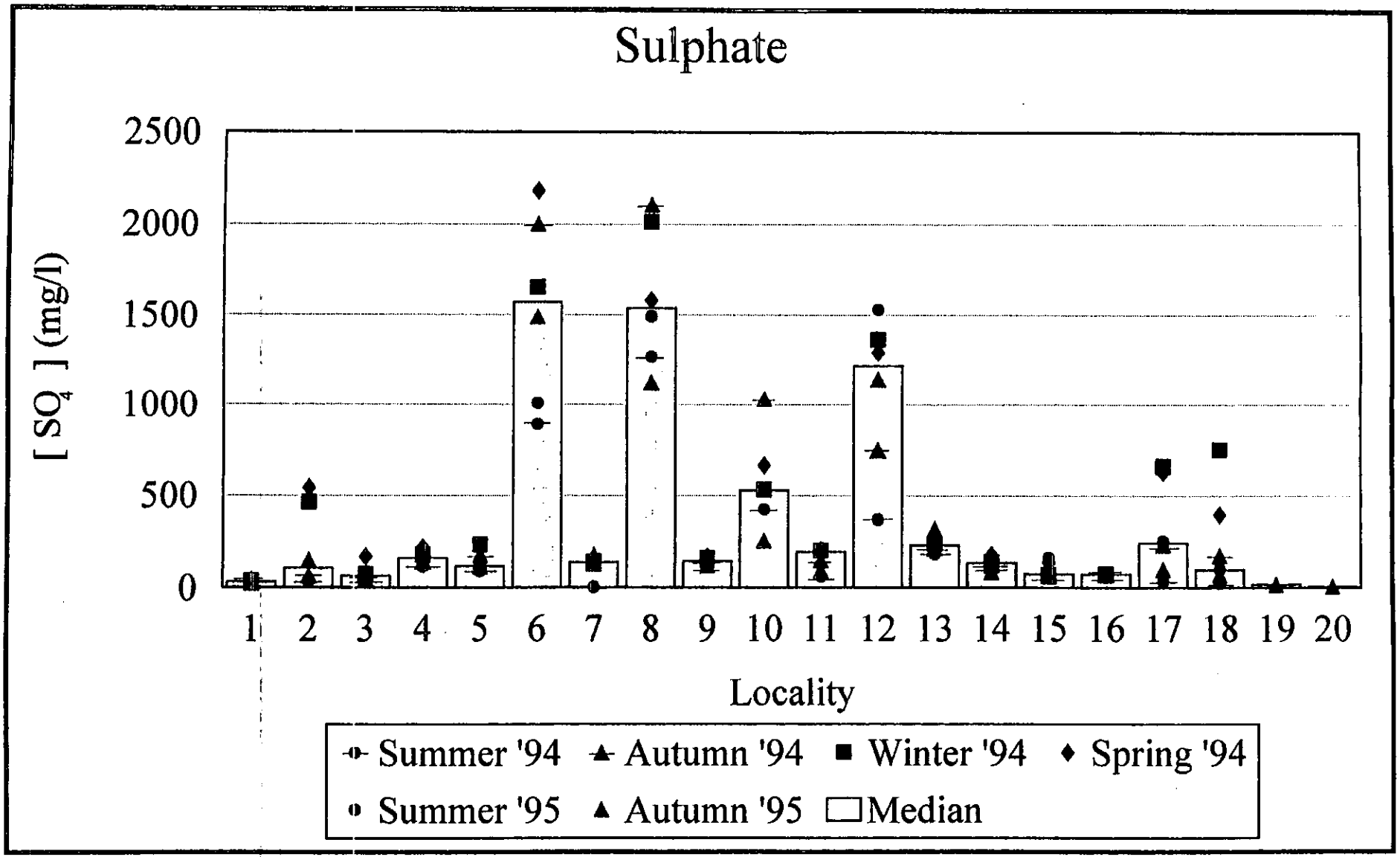

Figure 9.18: Spatial and temporal sulphate variation of the surface water at the selected localities in the Olifants River.

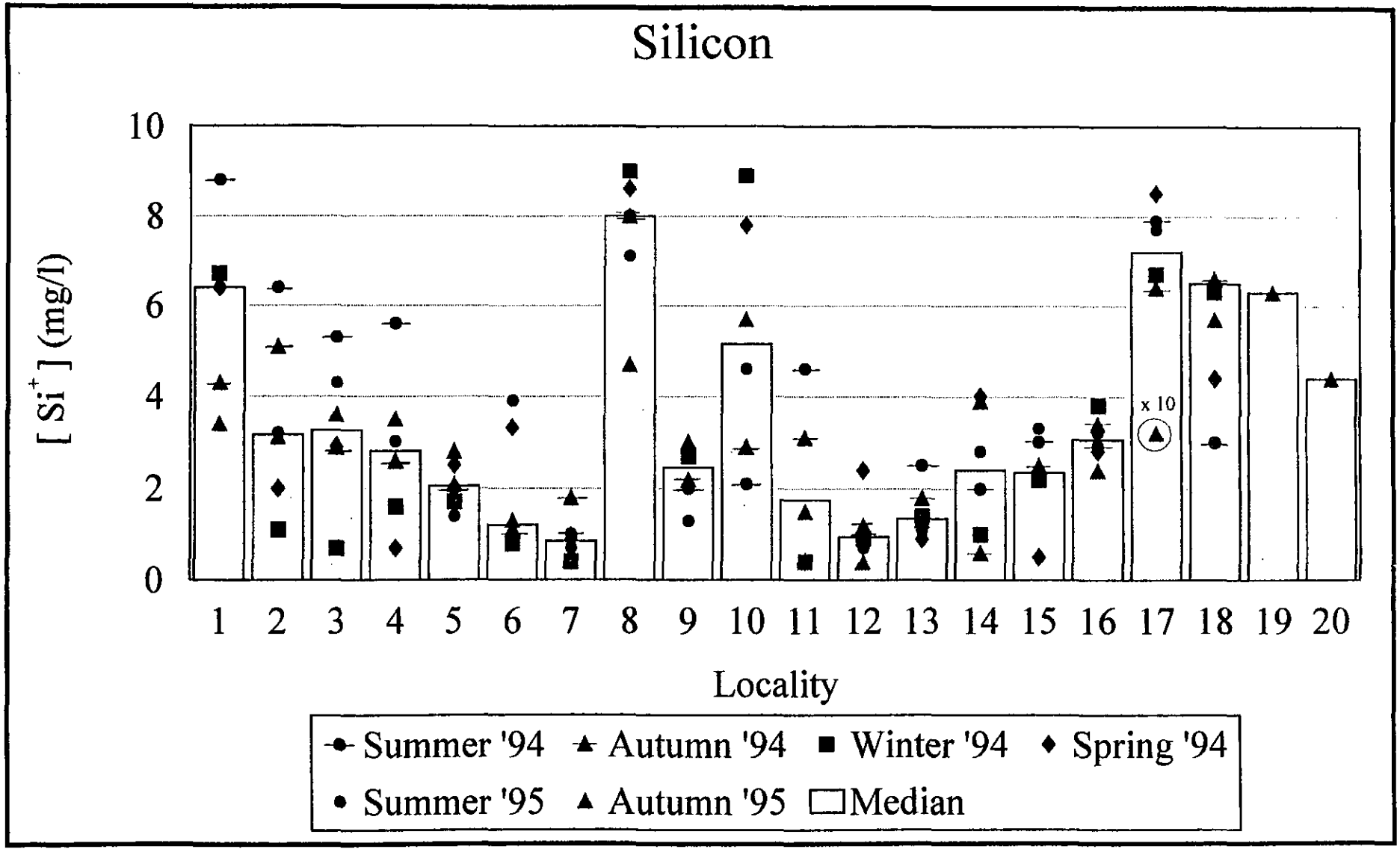

Figure 9.19: Spatial and temporal silicon variation of the surface water at the selected localities in the Olifants River.

Locality 20 (control) had very low sulphate concentrations when compared to most of the other localities sampled. The highest silicon levels (Fig. 9.19) were detected at locality 8 (8.0±1.5 mg/l), followed by localities 17 (7.2±1.8 mg/l), 18 (6.5±10 mg/l), 1 (6.4±1.9 mg/l) and 10 (5.1±2.6 mg/l). A very high level of silicon (29.9 mg/l) was detected at locality 18 during the summer 1994 survey. Locality 20 (control) had the lowest silicon concentrations (4.4 mg/l) of all sites sampled in the lower catchment.

9.1.4 *Discussion*

Many anthropogenic activities release pollutants into the environment and to its own detriment, the water ways are the main conveyers of such waste products on earth. The impact on the water quality of the pollutants entering an aquatic ecosystem is generally negative, resulting in an unhealthy and unstable ecosystem. Both the physical and chemical variables of the water quality, effects riverine biota in different ways. Depending on the variable in question, the ambient water quality as well as the organism involved (Table 9.1).

Under natural conditions the thermal characteristics of an aquatic ecosystems are reliant on the hydrological, climatical and structural features of its catchment. This natural variation in temperature is negatively impacted by anthropogenic activities which can decrease or increase the temperature. Decreased temperature is usually associated with releases of cold water from reservoirs while elevated temperatures generally occur from clearing of cover over streams, stream regulation and heated effluents from steam power generation plants (Heath, 1987; Dallas & Day, 1993). The South African Guidelines for aquatic ecosystems (DWAF., 1995) states that the water temperature should not be allowed to vary from background levels for a specific time and site by more than 2°C or 10%. The many power stations occurring in the upper catchment, however, have accepted a zero effluent discharge policy and thereby do not influence the water temperature of the Olifants River in the region. Temperature fluctuations within a short period of time could render problems for aquatic organisms (Table 9.1) and result in the loss of temperature sensitive species. Previous fish kills in the Olifants River inside the Kruger National Park have been reported due to decreased temperatures caused by extensive hail storms (Buermann *et al.*, 1985). Compensation releases from dams such as Witbank, Loskop, Middelburg and Phalaborwa barrage during low flowing periods might also result in sudden decreased temperatures and should be monitored in future. Surface water temperature seems to be of no concern at any of the selected localities. Temperature as a variable, should rather be seen as a factor influencing the toxicity and state of other constituents and toxins, as stated in Table 9.1 (Cairns *et al.*, 1975).

Gaseous oxygen (moderately soluble in H_2O) from the atmosphere dissolves in water and is also generated during photosynthesis by aquatic plants and phytoplankton (DWAF., 1995). Some physical characteristics of the river, such as the presence of water falls and rapids, also play a cardinal role in the amount of oxygen dissolved. Dissolved oxygen in water is essential and, in some cases, even the limiting factor for maintaining aquatic life (Ellis, 1989, see also Table 9.1). Low oxygen levels also create an increase in the metabolic rate of fish, causing an increased rate of water pumping over the gills and this increasing the amount of toxin in contact with the gill surface where it is absorbed (Alabaster & Lloyd, 1980). Large amounts of putricisable organic matter entering water from industrial or domestic wastes could utilize a large amount of the dissolved oxygen due to microbial respiration (Heath, 1987).

Table 9.1: The effects of some variables of concern on fish and on the aquatic ecosystem in general

Variable	Exposure	Effect on fish	General effects on aquatic ecosystem	Effects dependant on.....	References
Temperature	Acute	Mortality, metabolic malfunctions, including fluid electrolyte imbalance, alterations in gaseous exchange and osmoregulation, hypoxia of the central nervous system & inactivation of enzyme systems.	Reduced solubility of O_2 in water, increased microbial activity, increased metabolic rate of organisms, increased toxicity of and vulnerability to toxins, increased growth of sewage fungus.	Species, stage of development, acclimation temperature, dissolved O_2, pollution, season & extend to which environment is heated.	Alabaster & Lloyd, 1980; Heath, 1987; Dallas & Day, 1993; DWAF., 1995.
	Sub-lethal	Alterations in existing aquatic community > change in qualitative & quantitative composition of biota> population shifts. Influence on migration, spawning, growth, reproduction, fry-survival & egg-hatchability, acid-base balance of blood, cellular metabolic and membrane adaptations, low temperature dormancy & regional endothermy. Affects development of parasites & pathogenic bacteria.			
O_2-Saturation	Sub-lethal (Hypoxic)	Changes in behaviour, blood chemistry, growth rate & food intake. Hypoxia > necrosis, haemorrhage, hyperplasia, hypertrophy & hyperanaemia in the gills, liver, kidney & spleen. Reduced reproduction, spawning, growth, swimming speed, increase/decrease in metabolic rate, reduced blood oxygen demand. Increased susceptibility to poisoning.	Influence macro-invertebrates dependant on oxygen for respiration> reduced food sources of fish. Increases toxicity of pollutants to aquatic organisms.	Organism's dependance on water as a medium, species, life stage, life process (feeding, growth etc.), water temperature, salinity & atmospheric pressure.	Alabaster & Lloyd, 1980; Heath, 1987; Dallas & Day, 1993; DWAF., 1995.
	Acutely high levels	Gas bubble disease (O_2 bubbles surround gills)> mortality			

Table 9.1: (Continued)

Variable	Exposure	Effect on fish	General effects on aquatic ecosystem	Effects dependant on.....	References
Turbidity	Unnatural elevated levels in water (sub-lethal and lethal)	Reduced visibility> influence search for food & predator-pray interactions> change in community assemblage. Impairment of gill functioning (clogging) & decrease in O_2 uptake> suffocation. Reduced growth (reduce foraging efficiency & food availability). Reduced spawning success due to reduction in spawning habitats. Direct action on swimming ability. Reduced development of eggs and larvae (siltation), modify movement and migration. Increased susceptibility to diseases	Reduced light penetration> decrease in photosynthesis> decrease in primary production. Reduced water temperature. Settling out causes depletion of smothering & depletion of habitats. Adsorption of toxins onto particles.	Type of matter/particle causing the turbidity. Species sensitivity. Salinity of water.	Alabaster & Lloyd, 1980; Grobler *et al.*, 1987; Dallas & Day, 1993; DWAF., 1995.
pH	Sub-lethal	Increased uptake and toxicity of pollutants, influence ion exchange across body surfaces> influence ionic & osmotic balance>increased energy requirements> decreased growth & fecundity. Inhibition of spawning and embryo development. Unnatural migration. Reduced species diversity. Haematological changes= increased haematocrit, haemoglobin &/or red blood cell count. Hypersensitivity to bacteria, increased susceptibility to disease	Influence speciation of elements occurring in water. Alter species composition of community. Loss of blue-green algae. Decreased invertebrate diversity. Decrease in food quality.	Species sensitivity. Buffering capacity of water. Hardness, [Na^+ & Cl^-]. Age of fish.	Alabaster & Lloyd, 1980; Heath, 1987; Dallas & Day, 1993;
	Acute	Mortality due to H^+-ions competing with larger ions e.g. Na^+. Precipitation of mucus on gills> suffocation & precipitation of proteins within epithelial cells. Destruction of gill epithelium> suffocation.			

Table 9.1: (Continued)

Variable	Exposure	Effect on fish	General effects on aquatic ecosystem	Effects dependant on.....	References
TDS & EC	Sub-lethal	Affect metabolism. Decreased species diversity. Affects community structure, and ecological processes. Growth rate & life expectancy. Influence osmotic balance.	Effects water chemistry> influence on aquatic environment. Could decrease toxicity of metals to aquatic organisms. Influence microbial processes.	Species sensitivity. Rate of change. Stage of development and age of organism. Water temperature.	Hellawell, 1986; Dallas & Day, 1993; DWAF., 1995.
Fluoride	Sub-lethal	In *Catla catla* fry- inhibit protein synthesis, decrease glycogen and iron concentrations and alter lipid metabolism.	Play major role in deteriorating ecosystems.	Water hardness. Calcium concentration in water. Water temperature	Pillai & Mane, 1984; Smith *et al.*, 1985; DWAF., 1995.
	Chronic	Skeletal fluorosis			
	Acute (>20 mg/l)	Mortality			
Chloride and chlorine	Chronic and acute.	Avoidance behaviour, adverse changes in blood chemistry, decreased growth rate, restlessness preceding loss of equilibrium and death. Damage to gill epithelium > production of mucus > clogging of gill lamellae. Elevation in plasma potassium. Changes in behaviour, reproduction and spawning.	Can form various compounds, such as chloramines & stable chloro-organic compounds, which are harmful to fish. Influences other aquatic organism negatively.	Water pH, temperature and dissolved O_2. Presence of organic carbon & ammonia. Stage of development.	Cairns *et al.*, 1975; Alabaster & Lloyd, 1980. Heath, 1987; DWAF., 1995
Ammonia	Chronic	Reduction in hatching success, reduction in growth rate and morphological development, pathological changes in tissue of gills, liver and kidneys.		pH, temperature, dissolved O_2, CO_2 & TDS. Presence of other toxicants	DWAF., 1995.
	Acute	Loss of equilibrium, hyper excitability, increased breathing rate, increased cardiac output & O_2 intake. Convulsion, coma & death.			

TDS - Total Dissolved Salts EC - Electrical conductivity

Algal blooms, especially those occurring due to organic enrichment by agricultural and sewage pollution, may cause a considerable day-night fluctuations with a decrease in oxygen at night due to the combined respiration of plants and animals and an increase during the day due to photosynthesis. At locality 14 (Olifants River Lodge) a large amount of algae and aquatic macrophytes were observed during the summer 1994 survey, possibly causing the high levels of oxygen detected at this locality during that period. Extremely low levels of oxygen could, however, occur at night time when these plants and algae as well as the aquatic organisms utilize oxygen. This day-night fluctuation situation and low oxygen levels at night could be detrimental for aquatic biota at this site and should therefore be investigated. Median dissolved oxygen levels detected in the water of the selected sites were generally within the target range of 80 to 120 % saturation (DWAF., 1995). Only locality 6 (Boesman Spruit) had a median O_2-saturation below 60 % saturation. This could be stressful for aquatic organisms at this locality (Table 9.1), but the overall effect will depend on the duration of exposure and ambient water temperature.

Seasonal changes in dissolved oxygen concentrations detected during this study could possibly be ascribed to the changes in water temperature and biological productivity (DWAF., 1995). Increased solubility of oxygen with decreased water temperature (Ellis, 1989) were possibly the main reason for the high levels of oxygen generally detected during the winter 1994 survey. A decrease in the rate of oxygen consumption due to less activity during the colder seasons could also have played a minor role in this phenomenon (Saad, 1987). Fish are mostly capable of acclimatizing to different oxygen levels to a certain extent, but sudden fluctuations in dissolved oxygen could result in mortalities of sensitive fish (Alabaster & Lloyd, 1980). Bauermann *et al.* (1995) stated that fish being subjected to sudden elevated silt loads and associated decrease in oxygen levels, in the water of the lower Olifants River during scouring of the Phalaborwa Barrage, undergo oxygen related stress. Seasonal fluctuations detected in the oxygen levels of the selected sites during this study were of no real concern, assuming that there were gradual fluctuations over a long period of time. Areas with a large amount of algae and macrophytic growth are potential sites with high levels of nutrients and should therefore be investigated due to the fact that oxygen levels could become lethal at these sites.

Turbidity of surface waters is caused by suspended matter such as clay, silt, finely divided organic and inorganic matter, plankton and other microscopic organisms, as well as by soluble coloured organic compounds, such as fulvic, humic and tannic acids. The natural hydrological and geomorphological processes of a catchment play a major role in the turbidity of its surface waters (Wotton, 1994; DWAF., 1995). Soil particles derived from continuous and natural processes of wind and water erosion, enter the water ways and cause natural fluctuation. However, land use practices such as over-grazing, non-contour ploughing, removal of riparian vegetation and forestry operations accelerate erosion and result in increased turbidity levels (DWAF., 1995). These practices, and in particular poor agricultural practices in the middle catchment of the Olifants River, cause large amounts of silt to be washed into the Olifants River annually, which could be detrimental to the health of the aquatic ecosystem (Table 9.1).

The elevated levels of silt cause the siltation of dams, and in particular the lower catchment reservoirs which have to be flushed to regain storing capacity. The flushing of the Phalaborwa Barrage (locality 19) in the lower catchment releases great amounts of silt which

causes unacceptable levels of turbidity (>1 999 NTU). This was possibly the main contributor to the high levels of turbidity detected in the water of the lower catchment (Fig. 9.4). As mentioned, the increased silt loads during flushing causes decreased oxygen levels and gill clogging of fish, resulting in mortalities of fish downstream (Buermann *et al.*, 1995). The increased silt loads could also have other negative effects on the aquatic ecosystem such as habitat destruction. Moore *et al.* (1991) indicated that the critical value for turbidity of the Olifants River within the Kruger National Park is 20 NTU. This value was generally exceeded at localities 17 and 18, indicating that turbidity levels in this region are of concern.

Discharges from industrial, mining and other anthropogenic activities also causes increased turbidity of surface waters. This was obvious due to the difference in turbidity levels between the upper- (locality 5) and lower Boesman Spruit (locality 6). At these localities turbidity levels varied exceptionally even during low flowing periods due to the mining activities in the middle reaches of this stream (Fig. 9.4). Increased turbidity can sometimes be favourable to aquatic ecosystem by reducing algal blooms (Grobler *et al.*, 1987) and increasing protection from avian and piscine predators (Dallas & Day, 1993). Agricultural and mining activities in the Klein Olifants River catchment could be responsible for increased siltation of this river. Very low levels of turbidity were, however, detected at locality 13 (Middelburgdam) possibly due to the fact that it was a water source with low/no flow in which most suspended particles settled out to the sediment. It could also indicate that the turbidity of Klein Olifants River is presently not notably influenced by these anthropogenic activities upstream of this locality. Further investigations will have to be made to investigate the situation during high flow periods. As was expected, an obvious seasonal trend is evident in both the upper and lower catchments where turbidity levels in the water increased as the amount of runoff/rainfall in the region increased.

Geological and atmospheric influences determine the natural pH of a water body. Human impacts causing reduced pH are basically ascribed to acidic point-source effluents from industries and acid mine drainage entering the rivers. In some regions acid-rain contributes a great deal to acidification of the water courses. Events leading to an increased water pH are less common and are generally due to alkaline effluents from industries and anthropogenic eutrophication (Dallas & Day, 1993). Poorly buffered water can undergo rapid changes in pH with devastating effects on the aquatic organisms (Table 9.1). According to the results gained from this study, locality 8 (Suurstroom) had the lowest total alkalinity level, possibly due to the very low pH levels detected at this locality. This would also suggest that the water at this locality is unable to buffer the low pH water from the acid mine drainage resulting in a median pH value below the Canadian guideline value of 6.5 (Fig 9.5). An important feature of pH is that it plays a cardinal role in the determination of chemical species (and thus potential toxicity) in which numerous elements and molecules occur in water. The very low pH detected at locality 8 results in metals such as aluminium being mobilized, and making them more available to aquatic organisms. Generally, the water of the Olifants River is more alkaline than acidic (Fig. 9.5). The highest median alkalinity occurred at locality 1 indicating its buffering capacity which resulted in the stable pH level observed at this site. Locality 17 and 18 in the lower catchment also had high alkalinity levels compared to those of the other localities, indicating a good ability to buffer changes in pH. At locality 1, 9 and 10 the pH of the water sometimes exceeded the upper guideline limit (Fig. 9.4). Alabaster & Lloyd (1980) stated that higher pH levels are not as great a threat to aquatic organism as are low pH levels. Increased pH is, however, important as it creates more favourable conditions for

algal blooms, increased aquatic weed growth, and is thus a concern in areas with nutrient enrichment. The high level of temporal fluctuations in pH detected at locality 10 (Spook Spruit) could possibly be ascribed to acid mine drainage in its sub catchment. The pH at this site varied between acidic to alkaline and this could be detrimental to aquatic organism occurring in this reach of the river (Table 9.1).

Total dissolved salt (TDS) concentrations are a measure of all the salts dissolved in water and is usually directly proportional to the electrical conductivity (EC) of the water (Fig. 9.7 & 9.8). The EC of water refers to its ability to conduct an electrical current and is attributed to the presence of ions in the water that have a capacity to carry an electrical charge (CO_3^{2-}, HCO-, Cl-, SO_4^{2-}, NO_3-, Na^+, K^+, Ca^{2+} & Mg^{2+}). Natural processes such as geological weathering and atmospheric conditions contribute to the TDS of natural waters. Fluctuations of TDS occurring under natural conditions can be ascribed to the dissolution of rocks, soils and decomposing plant material (Dallas & Day, 1993; DWAF., 1995). Domestic and industrial discharges and surface runoff from urban, industrial and cultivated areas, together with evaporation, can increase natural levels of TDS to a great extent. These increased levels of TDS in the water could be of major concern to the health of the aquatic organisms (Table 9.1). Coal mining activities in the Boesman Spruit catchment could be the main reason for the immense differences in TDS and EC levels detected between the upper- (locality 5) and lower Boesman Spruit (locality 6). The highly polluted Suurstroom (locality 8) also had high levels of TDS and EC, indicating that polluted effluents have been entering this system. Some sources of pollution, possibly industrial effluents, are also responsible for elevated TDS and EC levels at localities 12 (Woesalleen Spruit) and 10 (Spook Spruit). Elevated TDS and EC levels have also been described as one of the major pollution concerns of the lower Olifants River catchment (Seymore *et al.*, 1994). Mines and industries, including Palabora Mining Company (PMC), Foskor and Fedmis, in the Phalaborwa region seem to be the main contributors of this type of pollution. Runoff from stock piles, waste dumps and seepage water from the tailing dams also enter the Selati River (CSIR, 1990). This river then confluences with the Olifants River before entering the Kruger National Park at locality 17 (Mamba weir). The impact of the water from the Selati was clearly visible in the difference between the TDS and EC concentrations measured between locality 17 and 19 (Phalaborwa Barrage), upstream of the Selati-Olifants confluence (Fig. 9.7 & 9.8). Moore *et al.* (1991) determined the critical value for TDS in the lower Olifants River to be 1 000 mg/l. This value was often exceeded, especially during low flow periods, and is still of concern.

Although sulphates themselves are non-toxic, in excess they form sulphuric acid which is a strong acid that reduces pH and can have devastating effects on aquatic ecosystems (Dallas & Day, 1993). Water seeping from coal mines (acid mine drainage) can cause high levels of sulphates in the receiving waters as sulphate is usually the dominant anion in mine drainage and is commonly used as an indicator of coal-mine drainage (Borchers *et al.*, 1991). Acid mine drainage occurring in the upper reaches of the Olifants River could therefore have played a major role in the elevated levels of sulphates detected at localities 2, 6, 8, 10 and 12. Sulphate also plays a major role in contributing to the levels of total dissolved salts, as was observed in the comparison between figures 9.7 and 9.18. A critical level of 300 mg/l was established for sulphate levels in the Olifants River inside the KNP by Moore *et al.* (1991). In the present investigation this level were exceeded at times, especially during low flow periods and could therefore be of concern for aquatic organisms in this ecosystem.

Chloride (Cl^-) being a major anion in many inland waters, is an essential component of living systems (involved in ionic, osmotic and the water balance of body fluids). Present in elevated levels it can effect living organisms by increasing the total dissolved salt concentrations (Table 9.1). Chlorine (Cl_2) on the other hand, is a greenish-yellow gaseous element that dissolves in water to form hydrochloric acid which is a strong acid that dissociates to form Cl^- and H^+ ions (Dallas & Day, 1993). Free chlorine in water is toxic and can be used as a disinfectant for removing odours from drinking water and for the destruction of pathogenic bacteria. It is therefore widely used in sewage and drinking water treatment as well as many industrial effluents which could cause increased and dangerous levels in receiving surface waters (Coetzee, 1996). Effluent containing ammonia, organic matter or cyanides convert chlorine into substances such as chloramines, which may be less toxic but more persistent than chlorine, thereby posing a long-term threat to aquatic life. Levels of chloride were very high at locality 17 (Mamba weir, KNP) due to the impact of the Selati River (confluencing with the Olifants River upstream of locality 17) containing elevated TDS levels caused by the impact of mining and industries in Phalaborwa. Chloride concentrations were generally lower during the low flowing period and indicate that the higher flows have a dilutent effect on these levels in the water. Levels of chlorine detected in the Olifants River exceed the proposed Guidelines for South African Aquatic ecosystems (DWAF., 1995) by a very large margin. A further study is therefore necessary to analyse historical data and to determine if the guideline values are realistic for the region, or if it must be adapted to create site specific guideline values.

Sodium and potassium play important roles in the ionic and osmotic water balance and muscle contraction of all animals, while sodium is also involved in the transmission of nervous impulses. They both function as contributors to TDS in the water and since potassium occurs in lower concentrations than does sodium, it can sometimes act as a limiting nutrient for plant growth (Dallas & Day, 1993). Although sodium is considered to be the least toxic metal cation (Hellawell, 1986), fish kills in the Olifants River have previously been associated with elevated sodium and potassium levels. The mortalities were, however, rather attributed to the elevated potassium levels than the sodium levels (Moore, 1990). In the upper Olifants River catchment, elevated median levels of sodium were observed at localities 6 (Lower Boesman Spruit) and 12 (Woesalleen Spruit). Elevated sodium concentrations measured in the water of coal mined areas of the United States of America have been reported by Borchers *et al.* (1991) and it can therefore be assumed that the coal mining in these areas were also directly responsible for these increased levels. There is a definite indication that locality 2 (Koring Spruit) is also influenced by some source of sodium containing pollutant because of the amount of sodium variation occurring over the study period at this site (Fig. 9.9). Similarly elevated potassium levels were also observed at localities 6 and 12, probably also due to coal mine pollution. High potassium levels were also detected at locality 8 (Suurstroom) indicating that the effluent entering this stream is of concern in this regard. The impact of the mine and industrial effluent entering the Selati River (as discussed for TDS and EC) is the main contributors for elevated levels of both sodium and potassium occurring in the lower catchment (localities 17 and 18).

Calcium and magnesium are both essential elements for living organisms because they are involved in the muscle contraction and nervous system. It is found in structural material such as bones and teeth and is vital for energy metabolism production and a variety of other biochemical interactions. Calcium is often the major cation in inland waters (Heath 1987;

Dallas & Day, 1993). Both these elements are determinants of the hardness of the water, which in turn usually determines the functional level of toxicity of toxins to aquatic organisms (Hellawell, 1986, Mason 1991). Natural processes are responsible for the input of these metals into surface waters, but significantly higher levels of these elements have been observed in coal mining areas (Borchers *et al.*, 1991). It is therefore assumed that the increased levels of magnesium and calcium detected at localities 2 (Koring Spruit), 6 (Lower Boesman Spruit), 8 (Suurstroom), 10 (Spook Spruit) and 12 (Woesalleen Spruit) are due to the coal mining and/or industrial activities in these areas. Elevated levels of these elements observed in the lower catchment are ascribed to the impact of the Selati River, being polluted by mining and industrial activities (Seymore *et al.*, 1994).

In nature, fluoride is normally found in combination with calcium, potassium and phosphates rather than as free fluorine gas. In natural waters, its concentration is dependant on geological variables such as the composition of the soils and rocks as well as climatic conditions, while anthropogenic activities such as industrial and agricultural pollution can lead to increased levels (Raubenheimer *et al.*, 1991). Elevated levels of fluoride in an aquatic ecosystem can be detrimental to the health of the aquatic organisms by influencing fish metabolism, causing skeletal fluorosis and even mortalities when present in extremely high levels (>20 mg/l) (Table 9.1). Fluoride concentrations are influenced the by water hardness, as high concentrations of calcium suppress fluoride concentrations through precipitation of insoluble calcium fluoride (Smith *et al.*, 1985). Locality 8 (Suurstroom) had exceedingly high levels of fluoride which probably contributed to the high levels detected at locality 9 (Olifants River), a few kilometres downstream. Locality 10 (Spook Spruit) is also influenced by elevated fluoride concentrations which is possibly due to mining activities in its subcatchment. In the Klein Olifants River, locality 13 (Middelburg Dam) seemed to be polluted by a fluoride containing source during some of the surveys (Fig. 9.13). An investigation will have to be conducted in an attempt to gain more knowledge concerning the natural levels of fluoride occurring in this area.

A study conducted by Raubenheimer *et al.* (1991) in the Kruger National Park (KNP) indicated that the Olifants River contained the highest levels of fluoride compared to all other major rivers in the KNP, indicating that monitoring should take place to evaluate this occurrence. The median level of fluoride detected at locality 17 (KNP) during this study was higher than 1 mg/l, which is higher than that recommended for drinking water (Raubenheimer *et al.*, 1991). If it is kept in mind that the water of the Olifants River is also used for human consumption, this could be of concern. The elevated levels could also be of concern to other aquatic organisms (especially hippopotami) or animals drinking the water. Moore *et al.* (1991) stated that the critical value for fluoride in the Olifants River inside the KNP is 1,5 mg/l. This level was exceeded during low flows and the source (the Selati River) should be reduced since it is negatively affecting the water quality of the Olifants River inside the Kruger National Park. The levels of fluoride detected in the Olifants River during this study exceeded the Target Water Quality Range (TWQR) of 0.05 mg/l and Chronic Effect Value (CEV) of 0,1 mg/l proposed for South African aquatic ecosystems (DWAF., 1995). This could be an indication that aquatic organisms are at present chronically exposed to fluoride in the Olifants River which could lead to various sub-lethal effects (Table 9.1). Fluoride concentrations were, however, much lower than the Acute Effect Value (AEV) of 7 mg/l (except at locality 8) indicating that aquatic organisms are not presently under direct threat of mortality (DWAF., 1995).

Various elements such as phosphorus, nitrogen, carbon and others are essential plant nutrients required for normal plant growth and reproduction. The natural input of these nutrients to water courses is determined by climatical factors (weathering, erosion, rainfall & variability of runoff) and catchment characteristics (surface geology & form). Elevated levels of these nutrients are, however, caused by anthropogenic activities such as point source effluent from sewage treatment works, industries and intensive animal enterprises, or diffuse sources such as agricultural runoff from excessive use of fertilizers, as well as from urban runoff. Most of these nutrients are not directly toxic to aquatic organisms (except nitrite & ammonia) even when in high concentrations. Nitrogen as nitrate (NO_3-) and phosphorus as phosphate (PO_4^{3-}) present in high concentrations due to nutrient enrichment of aquatic ecosystems could, however, result in excessive plant growth which can lead to an imbalance in the biological communities, particularly to an increase in plant communities and associated water quality deterioration (eutrophication). Lotic systems are reported to be less susceptible to nutrient enrichment than are lenthic systems because there is little retention in the moving water of rivers (Dallas & Day, 1993). This is evident in the results gained during this study at locality 20 (Nhlanganini Dam - a control site having little or no anthropogenic impacts) which contained high levels of nutrients compared to the other sites investigated. This locality was the most lenthic of all sites investigated and a large population of hippopotamus and various fish species also contributed to the input of nutrients to the water at this site.

In surface waters, phosphorus will be present in high concentrations, either as orthophosphates or as polyphosphates, which will in time revert to orthophosphates (Ellis, 1989). Orthophosphate or soluble reactive phosphate is phosphorus which is immediately available to aquatic biota and which can be transformed into an available form by natural processes (DWAF., 1995). In aquatic ecosystems, large proportions of phosphorus may be unavailable because it is adsorbed onto suspensoids or bound to particles such as iron, aluminium, calcium or organic polyphenols. During low flow periods, stream bed sediments act as a sink for phosphorus entering the stream at high concentrations, becoming available again during higher flows and/or anoxic conditions. High concentrations of phosphorus are likely to occur in waters that receive sewage and leaching or runoff from cultivated land. These sources were possibly the main contributors to high levels of phosphates detected at localities 3, 9, 11, and 14 in the upper catchment. Increased levels of orthophosphate (PO_4-P) detected at locality 17 (Mamba weir) in the lower Olifants River catchment can be attributed to the effect of the Selati River, which is known to be polluted with phosphates (CSIR., 1990; Seymore *et al.*, 1994). High levels of nitrite-nitrate were detected downstream of towns such as Davel (locality 1), Kriel (locality 3), Witbank (locality 9) and Middelburg (locality 14). These occurrences can possibly be ascribed to the impact of urban runoff, and sewage treatment effluents (Dallas & Day, 1993). Algal blooms and excessive aquatic plant growth were also witnessed in the Olifants River downstream of Witbank due to this nutrient enrichment. Ammonia is produced naturally by the biological degradation of nitrogenous matter and provides an essential link in the nitrogen cycle. Pollution could, however, be responsible for increased levels in surface waters such as is the case at locality 8 (Suurstroom). The pH at this locality is very low in comparison to the other localities sampled (Fig. 9.5). This results in the formation of high levels of the less toxic ammonium (under acidic condition) from the more toxic ammonia (under alkaline conditions) (Fig. 9.16). Increased temperature also causes an increase in the relative proportion of un-ionized ammonia in solution, and hence an increase in its toxicity to aquatic organisms. The pH of

the Olifants River is primarily alkaline (except for a few of its polluted tributaries) which caused the relatively low ammonium levels that were detected during this study. Due to the effect of the pH changes on ammonium, the acidification of this river system will cause elevated ammonia levels in the water, which could be detrimental to the aquatic life of this ecosystem (Table 9.1).

Silicon occurs as silica (SiO_2) and silicates in sand, sandstone and diatomaceous earth (Sharp, 1990; Manahan, 1993). Silica is a plant nutrient required for normal plant growth and reproduction (Dallas & Day, 1993). Industrially, silicon halohydrides (eg. SiH_2Cl_2 & $SiHCl_3$) are used as intermediates in the synthesis of organosilicon compounds and in the production of high-purity silicon for semi-conductors. Silicates and silica are furthermore used in glass, refractories and building material (Sharp, 1990). High median levels of Si were detected at localities 1, 8 and 10 in the upper catchment, and at localities 17 and 18 in the lower catchment. It therefore seems that some source of Si, is being released upstream of these localities.

In the present study, the results obtained for the lower Olifants River showed that most of the variables investigated were generally similar to, or slightly lower, than those observed by Seymore *et al.*(1994) for the period of 1990 to 1992. This could be an indication that there has been a slight improvement in the water quality of the lower Olifants River since 1992. Most variable values are, however, still high and above guideline values and further improvement is thus necessary. It is therefore stressed that a definite and urgent attempt should be made to address the poor water quality of the Selati River. Results also indicated that phosphates and nitrite-nitrate levels have increased since 1992. This could be an indication that increased levels of nutrients are entering the river, possibly from the sewage treatment works at Phalaborwa and Namakgale, as well as various informal settlements upstream of the KNP. This study indicated that the Olifants River is being affected by point and diffuse sources of pollution in many areas. A further investigation will have to be conducted in an attempt to identify the specific sources of pollution. These will then have to be addressed in an attempt to reduce the negative impacts on this aquatic ecosystem.

9.1.5 *References*

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9.2 Metals in the water and sediment at selected localities in the Olifants River, Mpumalanga.

9.2.1 *Introduction*

Metals enter surface water through natural processes including geological weathering and decomposition of biotic material. Anthropogenic activities can, however, greatly increase the input through diffuse and point sources. Increased anthropogenic inputs are usually associated with activities such as industrial development, increased agriculture, mining and urbanisation. Mining and industrial effluents are, however, the general sources of elevated metal concentrations in surface waters.

Over the past few decades the Olifants River catchment has experienced increased urbanisation, industrial development, agriculture and mining. This caused a great amount of pollution in the Olifants River via point and non-point sources. Of these pollution problems, elevated metal levels seem to be one of the most concerning in the Olifants River system (Grobler *et al.*, 1994; Seymore 1994). Acid mine drainage due to the extensive coal mining in the Olifants River upper catchment, additionally contributes to the elevated input of metals into the water through underground drainage and mobilisation of metals from soils. It also reduces the water pH that in turn can cause some metals (e.g. Al) to become more bioavailable to aquatic organisms (Klein *et al.*, 1975; Dallas & Day, 1993).

Metals entering a freshwater system could undergo various changes before temporary or final stability is reached. In an aqueous solution free metals could be complexed with water (hydrated) or could be associated with organic and inorganic matter through the processes of adsorption, chemical combination or complex formation (Förstner & Müller, 1973). These processes are primarily dependent on the ambient water quality that is the main determinant of metal speciation. Furthermore, the species of the metal occurring in the water plays an important role in the bioavailability and toxicity of that metal (Wade *et al.*, 1995). Concomitantly a toxic metal present in free ionic form will after complexation have a greatly reduced toxicity. The final toxicity of the metal is furthermore influenced by the interaction between pollutants, the developmental stage of the organism and interspecies variation in susceptibility to metals (Hellawell, 1986; Ellis, 1989).

Suspended and bed sediments are the most concentrated pool of metals occurring in an aquatic ecosystem. Sediments are composed of numerous individual layers, each of which corresponds to a distinct condition of water flow. Coarse layers represent bed load deposited during stronger currents, fine layers consist mainly of suspended load deposited during weaker or absent currents. Therefore, fine-grained sediments built up of a large number of individual layers should represent an average value for certain contaminants over a long period. This is especially true for metals that are strongly adsorbed to clay minerals and the organic fraction of the sediment (Förstner & Müller, 1973). Due to changes in the physico-chemical water quality such as decreased pH, there is a constant exchange of metals between the water and the sediment. Metals adsorbed onto sediments are furthermore transported into the food chain when these contaminated sediments are ingested with/as food particles by aquatic organisms.

Aquatic ecosystems contaminated with metals can be confirmed as such by examining the

water, sediment and organisms (Dallas & Day, 1993). This section of the study investigated the extent of pollution by various metals (Cu, Zn, Al, Fe, Ni, Mn, Pb and Cr) in the upper and lower catchment of the Olifants River, Mpumalanga.

9.2.2 *Materials and methods*

Seasonal surveys were conducted from summer 1994 to autumn 1995 at 18 selected localities in the Olifants River catchment, Mpumalanga. Sixteen of these sites were situated in the upper catchment and two in the lower catchment (Fig. 5.8). Two additional sites were sampled during the autumn 1995 survey namely locality 19 (Phalaborwa Barrage) to evaluate its present state of water quality and to asses the impact of the Selati River on the Olifants River, and locality 20 (Nhlanganini Dam) as a control site with relatively natural conditions (Fig.8.5).

A water sample was collected 10 cm below the surface and frozen until it could be subjected to metal analysis in a laboratory. After thawing of the samples in the laboratory, 50 ml of well-mixed sample was measured into 100 ml Erlenmeyer flasks. Ten ml of concentrated nitric acid (55%) and five ml of concentrated perchloric acid (70%) were added and the mixture was evaporated to between 2 and 5 ml on a hot plate until clear (Standard Methods, 1985). Each sample was then filtered (0.45 µm filter paper) and made up to 50 ml with distilled water and stored in clean 100 ml amber glass bottles for metal analysis. Before use, all glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 hours, rinsed in distilled water, acid-washed in 1 M HCl for another 24 hours and finally rinsed in distilled water (Giesy & Wiener, 1977).

A surface sediment sample (depth: 5 cm) was collected by means of a perspex corer (diameter: 5 cm). This sample was placed in pre-washed plastic containers and also frozen until further analysis. In the laboratory, sediment samples were thawed and dried in an oven at 50 °C for a period of 96 hours. This drying temperature ensures that no chemical or textural characteristics of clay particles that may occur in the sediment will be altered (Prof. T. Harmse, personal communication, 1994). From each sediment sample a 80 g subsample was placed in a Endecott-mechanical siever with a sieve rack consisting of sieves at 0.5 phi intervals (Phi is a factor of the grain size in micrometer on a logarithmic basis) (Folk & Ward, 1957). Sieve grid sizes with mesh ranging from 3 200 mm (-1.50 phi) to 0.0313 mm (4.75 phi) were used. Chemical analysis of three different size fractions (0.125 mm = 3 phi, 0.0625 mm = 4 phi and <0.0313 mm = < 4.75 phi) were done by digesting 1 g of each sample using a mixture of ten ml of concentrated nitric acid (55%) and five ml of concentrated perchloric acid (70%). Digestion was performed on a hot plate (200 to 250°C) for at least four hours or until the solution was clear. Each solution containing the digested sediment samples was allowed to cool before being filtered, using an acid resistant 0.45 µm filter paper and a vacuum pump. After filtration the filter system was rinsed with distilled water to remove all traces of metals. These samples were then accurately made up to 100 ml volumetric volume using doubly distilled water and stored in clean amber glass bottles for metal analysis.

A Varian atomic absorption spectrophotometer (Spectra AA-10) was used to detect the total metal concentration (Cu, Zn, Al, Fe, Ni, Mn, Pb and Cr) in water and sediment samples. Analytical standards were prepared from Holpro stock solutions. For the analysis of Aluminium, 0.5 ml of a 2.682 M potassium chloride (KCl) solution (200g KCl per litre

distilled water) was added to 50 ml sample in order to suppress ionisation of aluminium (Varian, 1989).

Detection limits for the various metals on the AAS were 0.003 µg/g for copper, 0.001 µg/g for zinc, 0.002 µg/g for iron, 0.1 µg/g for nickel, 0.002 µg/g for manganese, 0.01 µg/g for lead and 0.006 µg/g for chromium. The *Statgraphic 7* computer package was used for the calculation of general statistics (mean, median, etc.).

9.2.3 *Results*

Water

The metal concentrations detected during each survey at selected localities in the water of the Olifants River are presented in Figures 9.20 to 9.27. All concentrations refer to the total amount of metal detected in a surface water sample collected at a specific site. In the results reference is made to the upper catchment (localities 1 to 16) and the lower catchment (locality 17 to 20) of the Olifants River.

The copper concentrations in the water (Fig. 9.20) of the upper catchment ranged from 1 µg/l (locality 5-autumn 1994) to 60 µg/l (locality 8-winter 1994), and in the lower catchment from 8 µg/l (locality 19-autumn 1994) to 34 µg/l (locality 18-summer 1994). Locality 8 (29±17 µg/l) had the highest median copper concentration of all selected sites. Other sites in the upper catchment with high median levels of copper were localities 3 (24±15 µg/l), 7 (19±14 µg/l), 11 (17±21 µg/l), 13 (18±3 µg/l) and 14 (22±9 µg/l). In the lower catchment locality 18 had the highest median Cu concentration (17±8 µg/l) followed by locality 17 (13±4 µg/l). High levels of copper were also detected during specific surveys at localities 3 (49 & 41 µg/l), 4 (50 µg/l), 6 (38 µg/l), 7 (49 µg/l), 8 (60 & 54 µg/l), 11 (65 µg/l), and 18 (34 µg/l). Large temporal fluctuations were detected at localities 3, 6, 7, 8 and 11, while the copper concentrations remained relatively constant over time at localities 9, 10, 13 and 16.

Zinc concentrations in the water (Fig. 9.21 ranged) from 2 µg/l (locality 1-summer 1994) to 5138 µg/l (locality 8-winter 1994) in the upper catchment, whilst the levels in the lower catchment were between 9 µg/l (locality 18-winter 1994) and 472 µg/l (locality 18-spring 1994). The highest median level of zinc was observed for locality 8 (2374±1423 µg/l), which was much higher than all other sites. High Zn levels were detected during specific surveys at localities 3 (689 µg/l), 5 (680 & 722 µg/l), 10 (779 µg/l) 12 (314 µg/l), 15 (384 µg/l) and 18 (472 µg/l). Great temporal fluctuations in zinc concentrations were detected at localities 3, 5, 8 and 10.

Total aluminium concentrations in the water (Fig. 9.22) of the upper catchment localities ranged from 80 µg/l (locality 13-autumn 1994) to 43 680 µg/l (locality 8-winter 1994) and in the lower catchment from 94 µg/l (locality 18-spring 1994) to 3183 µg/l (locality 18-autumn 1994). Locality 8 (26 370±11 839 µg/l) had the highest median Al concentration during this study. Other sites with relatively high Al levels were localities 2 (1 080 ±2 096 µg/l), 17 (1 769±1187 µg/l) and 19 (888 µg/l). In the lower catchment, high values were detected at both localities 17 and 18 during the summer 1994 and autumn 1995 surveys. Great temporal fluctuations were detected at most sites investigated, particularly at localities 1 to 5, 8 and 15 to 18 but localities 9, 10, 12, 13 and 14 had very little seasonal aluminium concentration variation.

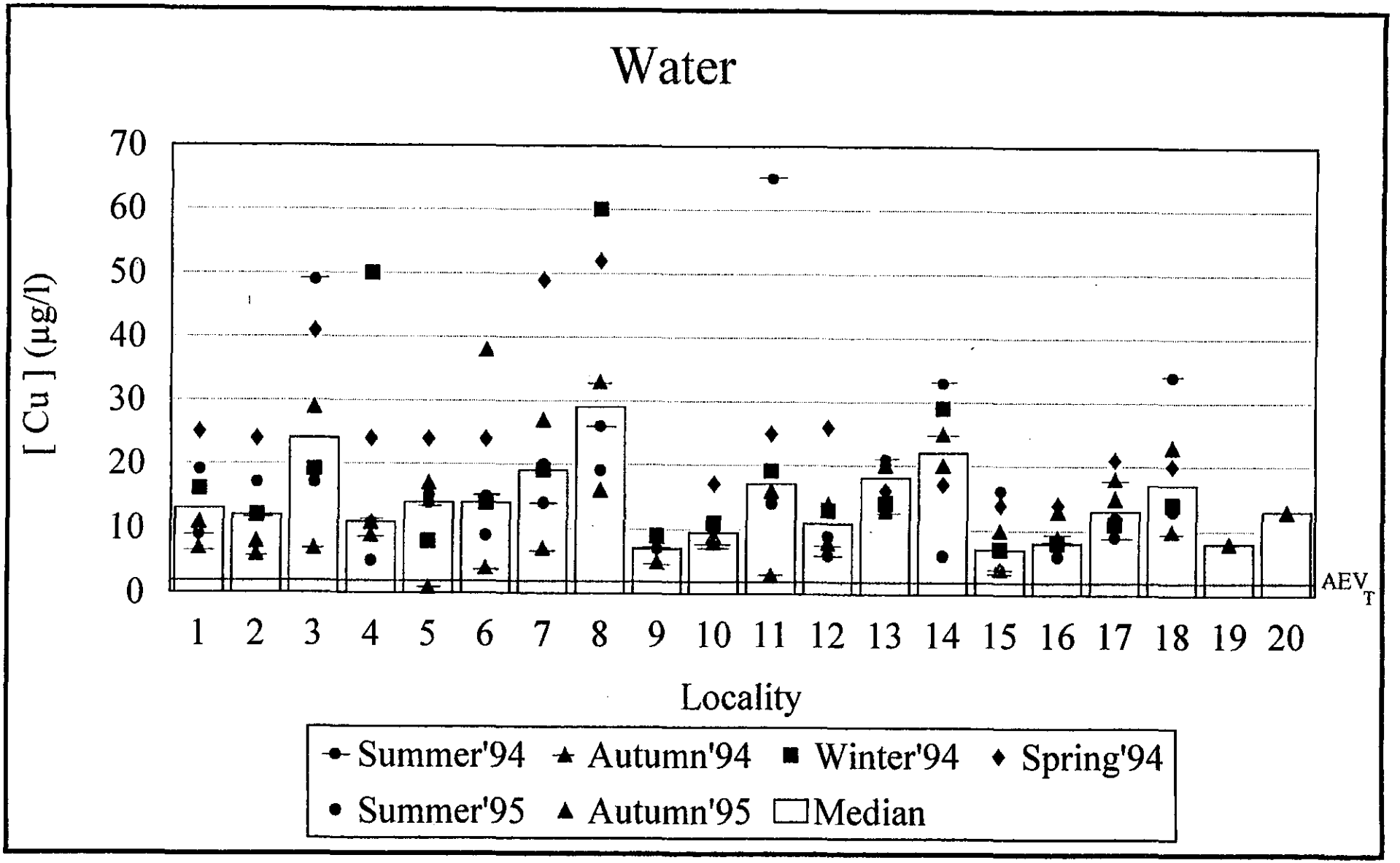

Figure 9.20: Spatial and temporal total copper variation at the selected localities in the Olifants River. AEV_T = Acute Effect Value for total copper concentrations in South African aquatic ecosystems (DWAF., 1995).

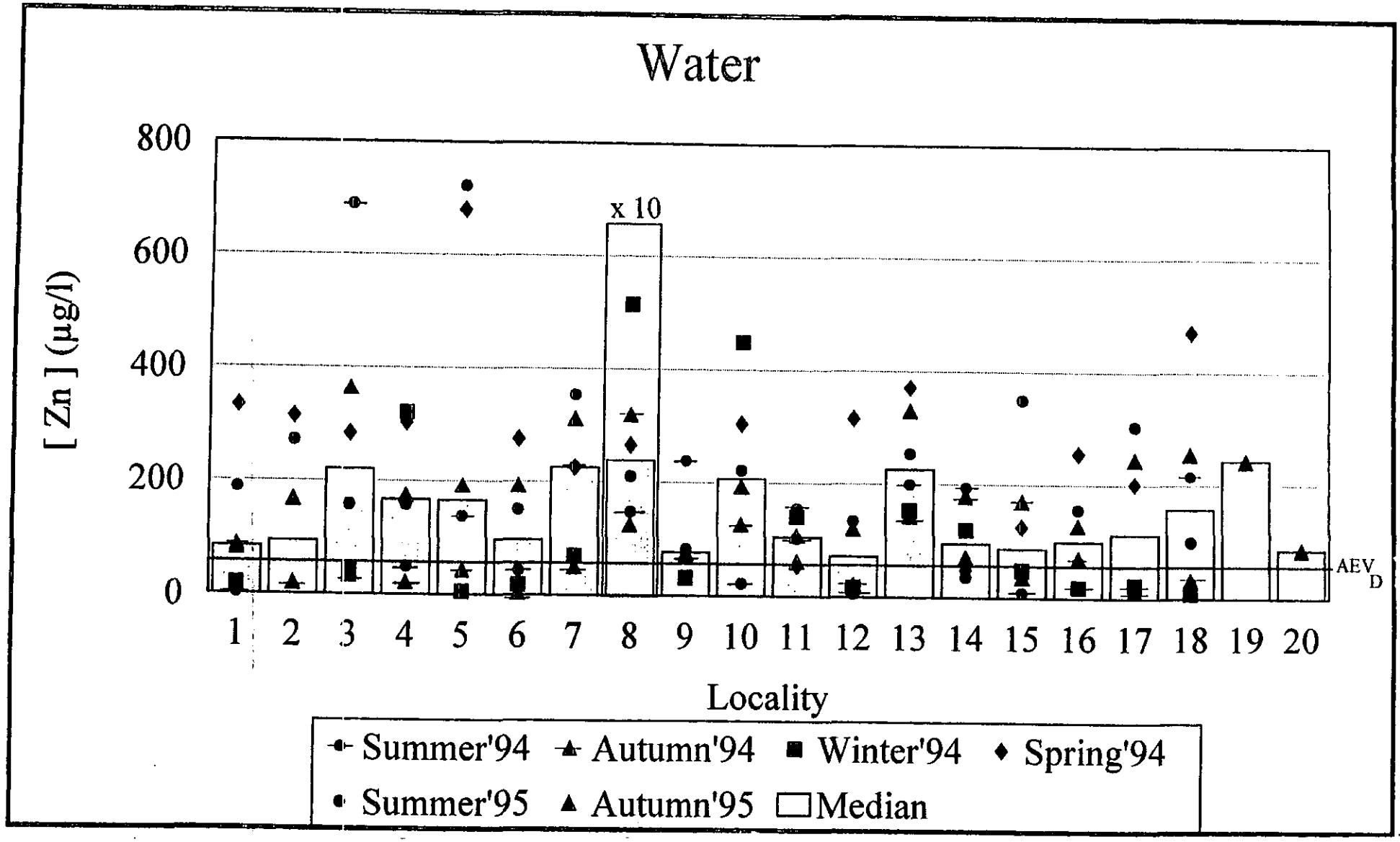

Figure 9.21: Spatial and temporal total zinc variation at the selected localities in the Olifants River. AEV_D = Acute Effect Value for dissolved zinc concentrations in South African aquatic ecosystems (DWAF., 1995).

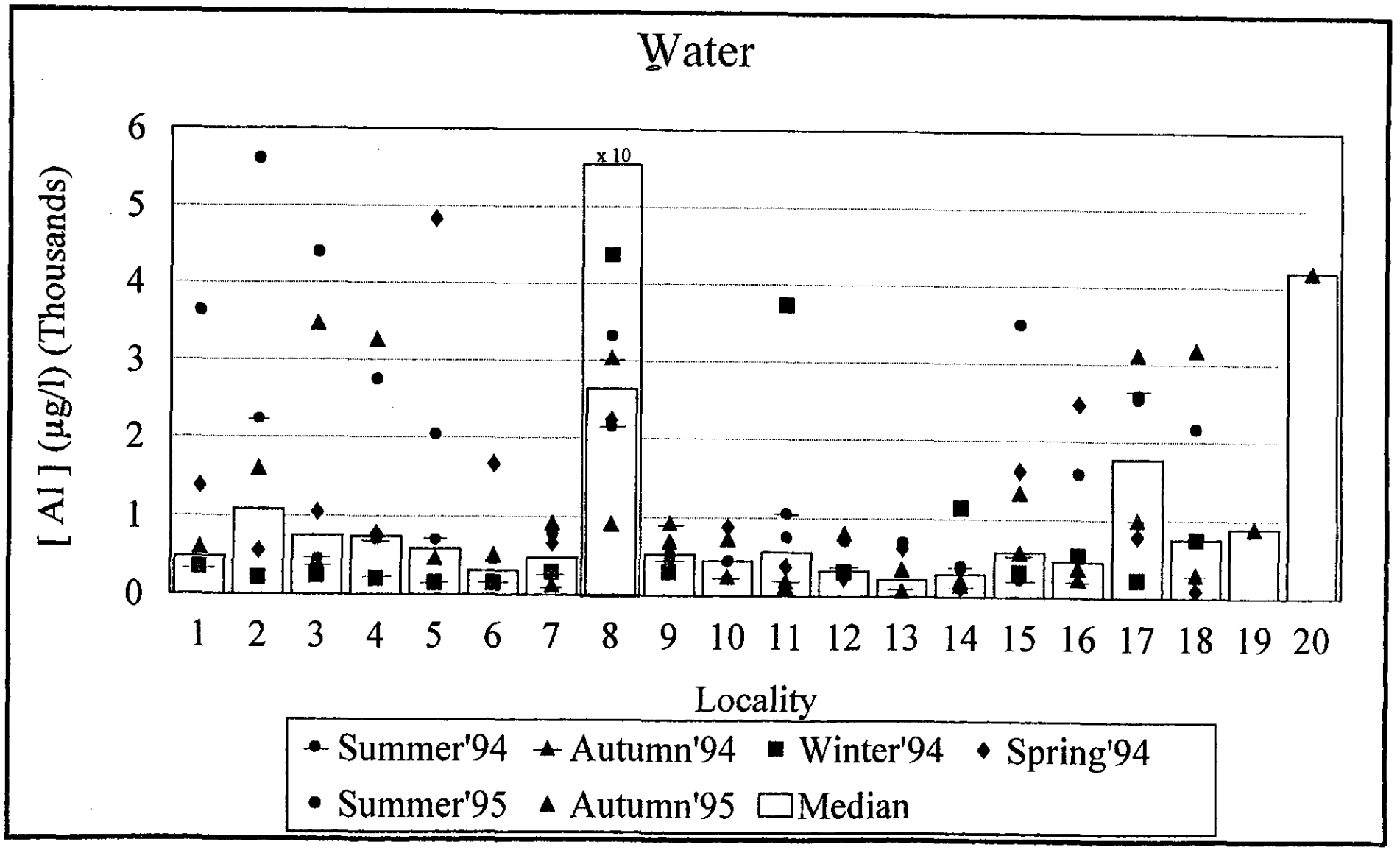

Figure 9.22: Spatial and temporal total aluminium variation at the selected localities in the Olifants River.

Iron concentrations in the water (Fig. 9.23) in the upper catchment ranged from 580 µg/l (locality 5-autumn 1995) to 8 910 µg/l (locality 15-spring 1994), while levels in the lower catchment were between 760 µg/l (locality 17-winter 1994) and 3 280 µg/l (locality 17-spring 1994). The highest median iron concentrations were detected at localities 17 (2 645±1 007 µg/l) and 18 (2 480±1 079 µg/l). Iron concentrations fluctuated greatly between surveys at all sites except localities 7, 9, 10, 12, 13, 14 and 16. In the upper catchment iron levels were generally low from summer to winter 1994, after which levels increased to spring and summer 1995. In the lower catchment levels were also low during autumn and spring 1994 and higher during all other surveys. Locality 20 (control) had relatively high levels of Fe (3 480 µg/l) when compared to the other sites investigated.

Nickel concentrations in the water (Fig. 9.24) of the upper catchment ranged from 35 µg/l (locality 11-summer 1995) to 1916 µg/l (locality 8-winter 1994), and in the lower catchment from 64 µg/l (locality 18-spring 1994) to 242 µg/l (locality 18-summer 1994). Locality 8 (1153±401 µg/l) had an exceedingly high median Ni level compared to the other sites investigated. Other sites with relatively high median levels of Ni were localities 3 (209±97 µg/l), 7 (212±100 µg/l), 10 (232±184 µg/l), 13 (204±121 µg/l) and 14 (214±94 µg/l). In general, the lowest levels of nickel were detected during the spring 1994 surveys in the upper and lower catchment.

Manganese concentrations in the water (Figure 9.25) of the upper catchment ranged from 14 µg/l (locality 11-summer 1995) to 35 040 µg/l (locality 8-autumn 1994). In the lower catchment, levels varied between 29 µg/l (locality 17-winter 1994) and 377 µg/l (locality 18-summer 1994). Localities 8 (27 400±7 803 µg/l) and 10 (2 610±8 483 µg/l) had extremely high median levels of manganese when compared to the other sites. Sites with exceedingly high levels of manganese during certain surveys were localities 1 (604 µg/l), 2 (708 µg/l), 4 (1 177 & 1 084 µg/l), 5 (1 019 µg/l), 6 (1 646 & 4 665 µg/l), 12 (935 µg/l), 15 (531 µg/l) and 16 (1 188 & 1 442 µg/l).

Lead concentrations in the water (Fig. 9.26) of the upper catchment ranged from below detection limit (localities 1, 2, 9, 12 & 15) to 284 µg/l (locality 13-autumn 1995) and in the lower catchment from below detection limit (locality 17-summer 1994) to 206 µg/l (locality 17-autumn 1995). Locality 13 (173±84 µg/l) had the highest median lead concentration. Other sites with relatively high median lead levels were localities 3 (131±55 µg/l), 7 (136±61 µg/l), 11 (162±60 µg/l) and 14 (164±84 µg/l). Lead levels in the water indicated temporal fluctuations at all localities. In the upper catchment lead levels were generally high during spring 1994 and summer 1994 and 1995, while low during the other surveys.

Chromium concentrations in the water (Fig. 9.27) varied between 104 µg/l (locality 4-winter 1994) and 1 022 µg/l (locality 11-autumn 1995) in the upper catchment, and between 136 µg/l (locality 17-summer 1995) and 404 µg/l (locality 18-summer 1994) in the lower catchment. Temporal fluctuations were also evident at these localities. In the upper catchment Cr levels were generally low during autumn 1995, whilst in the lower catchment during summer 1995.

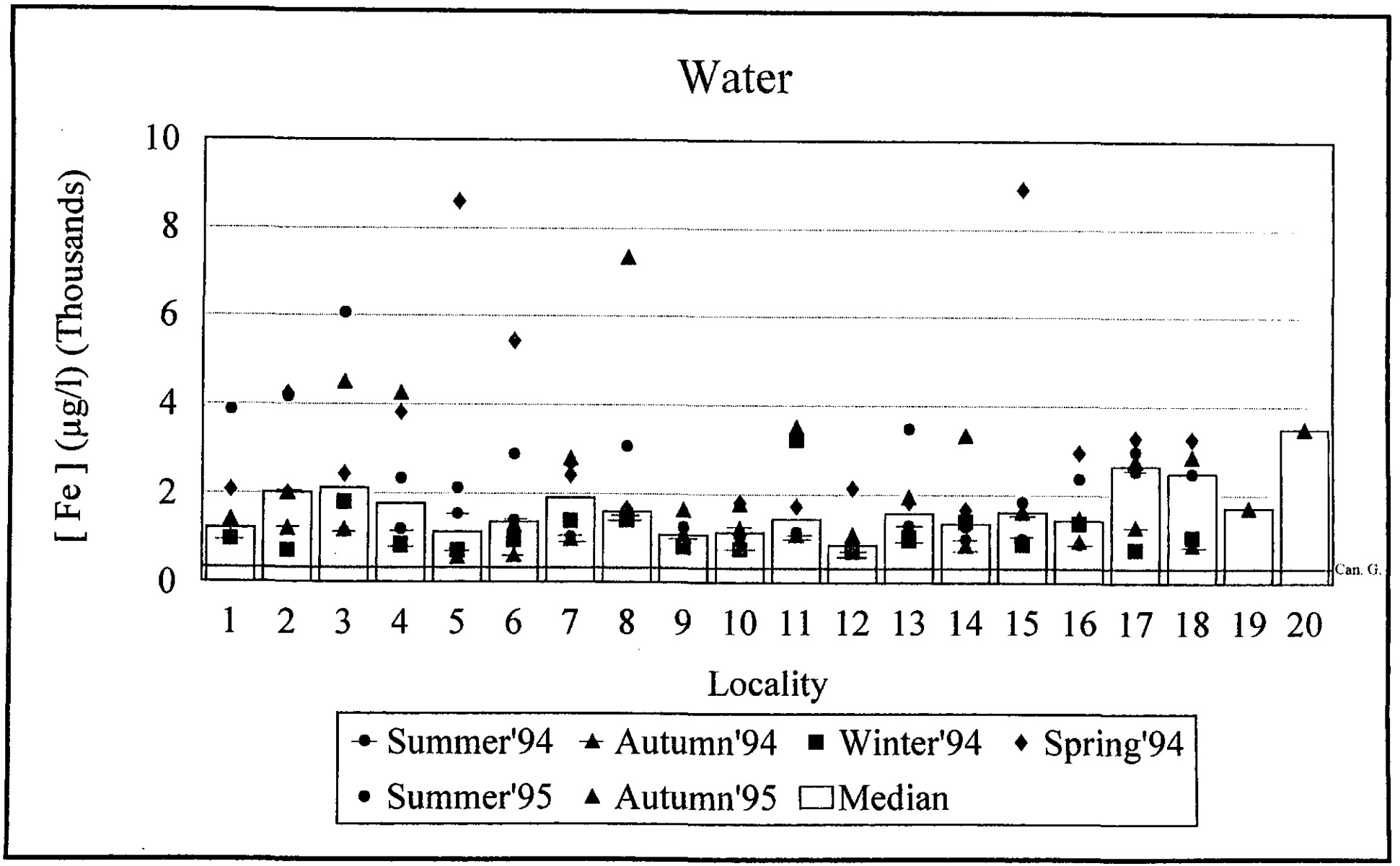

Figure 9.23: Spatial and temporal total iron variation at the selected localities in the Olifants River. Can. G. = Canadian Guideline Value for aquatic ecosystems (Environment Canada, 1987).

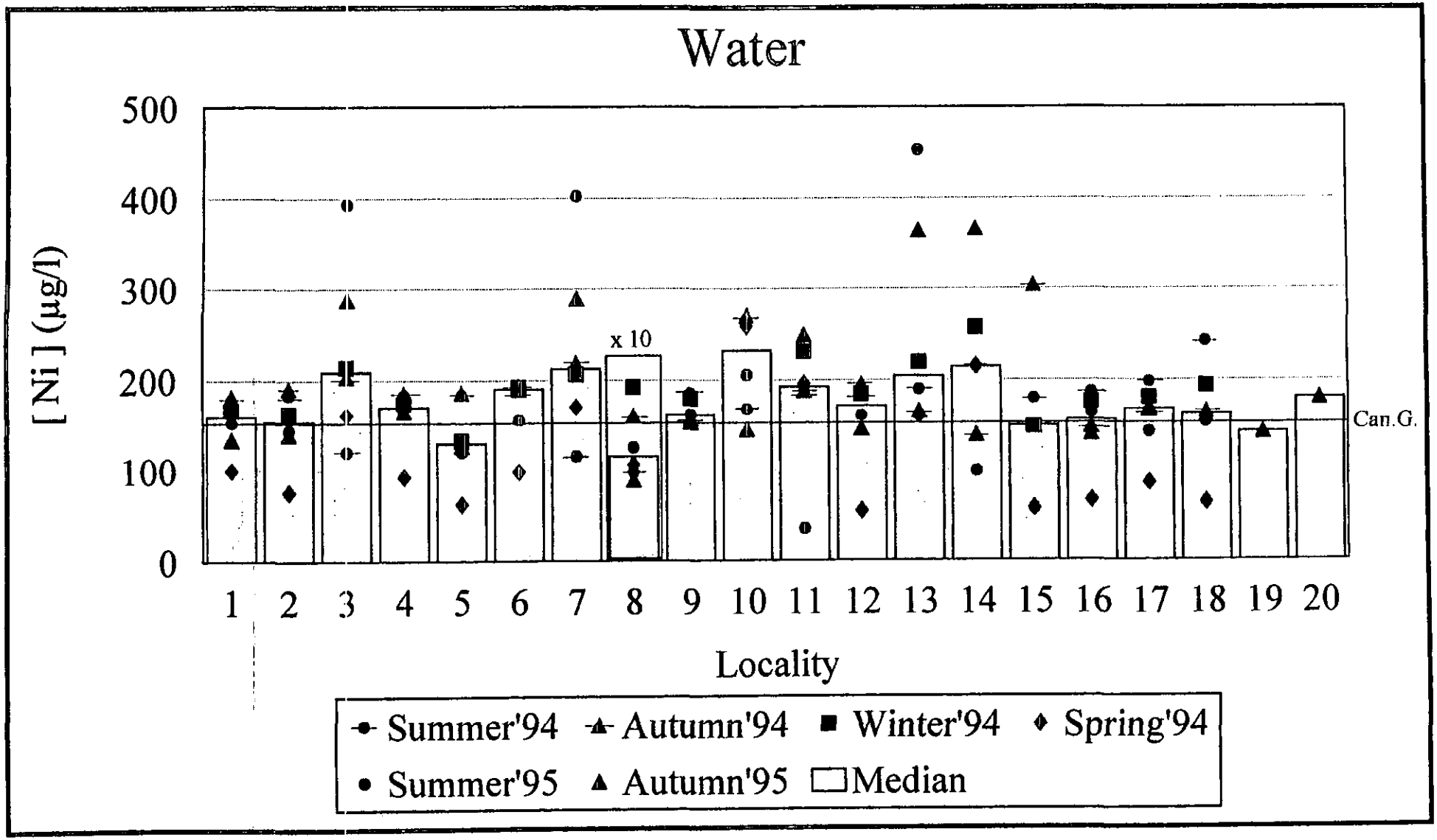

Figure 9.24: Spatial and temporal total nickel variation at the selected localities in the Olifants River. Can. G. = Canadian Guideline value for Ni in hard water systems. (Environment Canada, 1987).

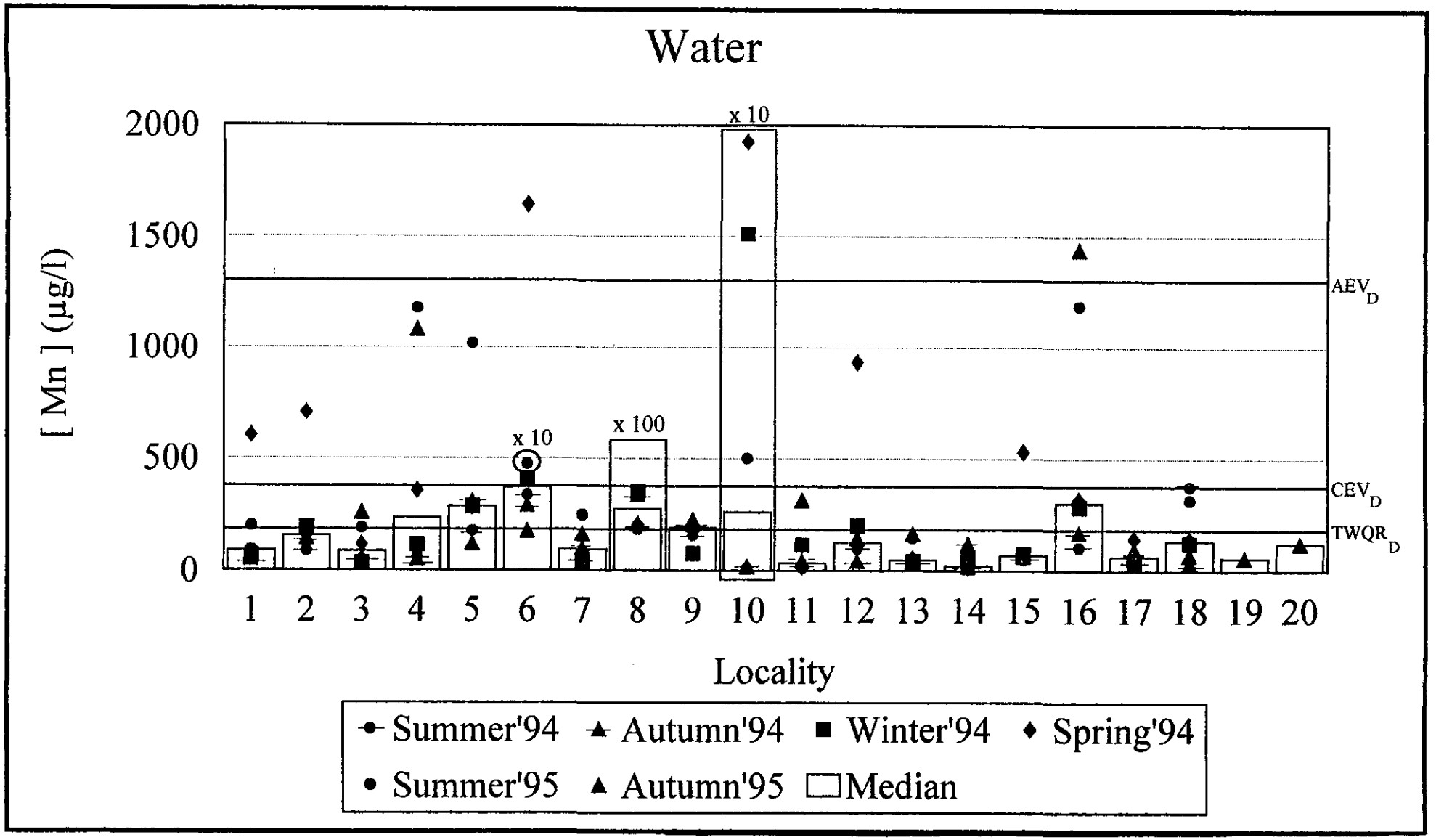

Figure 9.25: Spatial and temporal total manganese variation at the selected localities in the Olifants River. $TWQR_D$ = Target Water Quality Range, CEV_D = Chronic Effect Value & AEV_D = Acute Effect Value of dissolved Mn proposed for South African aquatic ecosystems (DWAF., 1995).

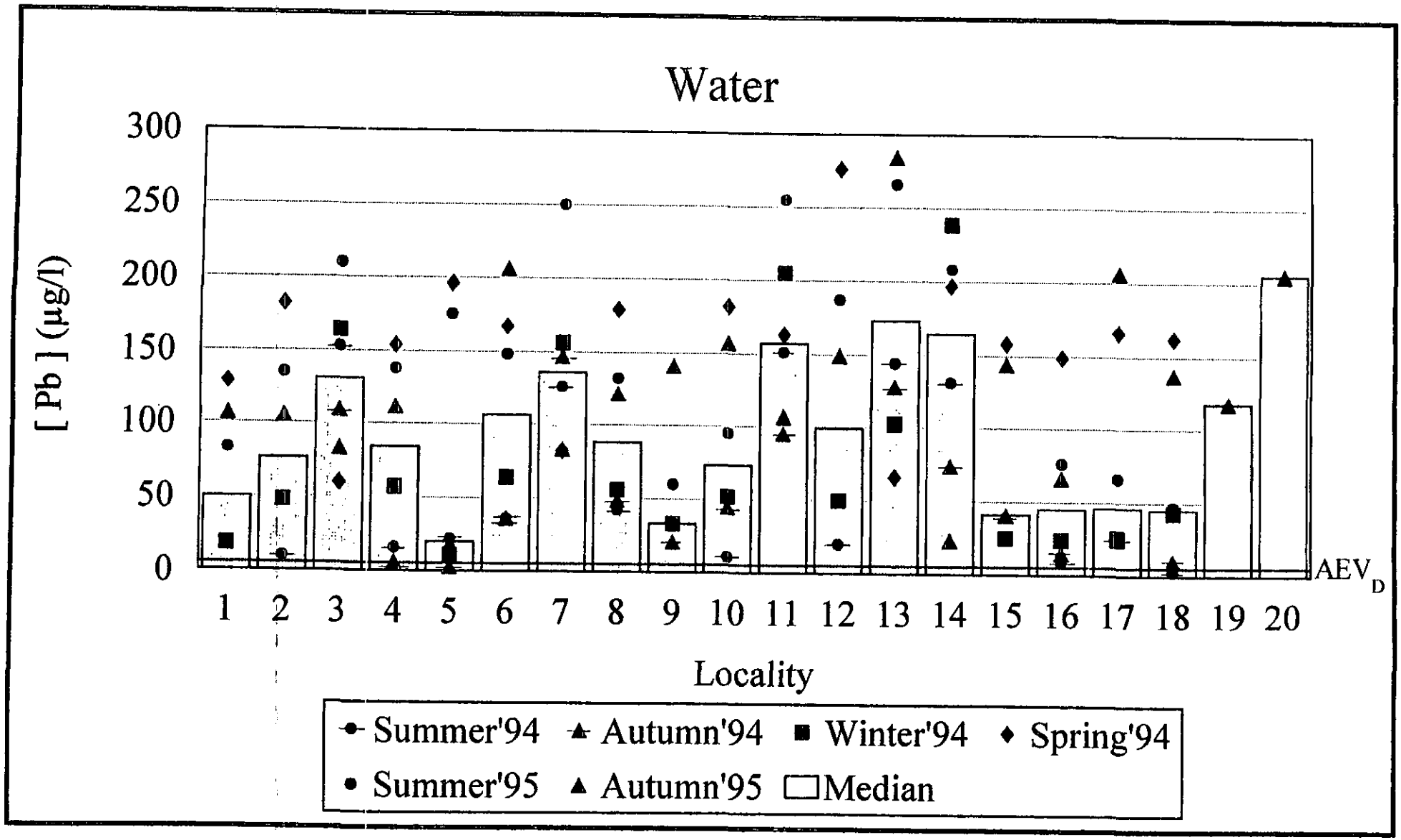

Figure 26: Spatial and tempral total lead variation at the selected localities in the Olifants River. AEV_D = Acute Effect Value proposed for South African aquatic ecosystems (DWAF., 1995).

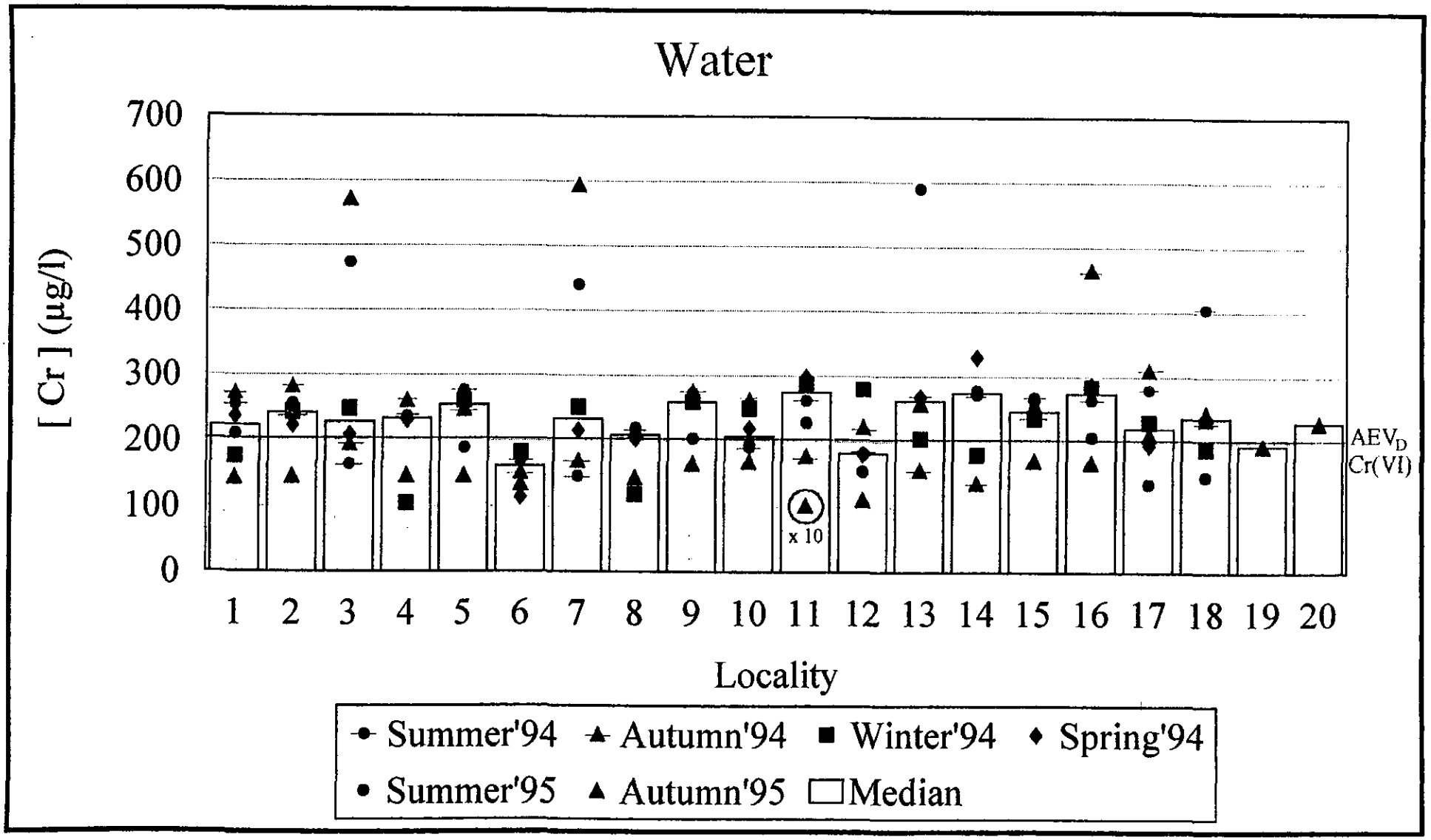

Figure 9.27: Spatial and temporal total chromium variation at the selected localities in the Olifants River. AEV_D = Acute Effect Value for dissolved Cr(VI) proposed for South African aquatic ecos16

Sediment

Metal concentrations detected in the sediment samples collected seasonally from the selected sampling sites (Fig. 8.5) are presented in Figures 9.28 to 9.35. These small particles are the most available for uptake by aquatic organisms and therefore of most importance in toxicological studies. Concentrations therefore refer mostly to a particle size of smaller than 0.0313 mm (fine silt and fine clay) or in some cases to sizes of 0.0625 mm (coarse silt) and 0.125 mm (fine sand). Distinction is also made between the upper catchment (localities 1 to 16) and the lower catchment (localities 17 to 19) of the Olifants River.

Copper concentrations in the sediment (Fig. 9.28) in the upper catchment ranged from 7 µg/g (locality 9-winter 1994) to 215 µg/g (locality 1-spring 1994), whilst in the lower catchment it ranged from 14 µg/g (locality 18-summer 1995) to 126 µg/g (locality 17-winter 1994). The highest mean sediment copper concentration was detected at locality 10 (124±50 µg/g). Sites with low sediment copper concentrations were localities 2 (38±27 µg/g), 3 (23±10 µg/g), 9 (52±40 µg/g) and 18 (38±17). Copper concentrations detected in the sediment of locality 20 (control) were high and exceeded the levels detected at many of the sites in the catchment.

Zinc concentrations in the sediment (Fig. 9.29) of the upper catchment ranged from 22 µg/g (locality 3-summer 1994) to 13 410 µg/g (locality 10-summer 1995), and in the lower catchment, values were between 52 µg/g (locality 17-summer 1994) and 337 µg/g (locality 17-autumn 1995). Extremely high mean levels of Zn were detected in the sediments at locality 10 (3 895±4 760 µg/g), 11 (919±946 µg/g) and 12 (2 616±1 361). Low mean concentrations and little seasonal fluctuations were observed in the Zn levels detected at localities 1 (50±12 µg/g), 2 (52±19 µg/g) and 3 (39±19 µg/g).

Aluminium concentrations in the sediment (Fig. 9.30) detected in the upper catchment were between 8 839 µg/g (locality 13-autumn 1994) and 263 898 µg/g (locality 13-autumn 1994), and in the lower catchment it ranged from 10 119 µg/g (locality 18-autumn 1994) to 73 032 µg/g (locality 17-spring 1994). High mean Al concentrations were detected at localities 5 (87 423±39 321 µg/g), 6 (83 332±42 461 µg/g), 8 (80 691±33 067 µg/g) and 13 (78 059±92652 µg/g). Sites with low mean Al concentrations and little seasonal variation (compared to the other sites) were localities 2, 3, 7, 11, 14, 16 and 18. Locality 20 (control) had relatively low Al levels compared to the other sites.

Iron concentrations in the sediments (Fig 9.31) varied between 7 548 µg/g (locality 13-autumn 1995) and 160 078 µg/g (locality 6-winter 1994) in the upper catchment, and ranged from 11 638 µg/g (locality 18-autumn 1994) to 64 689 (locality 17-summer 1995) in the lower catchment. Sites with high mean iron concentrations were localities 6 (79 517±41 791 µg/g), 8 (61 489±36 473), 10 (53 922±4 880 µg/g), 12 (48 273±17 524 µg/g), 15 (44 591±15 336 µg/g) and 17 (47 880±12269 µg/g). Localities 2 (18 619±7 373 µg/g) and 3 (16 760±5 585 µg/g) had the lowest Fe concentrations of all the sites. Iron concentrations in the sediments at localities 2, 3 and 7 stayed relatively constant over the study period. Locality 20 (control) had relatively high Fe levels compared to most of the sites in the catchment.

Concentrations of nickel in the sediment (Fig. 9.32) of the upper catchment ranged from 15

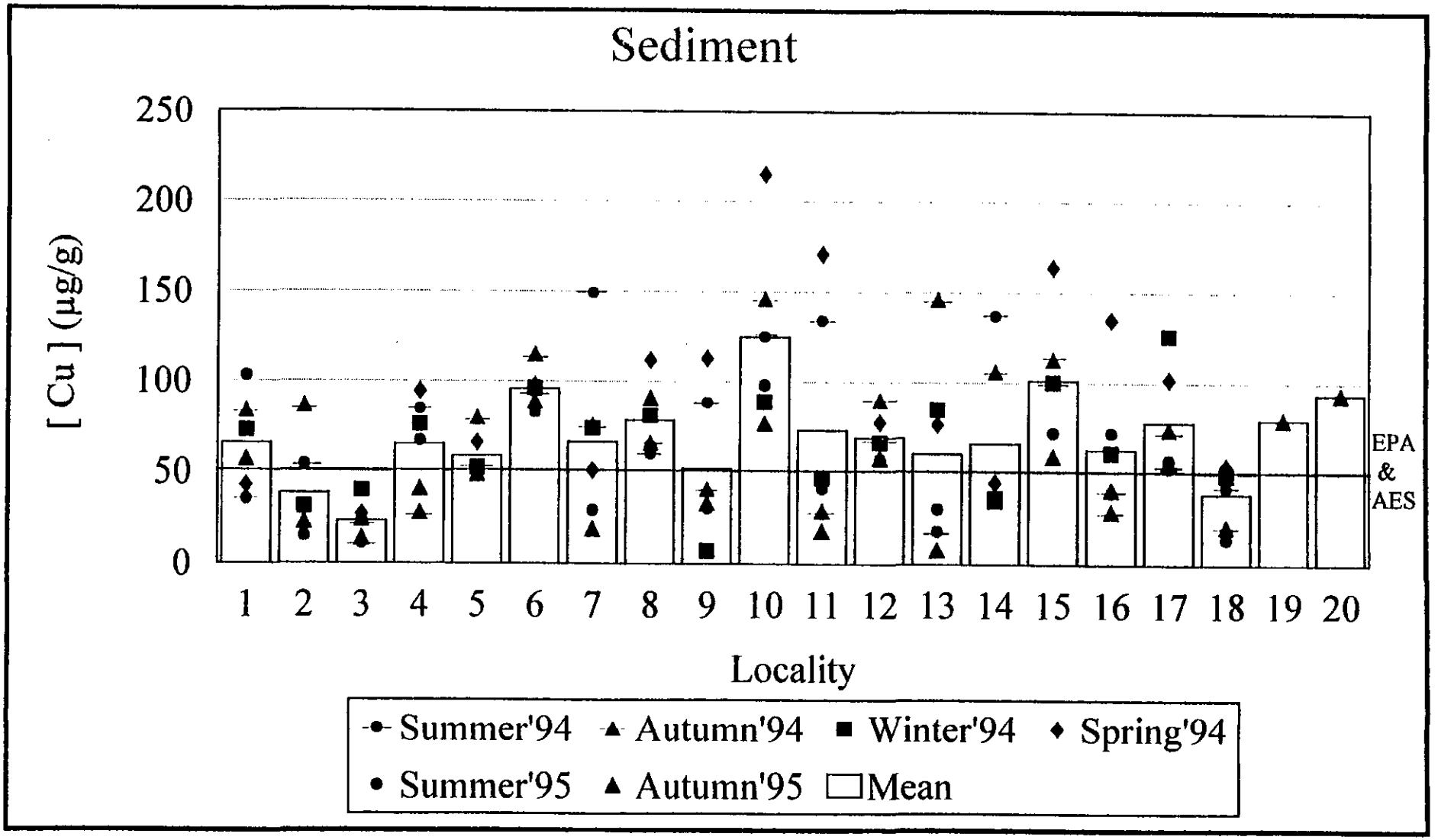

Figure 9.28: Spatial and temporal variation of the sediment copper concentration at the selected localities in the Olifants River. EPA = Maximum Cu level according to EPA toxicity classification and AES = Cu concentration of Average Earth Sediment (from Steenkamp *et al.*, 1994).

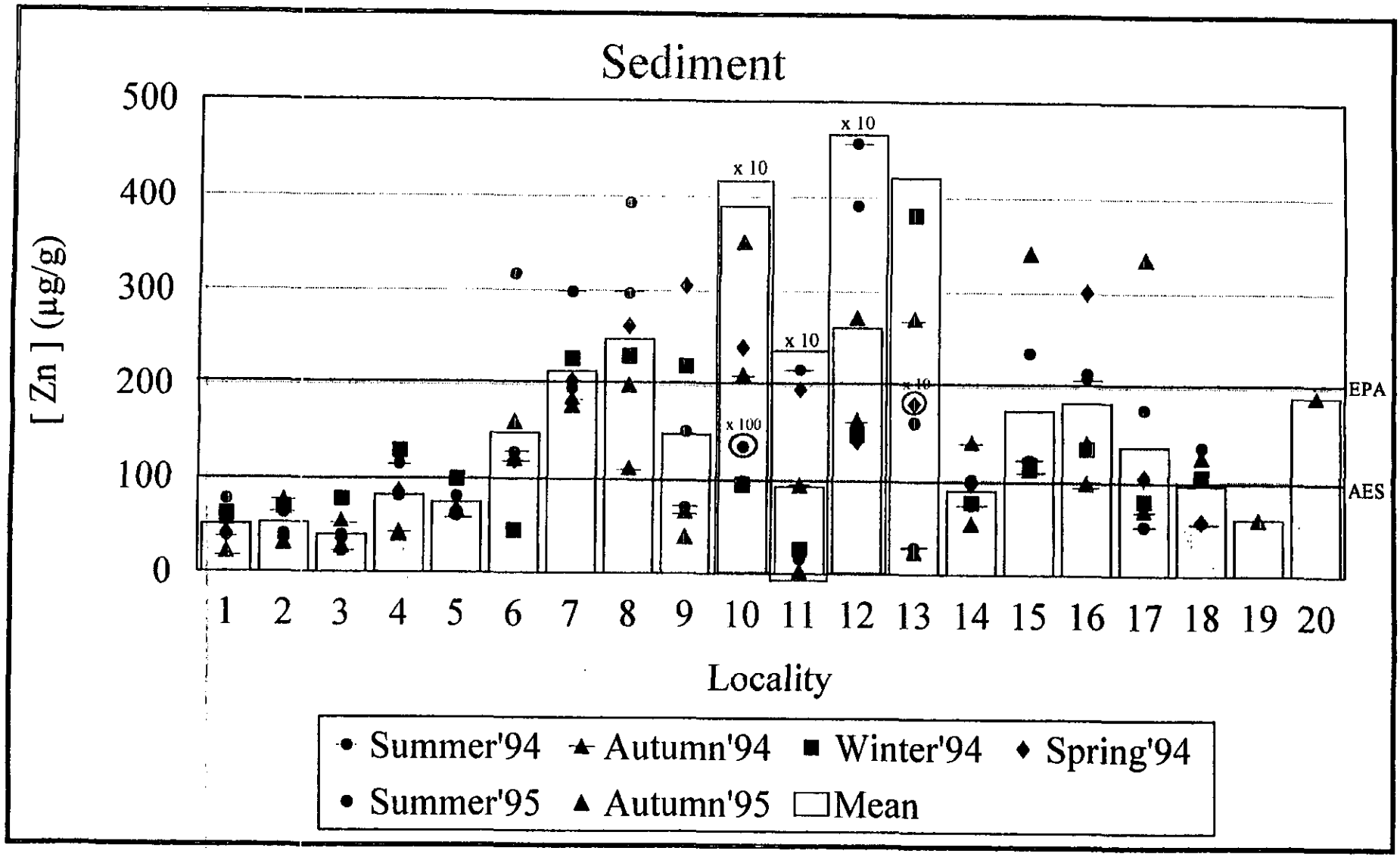

Figure 9.29: Spatial and temporal variation of the sediment zinc concentration at the selected localities in the Olifants River. EPA = Maximum Zn level according to EPA toxicity classification and AES = Zn concentrations of Average Earth Sediment (from September *et al.*, 1994).

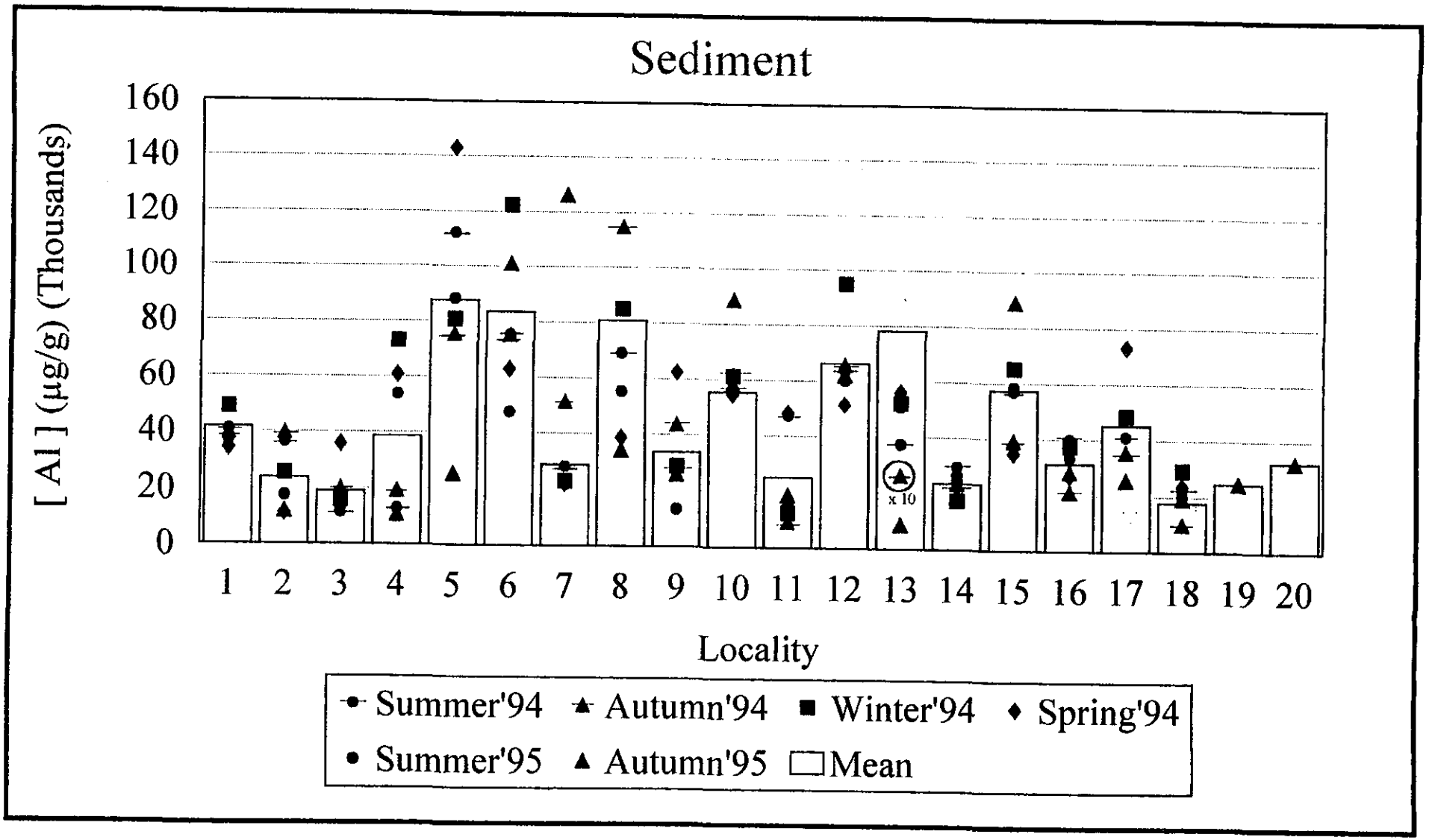

Figure 9.30: Spatial and temporal variation of the sediment aluminium concentration of the selected localities in the Olifants River.

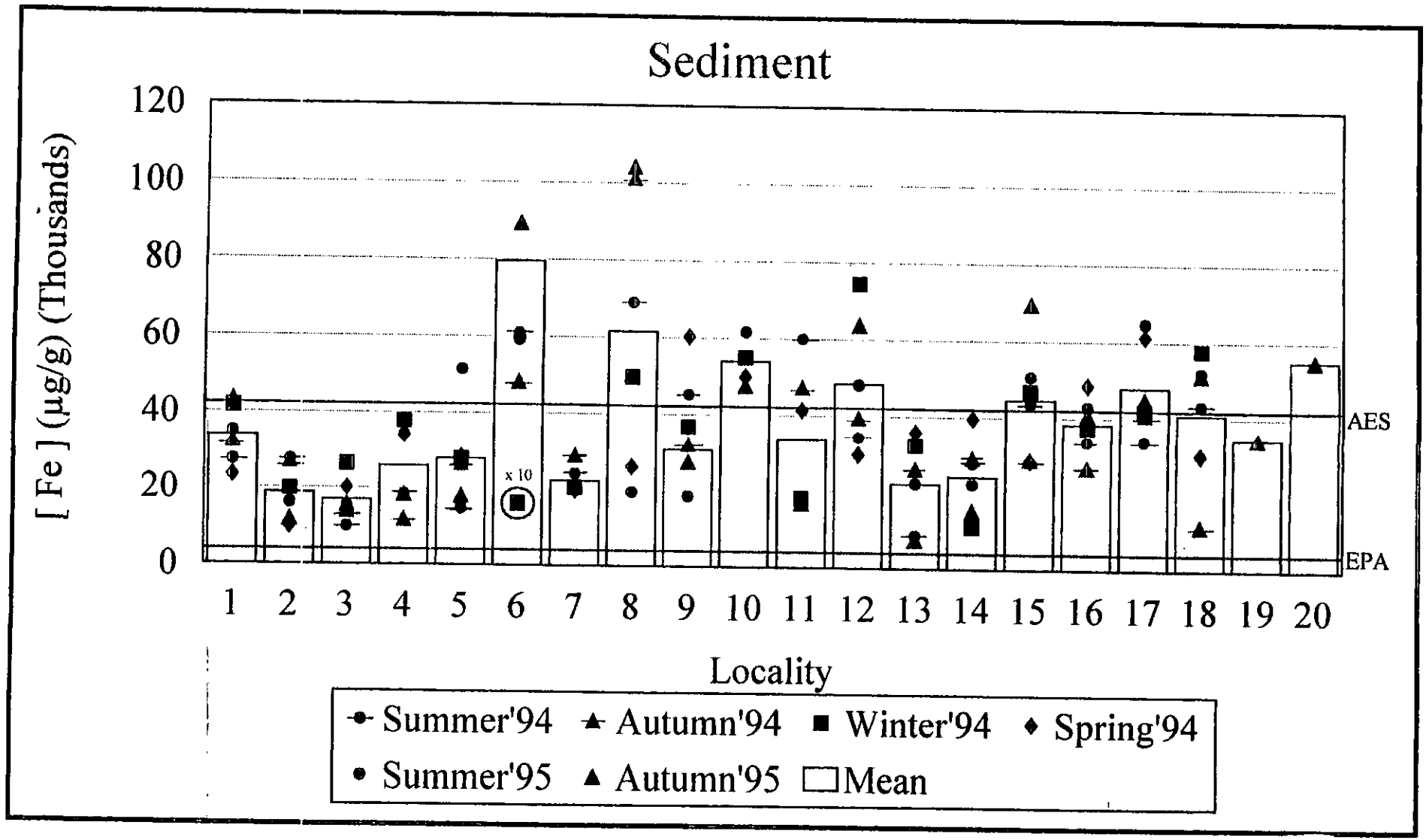

Figure 9.31: Spatial and temporal variation of the sediment iron concentration at the selected localities in the Olifants River. EPA = Maximum Fe level according to EPA toxicity classification and AES = Iron concentration in Average Earth Sediment (from Steenkamp *et al.*, 1994).

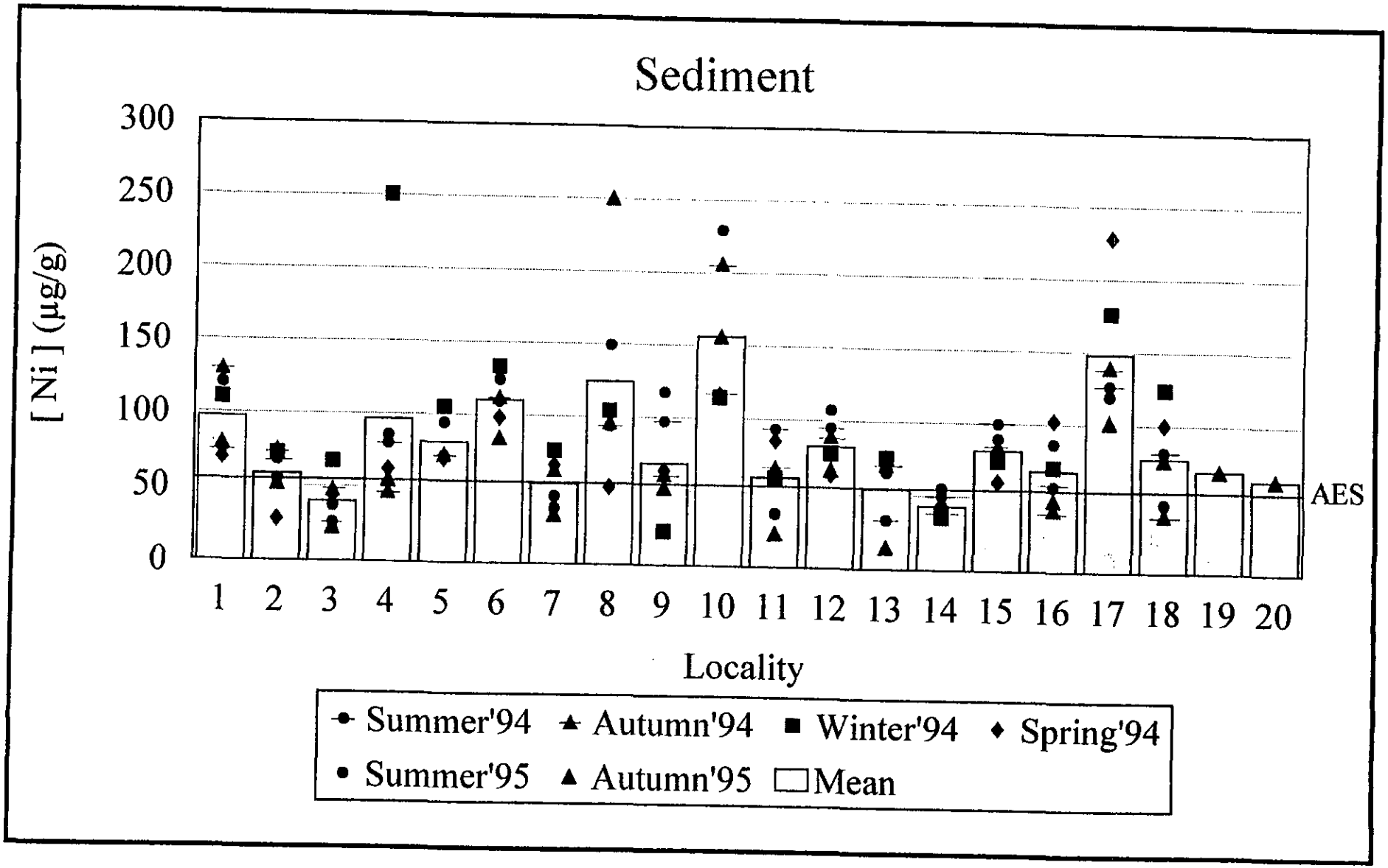

Figure 9.32: Spatial and temporal variation of the sediment nickel concentration at the selected localities in the Olifants River. AES = Nickel concentration in Average Earth Sediment (from Steenkamp *et al.*, 1994).

µg/g (locality 13-autumn 1995) to 250 µg/g (locality 4-winter 1994 & locality 8-autumn 1995), and in the lower catchment the Ni levels were between 39 µg/g (locality 18-autumn 1994) and 228 µg/g (spring-1994). Relatively low mean Ni levels and temporal fluctuations in Ni concentrations were observed in the sediment at localities 3 (40±16 µg/g) and 14 (43±7 µg/g). Levels of Ni detected at locality 20 (control) were relatively low when compared to most of the other sites.

Manganese concentrations in the sediment (Fig. 9.33) of the upper catchment varied from 31 µg/g (locality 13-autumn 1995) to 13 410 µg/g (locality 10-summer 1995), and from 255 µg/g (locality 18-summer 1994) to 797 µg/g (locality 17-winter 1994) in the lower catchment. Very high mean levels of Mn were detected at localities 10 (3 895±4 760 µg/g) and 12 (2 616±1 361 µg/g) and to a lesser degree at localities 1 (1 287±611 µg/g) and 11 (938±926 µg/g). Manganese levels detected in the sediment at locality 20 (control) were generally within close range of those detected at most sites.

Lead concentrations in the sediment (Fig. 9.34) of the upper catchment ranged from 2 µg/g (locality 9-winter 1994) to 163 µg/g (locality 8-autumn 1995), and in the lower catchment concentrations varied between 8 µg/g (locality 18-autumn 1995) and 30 µg/g (locality 17-winter 1994). The highest mean lead concentration was detected at locality 8 (106±48 µg/g), followed by localities 6 (58±10 µg/g), 10 (55±10 µg/g) and 12 (50±5 µg/g). Seasonal fluctuations were generally small at most localities with the highest concentration detected during the winter 1994 survey at various localities. Locality 20 (control) had a very low Pb concentration in the sediment compared to the other sites.

Concentrations of chromium in the sediment (Fig. 9.35) of the upper catchment ranged from 28 µg/g (locality 13-autumn 1995) to 391 µg/g (locality 10-summer 1995), and in the lower catchment from 123 µg/g (locality 18-spring 1994) to 1290 µg/g (locality 18-summer 1995). The highest mean Cr level was detected in the sediment at locality 18 (521±473 µg/g), followed by 17 (305±124 µg/g) and 10 (209±118 µg/g). Locality 3 (55±17 µg/g) had the lowest chromium concentration as well as the slightest variation between seasons. The level of Cr detected in the sediment at locality 20 (control) was relatively high when compared to most of the sites in the upper catchment, but less than the lower catchment sites.

9.2.4 *Discussion*

Many metals such as Cu, Fe, Zn, Mn, Co and Cr are required in trace amounts for normal physiological functions in mammals, and it is assumed that they have similar functions in fish. In both fish and mammals, altered physiological function result when one or more of these metals reach sufficiently high concentrations in body cells (Heath, 1987). The exposure of fish to metal-polluted waters can also cause changes to the community (e.g. species richness, species composition, and biotic interactions) as well as physiological changes (e.g. respiration, reproduction and metabolism). The general affect of metals on fish is summarised in Table 9.2 to give an indication of the extent to which aquatic organisms can be influenced by metal pollution. It must, however, be stressed that various chemical and physical attributes of the water column (discussed in Section 9.1) modify the toxicity and uptake of metals by aquatic organisms (Dallas *et al.*, 1993). It is usually the ionic forms that produce the immediate mortalities, while complexed metal compounds tend to act by accumulation in the body tissue over a considerably longer period

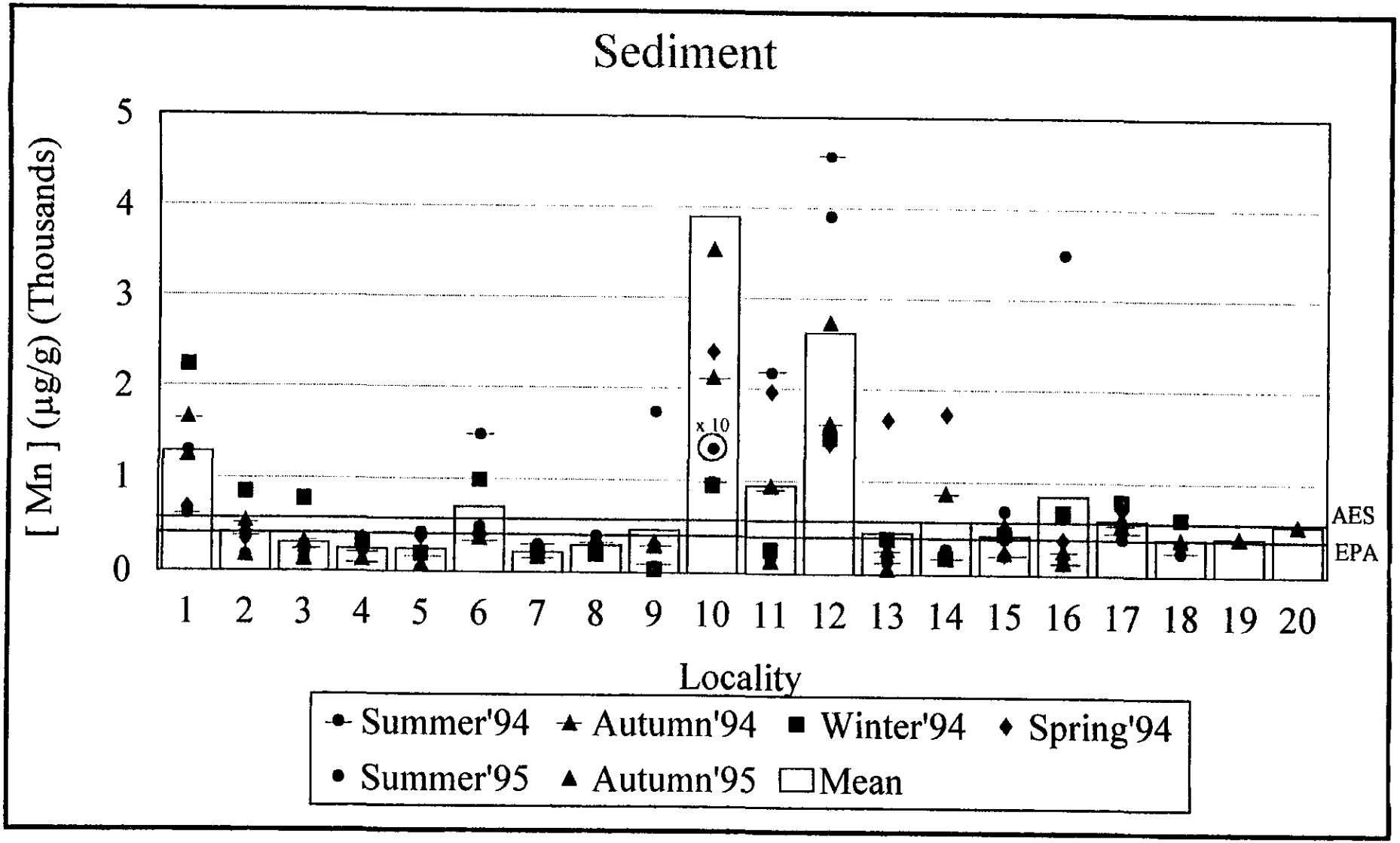

Figure 9.33: Spatial and temporal variation of the sediment manganese concentration at the selected localities in the Olifants River. EPA = Maximum Mn level according to EPA toxicity classification and AES = Mn concentration in Average Earth Sediment (from Steenkamp *et al.*, 1994).

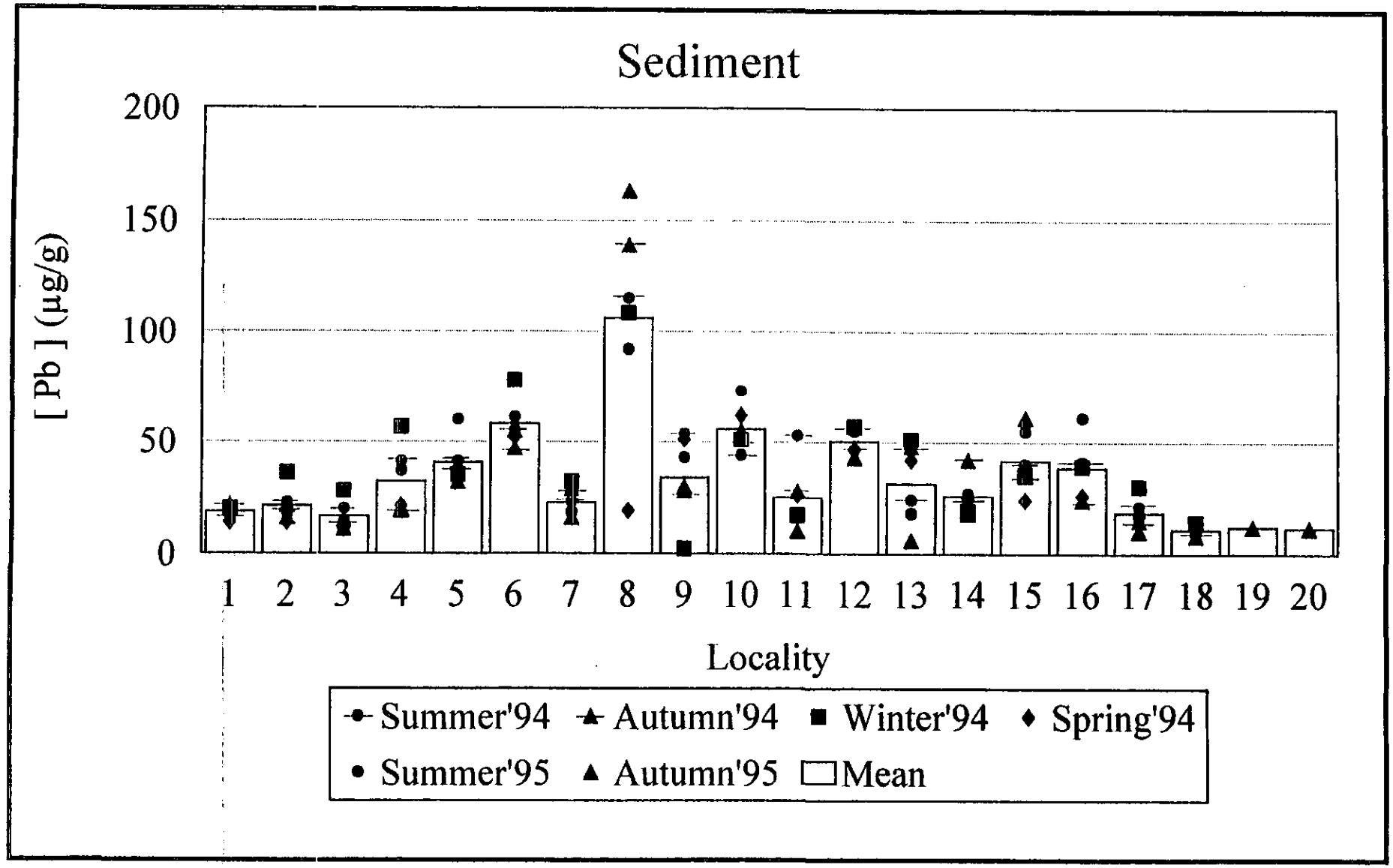

Figure 9.34: Spatial and temporal variation of the sediment lead concentration of the selected localities in the Olifants River.

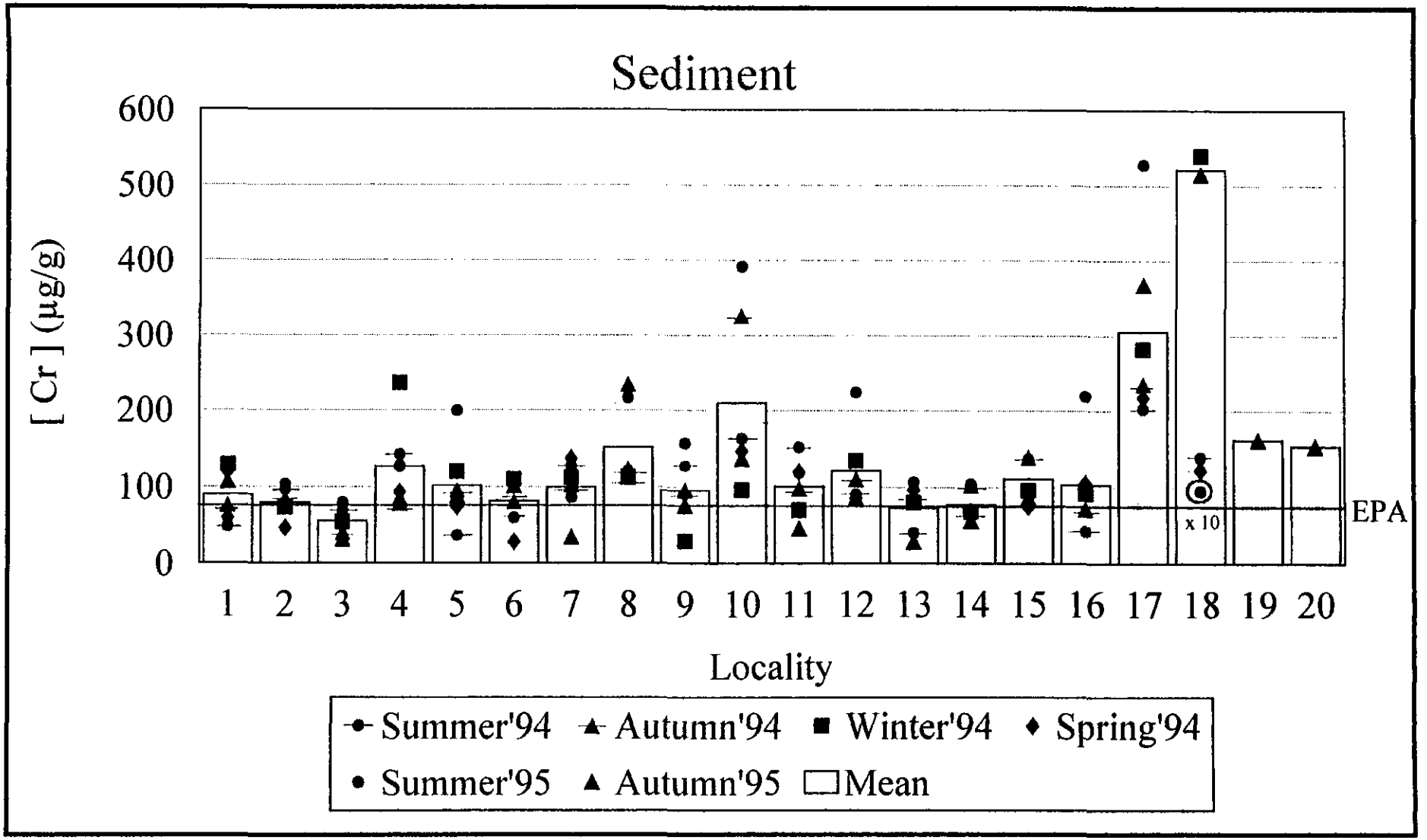

Figure 9.35: Spatial and temporal variation of the sediment chromium concentration at the selected localities in the Olifants River. EPA = Maximum Cr level according to EPA toxicity classification (from Steenkamp *et al.*, 1994).

Table 9.2: Summary of the general effects of various metals on fish.

Metal	Concentration	Effect	Toxicity dependant on..	General	Reference
Al	TWQR= 0,05µg/l CEV= 1 µg/l AEV= 10 µg/l	Interference with ionic & osmotic balance as well as respiratory problems due to coagulation of mucus on the gills. Interferes with Ca metabolism and ion exchange sites especially those involved with sodium homeostasis > Neuromuscular dysfunction.	Species and life stage of organism, [Ca] and pH.	More toxic in acidic waters. Non-critical element.	DWAF., 1995.
Cr(VI)	TWQR= 7 µg/l CEV= 14 µg/l AEV= 200 µg/l	Reduced growth	Species of Cr present.	Fish most resistant of aquatic organisms.	DWAF., 1995.
Cu	TWQR= 0.2 µg/l CEV= 0,53 µg/l AEV= 1,6 µg/l	Forms stable co-ordinate bonds in proteins where it functions as a catalyst in redox reactions.	Water hardness, dissolved oxygen, other metals, alkalinity, exposure time & life stage.	Early life stages more sensitive.	DWAF., 1995.
	*	Upset osmoregulation, effects metabolism			Heath, 1987.
	*	Precipitation of mucus on the gills, hyperplasia and hypertrophy of gill lamellae. Hepatic and renal disorders. Effects enzyme activity. Influence behaviour.	Temperature, dissolved oxygen, pH, hardness, salinity, organic substances,, suspended solids, age of fish		Alabaster *et al.*, 1980.
	Total [Cu] = 13 µg/l.	Increased growth in length & weight, Increased fecundity, earlier age of maturation, reduced spawning success, reduced larval & egg survival, smaller egg size, reduced longevity.	Dissolved organic matter	Results based on White suckers (Catastoma commersoni)	McFarelane & Franzin, 1978.
Fe	*	Inhibits various enzymes	Ferrous or ferric state	Limited toxicity and bio-availability.	DWAF., 1995.
	*	Inhibiting action on diffusion across the respiratory membrane. Damage of respiratory epithelial tissue on gills. Increased mucuc excretion and operculum movement.		Relatively low-toxicity in fish.	Van Rensburg 1989

Table 9.2: (Continued)

Metal	Concentration	Effect	Toxicity dependant on..	General	Reference
Mn	TWQR= 180 µg/l CEV= 370 µg/l AEV= 1300 µg/l	Skeletal deformities and reduced reproductive capabilities. Disturbance in various metabolic pathways.	Changes in redox potential, dissolved oxygen, pH and organic matter	Essential micro-nutrient.	DWAF., 1995.
	*	Alters liver glycogen and blood glucose levels. Disturbed physiology & reproductive impairment. Opaque eyes and haemorrhaging of fins. Loss of balance.			Seymore, 1994.
Ni	64 000 µg/l $NiSO_4$	Degenerative alterations of gonads.		Results based on Giant Gaurami (Colisa fasciatus).	Nath & Kumar, 1990.
		Gill damage= hypertrophy of respiratory & mucus cells, separation of epithelial layer from pillar cell system, cauterization & slaughing, extensive necrosis of epithelium, hyperplasia.			Nath & Kumar, 1989.
Pb	TWQR= 0,2 µg/l CEV= 0,5 µg/l AEV= 4 µg/l	Interferes with haemoglobin through interaction with iron. Affects membrane permeability. Inhibits some enzymes involved in energy metabolism. Spinal deformities. Coagulation of mucus over gills and entire body > suffocation.	Water hardness	Physiologically non-essential element.	DWAF., 1995.
Zn	TWQR= 2 µg/l CEV= 3,6 µg/l AEV= 36 µg/l	Formation of insoluble compounds in the mucus covering the gills. Depressed white blood cell-thrombocyte counts. Oedema & liver necrosis. Affects osmoregulation	Water hardness, [Cu], synergism and antagonism with other metals.	Essential trace-element.	DWAF., 1995.
	*	Destroy gill epithelial tissue. Chronic effects on various organs and enzyme systems. Darkening of colour. Increased activity. Vertebral damage. Inhibition of reproduction, reduced growth and behavioural changes.	Temperature, dissolved oxygen, pH, water hardness, salinity, organic matter, suspended solids, age and size		Alabaster *et al.*, 1980.
	Total [Zn] = 245 µg/l	Increased growth in length & weight, Increased fecundity, earlier age of maturation, reduced spawning success, reduced larval & egg survival, smaller egg size, reduced longevity.	Dissolved organic matter	Results based on White suckers (Catastoma commersoni)	McFarelane & Franzin, 1978.

TWQR= Target Water Quality Range (No Effect Level) CEV= Chronic Effect Value AEV= Acute Effect Value * = Not specified

(Ellis, 1989). It is stressed that this discussion is based on the total levels of a specific metal, detected at a locality and no reference is made to the specific species of the metals present.

Results from this study indicate that there are a few areas of immediate concern in the Olifants River catchment regarding metal pollution. Locality 3 (Steenkool Spruit) had elevated levels of Cu, Zn, Fe, Ni and Pb (Fig. 9.38 to 9.39). Coal mining in the upper reaches of the Steenkool Spruit could possibly have led to the occurrence of these elevated metal levels. Seepage water from active and/or abandoned coal mines is known to effect the water quality of surface and ground water (Borchers *et al.*, 1991). These effects are usually linked to changes in pH and metal levels since the seepage water is characterised by low pH and high concentrations of pollutants, and in particular metals. Acid mine drainage in particular can cause high levels of Fe in the receiving waters due to the mineral pyrite (FeS_2) being oxidised by air, water and chemosynthetic bacteria (Dallas & Day, 1993). Locality 10 (Spook Spruit) seems to be polluted by some source containing elevated Mn and Ni levels. Manganese is known to be used and discarded by many industries while acid mine drainage also releases a large amount of manganese (DWAF., 1995). Again, these levels of elevated metals can be ascribed to coal mining in the upper reaches of the Spook Spruit. The impact of coal mining was also clearly illustrated by the difference in Fe, Ni, Mn and Pb concentrations between the upper Boesman Spruit (locality 5) and the lower Boesman Spruit (locality 6) as coal mining occurs in the middle reaches of this stream (Fig. 9.37 to 9.39).

Locality 8 (Suurstroom) is a severely impacted site that had levels of Zn, Al, Ni, Mn and to a lesser degree Cu, exceeding all other sites by far (Fig. 9.16 to 9.18). The pH at this locality was also very low (3.9 ± 1) in comparison to the other sites. It must be stressed that low and/or fluctuations in water pH were also recorded at localities 3, 6, and 10 (Section 9.1, Fig. 9.5). The low pH not only impacts aquatic life directly negatively but also influences the bioavailability of metals, and especially aluminium (Ormerod *et al.*, 1987). These increased metals can have various deleterious effects on the biota of the surface waters receiving the acid mine drainage, as mentioned in Table 9.2. As the upper catchment of the Olifants River is known to contain a large amount of operational and abandoned coal mines, it can be assumed that these mines have a very negative impact on surface water quality. This is clearly shown by the water quality detected at locality 8 being mainly influenced by acid mine drainage. High levels of copper, iron, lead and chromium occurring at locality 11 (Olifants River Lodge) could be due to the impact of the polluted Suurstroom and Spook Spruit, confluencing with the Olifants River upstream of this locality, as well as other mine polluted sources in its upper reaches. Industrial effluents as well as combined sewage purification works from the Witbank area could also contribute to these elevated levels of metals at this site, especially during low flows when there are limited water releases from the Witbank Dam.

The Klein Olifants River is also influenced by sources of metal pollution. A progressive increase in mean copper and chromium concentration was witnessed in the water of the Olifants River moving downstream from locality 12 to 14 (Fig. 9.36 & 9.39). Nickel and lead levels detected in the water of the Klein Olifants River were also generally high. The elevated metal levels could possibly be due to sewage treatment effluent which

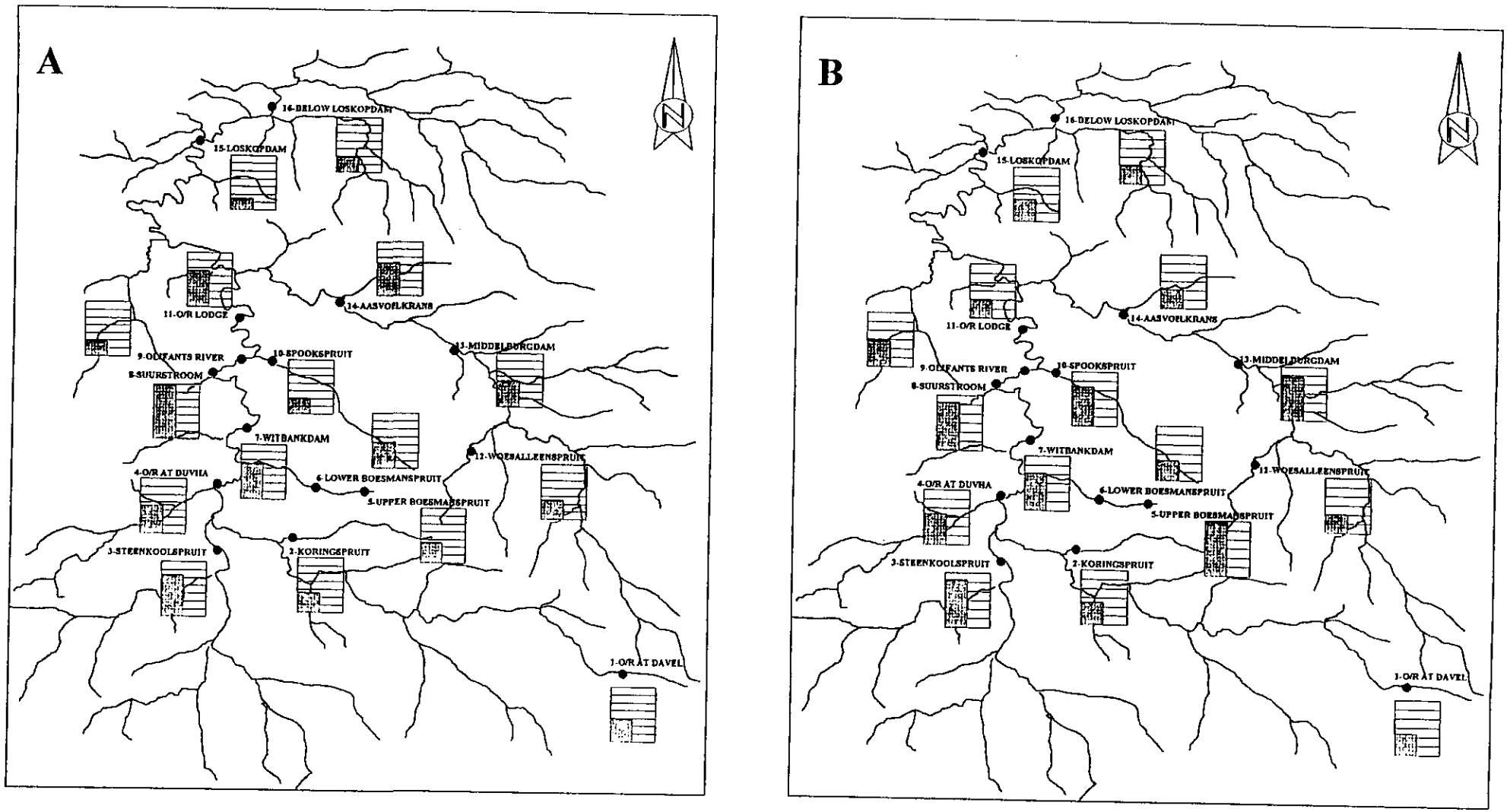

Figure 9.36: Mean levels of total copper (A) and zinc (B) detected in the water of the selected localities in the upper catchment. Increments for Cu = 5 µg/l and for Zn = 50 µg/l. (The mean zinc value at locality 8 should be multiplied by 10).

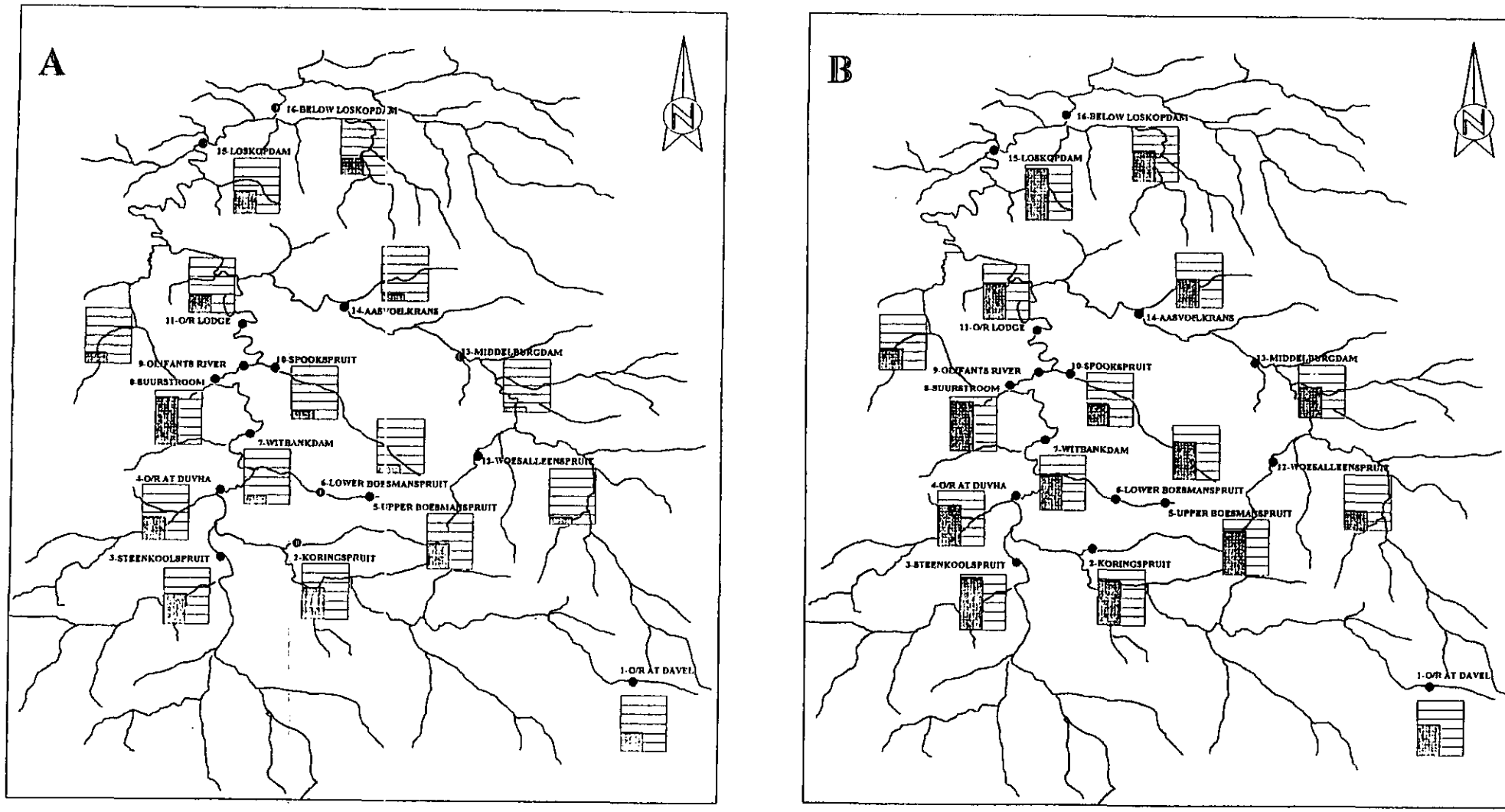

Figure 9.37: Mean levels of total aluminium (A) and iron (B) detected in the water of the selected localities in the upper catchment. Increments for Al and Fe = 500 µg/l. (The mean aluminium value at locality 8 should be multiplied by 10).

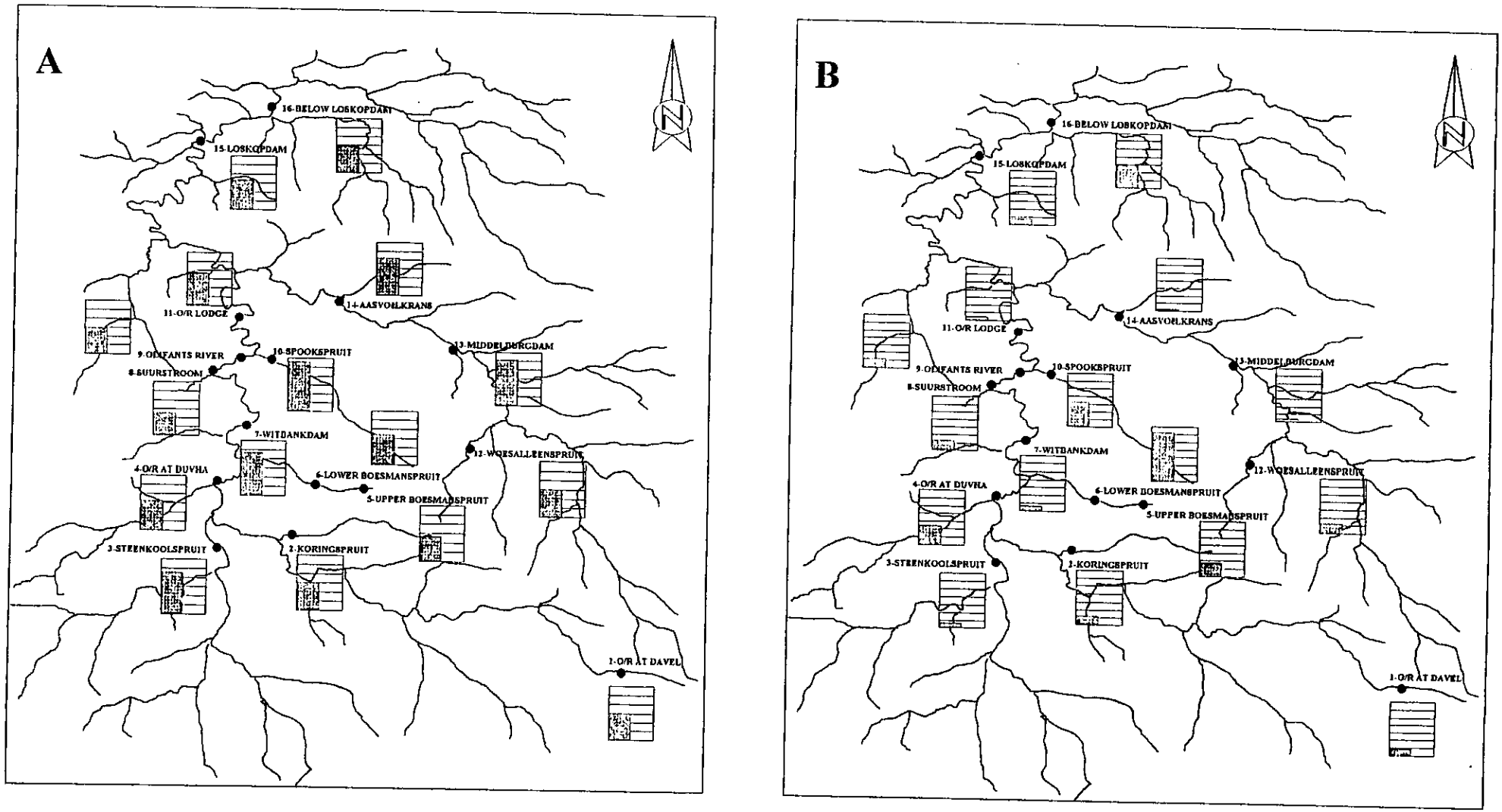

Figure 9.38: Mean levels of total nickel (A) and manganese (B) detected in the water of the selected localities in the upper catchment. Increments for Ni = 50 µg/l and for Mn = 200 µg/l. (The mean Ni value at locality 8 and Mn value at locality 10 should be multiplied by 10, and the mean Mn level at locality 8 by 100).

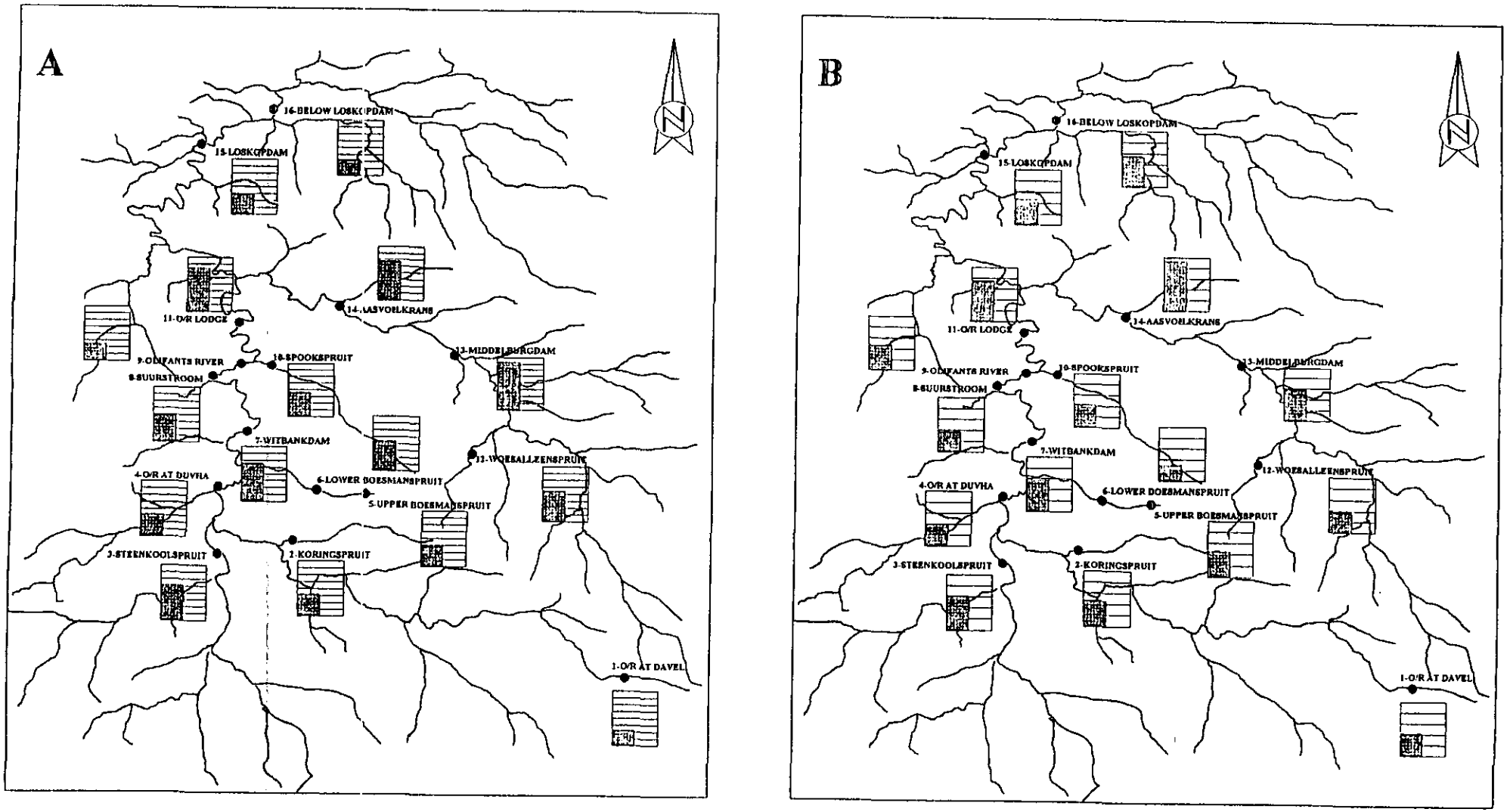

Figure 9.39: Mean levels of total lead (A) and chromium (B) detected in the water of the selected localities in the upper catchment. Increments for Pb = 25 µg/l and for Cr = 100 µg/l.

contains industrial effluent discharges, urban runoff from Middelburg town and possible illegal discharge of industrial effluent may also contribute to these levels (Coetzee, 1996). The contribution of each of these sources should be quantified in future. Coal mining in the Woesalleen Spruit (locality 12) is another contributor to these sources of elevated metals occurring in the Klein Olifants River.

Some dams investigated in this study contained relatively high levels of metals and it seems that they act as a sink for metal pollution occurring in their sub catchments. Elevated levels of Cu, Zn, Fe, Ni, Pb and Cr detected at locality 7 (Witbank Dam) could be ascribed to the input of polluted water (due to coal mining) from the Steenkool Spruit and Boesman Spruit (as discussed above). Locality 13 (Middelburg Dam) contained high levels of Zn, Fe, Ni, Pb and Cr, possibly due to mining influences in its upper reaches (Woesalleen Spruit). Engelbrecht (1992) stated that the Loskop Dam acts as a sink for metals upstream of this locality. Levels of metals detected in the water at this locality during this survey were, however, usually similar to or lower than those detected in the upper reaches. It could be possible that the concentrations of metals are diluted by the time they enter the dam. These metals will be adsorbed onto the suspended sediment particles and settled out in the lotic environment, therefore being incorporated in the lake sediment. The levels of metals in the sediment were in some cases relatively high when compared to those of the localities upstream of locality 15. Due to the fact that the subcatchment responsible for the runoff at locality 15 is being influenced by various negative impacts, this locality will have to be monitored in future to prevent potential progressive degradation.

In the lower catchment of the Olifants River, elevated levels of most metals investigated occurred at locality 17 (Mamba weir, KNP). The Selati River, one of the tributaries of the lower Olifants River has been shown to be highly polluted by metals in previous studies (CSIR., 1990; Seymore *et al.* 1994). Mining and industrial activities occurring in its subcatchment are responsible for the point and diffuse effluents entering the river, resulting in poor and unacceptable water quality (see Section 9.1.1). After the confluence of the Selati River with the Olifants River it enters the Kruger National Park causing a reduction in the water quality, this is especially evident during low flows. The negative impact of the Selati River on the Olifants River is also evident when comparing the metal levels at locality 19 (upstream of Olifants-Selati confluence) and locality 17 (downstream of the Olifants-Selati confluence). These results add evidence to previous studies that stressed the negative impact of the poor quality of the Selati River on the Olifants River. In accordance with the results of Seymore *et al.* (1994), levels of Cu, Zn, Mn and Cr detected at Locality 18 (Balule) were higher than those detected at locality 17, approximately 40 km upstream. As the stretch of the river between these localities lies within the Kruger National Park little or no anthropogenic impacts could have been responsible for this phenomenon. Seymore *et al.* (1994) attributed it to the frequent occurrence of reed beds in the vicinity of locality 18, which accumulate the metals and release them again when decaying occurs. Natural geological weathering could also have contributed as various tributaries such as the Timbavati River join the Olifants River before locality 18. These natural levels of metals could therefore also have been added to the concentrations in the water from locality 17 (due to anthropogenic and natural sources) thus resulting in higher concentrations. The levels of metals occurring in the seasonal Timbavati River should be investigated to ascertain its potential effect on the water quality at locality 18. There is also a possibility that polluted ground water and other smaller streams add metals to the Olifants River below locality 17 (Mamba weir), contributing to the high levels of some metals detected at

locality 18 (Balule).

Temporal trends in water metal concentrations varied to a great extent between different metals as well as between different localities. A few general trends were, however, evident. The copper and zinc levels detected in the water of most of the upper catchment localities were the lowest during the autumn 1994 survey. Rains that occurred during the previous wet season could have caused dilution of these metals as well as the cleansing of these localities by the increased amount of waters running through it. The highest levels of Cu, Zn and Mn were generally detected during the spring survey of 1994 and could indicate that the input of these metals into the river were increased due to rainfall previous to the survey. Pollutants accumulating during the dry season will be washed into the river by the first rains. The hydrogen ion concentration will also increase with rainfall, thereby slightly increasing the solubility of metals in the water and resulting in elevated metal concentrations (Seymore *et al.*, 1994). Brown (1977) also indicated that levels of Cu and Zn in the water of the river Hayle increased with increasing flows. Metal levels detected in the water of the Olifants River catchment were also generally higher during summer and autumn 1995 than during the same period in 1994. Rainfall was much higher during the 1994 period and it could therefore be assumed that metal concentrations detected during this phase were decreased through dilution. Furthermore, the variation in discharge rates of industrial, mining and sewage waste entering the river in different months could also have contributed to the temporal fluctuations observed (Saad *et al.*, 1981). Large temporal fluctuations observed at many of the sites investigated could render problems for aquatic organisms occurring at those sites. Many aquatic animals including fish can acclimatise to potential toxic levels of metals in water and could thus survive sub-lethal chronical exposure to pollutants in their environment (Klaverkamp *et al.*, 1984). If the levels of such pollutants are, however, drastically fluctuated over a short period of time, it could have detrimental impacts on the survival of such organisms.

The copper, iron, manganese and lead levels detected at localities 17 (Mamba weir), 18 (Balule) and 19 (Phalaborwa Barrage) were lower than the levels detected by Seymore *et al.* (1994) at the same localities during the period 1990 to 1992. It therefore seems that an attempt has been made by the dischargers of effluents in this region to lower the metal concentration input into the lower Olifants River. Chromium, nickel, and to a lesser degree zinc are presently of major concern as their levels have increased since the study by Seymore *et al.* (1994). As many of the metals still exceed the guideline values proposed for aquatic ecosystems (DWAF., 1995) by a large margin, it is recommended that the levels of all metals should be decreased further in future. Levels of metals in the water at locality 15 (Loskop Dam) were generally higher than those detected by Grobler *et al.* (1994) at the same locality. These results are, however, based on a single sample collected during December 1990 and not near the inflow, which render it of limited value for comparisons. Although not directly comparable, it is interesting to note that all metals investigated (except copper) occurred in higher concentrations in the upper and lower catchment of the Olifants River than in the Germiston Lake (Gauteng), which has in the past been influenced by mining and industrial activities (Du Preez, 1995). These comparisons clearly indicate that the Olifants River is at present a metal-polluted system with unacceptable levels of most investigated metals occurring throughout its catchment. It is therefore stressed that further investigations should be conducted to investigate the metal loads in the areas indicated to be of concern.

Metal concentrations detected in the water of the Olifants River during this study were evaluated through the use of South African Water Quality Guidelines for Aquatic Ecosystems (DWAF., 1995) and the Canadian Water Quality Guidelines (Environment Canada, 1987). The first mentioned guidelines (South African) are stated as the following three criteria. The Target Water Quality Range (TWQR) is equal to the No Effect Range for a certain variable and should be seen as a management objective for protecting the health and integrity of aquatic ecosystems. Chronic Effect Values (CEV) are defined as the concentration of a variable at which there is expected to be a significant risk (5% or less) of measurable chronic effects to the sensitive organisms in the aquatic ecosystem. Thirdly, the Acute Effect Value (AEV) refers to the concentration or level of a constituent above which there is expected to be a significant risk (5% or less) of acute toxic effects to sensitive organisms in the aquatic population. Copper, zinc, aluminium and lead concentrations detected in the water at all localities, exceeded all South African guidelines (DWAF., 1995) by a large margin. As mentioned above, it should be stressed that the guidelines for Zn, Al and Pb refer to dissolved metal concentrations while total metal concentrations were measured during this study. Total chromium levels also mostly exceeded the South African guidelines (DWAF., 1995) set for dissolved Cr(III) and Cr(VI). The only sites with median Cr concentrations below the Cr(VI) AEV were localities 6 (lower Boesman Spruit) and 12 (Woesalleen Spruit). The median of the total manganese concentrations detected in the Olifants River were mostly below the CEV and in close range of the TWQR. The only exceptions were localities 8 (Suurstroom) and 10 (Spook Spruit) which exceeded even the AEV. Iron levels detected at all sites exceeded the Canadian guideline value for Fe in the environment (Environment Canada, 1987) by a large margin. Nickel levels were, however, usually in close range of the Canadian guidelines. Localities 5 (Upper Boesman Spruit) and 15 (Loskop Dam) had median Ni levels below the guideline, while Ni levels detected at locality 8 (Suurstroom) were exceedingly higher than the guideline value.

If the type of metal occurring (total or dissolved) is ignored, the above mentioned results would suggest that fish populations and especially sensitive species occurring at many of the sites are at this stage being subjected to chronic and acute exposure to these metals. The fact that fish still occur at many of these sites (Table 9.3) could be ascribed to the fact that the toxicity of most of these metals is reduced by factors related to the water quality (especially hardness and alkalinity) of the Olifants River water (Erickson *et al.*, 1996). Most guidelines referred to the dissolved metal concentrations in the water while this study investigated the total amount of a specific metal in the water column. It is therefore proposed that future studies should focus on dissolved metal levels in an attempt to use the guidelines in the evaluation of such systems. This could also enable scientists to continually evaluate the guidelines and adapt them where necessary for local conditions.

Eventually most soluble metals reach the sediment where they become bonded to various components of the sediment such as organic matter and clay minerals (Förstner & Wittmann, 1983). Suspended solids such as clays are furthermore important in the transport and availability of metals because of their cation exchange capacity (Turekian & Scott, 1967). It is generally accepted that the top sediment layers reflect the current water quality of the system and that fine-grained sediments bind metals more efficiently

than do coarse-grained sediments (Giesy & Briese, 1977; Coetzee, 1993). Metals in the finer sediment particles are sometimes in suspension with the water and usually form the top layer of the sediment and are therefore more available for uptake by aquatic organisms. Contaminants such as metals in the sediment can be bioaccumulated by benthic organisms from where they can be taken up into the food chain, contaminating fish, wildlife and humans (Burton, 1992). The determination of metals in sediments and in particular the

Table 9.3 Fish species captured at selected sampling sites.

Locality	Specie Scientific Name	Common Name
7: Witbank Dam	*Clarias gariepinus* *Cyprinus carpio* *Labeo umbratus*	Sharptooth catfish Carp Moggel
11: Olifants River Lodge	*Barbus marequensis* *Clarias gariepinus* *Cyprinus carpio* *Labeo umbratus* *Micropterus salmoides*	Largescale yellowfish Sharptooth catfish Carp Moggel Largemouth bass
13: Middelburg Dam	*Clarias gariepinus* *Cyprinus carpio* *Labeo umbratus* *Micropterus salmoides*	Sharptooth catfish Carp Moggel Largemouth bass
14: Klein Olifants River	*Barbus marequensis* *Clarias gariepinus* *Cyprinus carpio* *Labeo umbratus* *Micropterus salmoides*	Largescale yellowfish Sharptooth catfish Carp Moggel Largemouth bass
16: Loskop Dam	*Clarias gariepinus* *Labeo rosae* *Oreochromis mossambicus* *Schilbe intermidius*	Sharptooth catfish Rednose labeo Mozambique tilapia Silver catfish
17: Mamba Weir	*Barbus marequensis* *Clarias gariepinus* *Labeo rosae* *Oreochromis mosssambicus* *Labeo ruddi* *Labeo congoro* *Barbus trimaculatus* *Schilbe intermidius* *Synodontis zambezense*	Largescale yellowfish Sharptooth catfish Rednose labeo Mozambique tilapia Silver labeo Purple labeo Threespot barb Silver catfish Brown squeaker
19: Phalaborwa Barrage	*Oreochromis mossambicus*	Mozambique tilapia
20: Nhlanganini Dam	*Clarias gariepinus* *Oreochromis mossambicus*	Sharptooth catfish Mozambique tilapia

species in which they occur can provide information that is essential for proper risk assessment to long-term conservation and management of natural water systems (Pardo *et al.*, 1990; Coetzee, 1993).

Levels of Cu, Zn, Ni, Mn and Pb detected in the sediments of most sites in the Olifants River catchment were lower than those detected by Du Preez (1995) in the mine polluted Germiston Lake (Gauteng). This could be due to the fact that a large amount of the sediment occurring in Germiston Lake is minesand/silt that washed into the Lake during rains (Du Preez, 1995). Metal levels in the water were, however, generally higher in the Olifants River than in the Germiston Lake. Metals tend to precipitate out of the water column and get adsorbed to sediment particles to a higher degree in hard waters (Ormerod *et al.*, 1987). The higher level of water hardness in the Germiston Lake could also have attributed to higher levels of metals in sediment and lower levels in water of the lake when compared to the Olifants River system. Various other factors such as pH and $CaCO_3$ are furthermore responsible for the bioavailability and occurrence of species of metals in the water column which will determine its final impact on aquatic organisms (Wade *et al.*, 1995).

Metal levels detected in the sediment at localities 17 (Mamba weir) and 18 (Balule) were generally higher than those detected by Seymore *et al.* (1994) at the same localities during the period 1990 to 1992. A large increase occurred in the zinc, nickel, chromium and iron sediment concentrations since the study by Seymore *et al.* (1994). Zinc, nickel and chromium increases were also detected in the water, which could be directly responsible for the increased levels in the sediments. The higher levels of iron could be ascribed to the combined result of both anthropogenic inputs as well as the weathering of the underlying rocks that are also known to produce iron. High copper levels in the sediments at locality 20 (control) compared to those of the other sites could be ascribed to the impact of air pollution from the mining industry in the Phalaborwa region. Very high levels of copper depositioning occurs in this area due to emissions from the mines (Dr. Grobler, personal communication, 1996). This copper seems to have entered locality 20 (Nhlanganini Dam) causing relatively high levels in the water (Fig. 9.20). Copper then precipitates out to the sediments due to the stagnant situation of this locality, explaining the high levels detected in the sediments (Fig. 9.28). Copper is furthermore known to be especially associated with the organic and humic matter in water and sediments (Paul & Pillai, 1983; Pardo *et al.*, 1990). A large population of hippopotamus and various fish species occur in this dam with a relatively low water level. The water in this dam is almost always stagnant and a great amount of organic material is therefore accumulated in its waters and sediments. This could also have contributed to the elevated copper levels detected at this locality. It should, however, be stressed that the results of locality 20 are based on a single water and sediment sample which limit the reliability of results to some extent. The general background levels and the relative contribution of air pollution, ground water seepage and weathering should be investigated. No general seasonal trends for metal concentrations in the sediments could be observed. It seems that seasonal

variation in metal concentrations are not reflected by the sediment but that it rather gives an indication of long term metal pollution at a specific site.

Iron levels detected in the sediment at all sites investigated during this study exceeded the EPA guideline value (Steenkamp *et al.*, 1994) with a very large margin (Fig. 9.31). Copper and Cr levels were generally higher than the EPA guideline levels whilst manganese and zinc levels showed the opposite trend. Some sites such as localities 8, 10, 11, 12 and 13, however, generally exceeded the guideline values for these metals. Copper and nickel levels were generally higher and manganese and iron lower than the levels of these metals in average earth sediment (Steenkamp *et al.*, 1994). Localities 1 to 5, 14 and 18 had mean levels of Zn lower than those of average earth sediments did. However, critical evaluation of the degree of contamination at these sites is hampered by the availability of historic data and especially background metal levels in the sediments of the region. Since many of these sites have been impacted before any analysis was performed, the precise determination of background levels is not possible. Some idea may be obtained by the calculation of the possible metal contribution by the natural geology and should be investigated.

9.2.5 *References*

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9.3 Metal bioaccumulation in tissues of fish from selected sites in the Olifants River.

9.3.1 *Introduction*

In aquatic ecosystems, metals are common contaminants and because of their persistence, toxicity, tendency to bioaccumulate and their general availability from point and/or diffuse sources, they are considered hazardous to aquatic life (Athison *et al.* 1987). However, metals play an important role in these systems but there are narrow and predetermined boundaries of tolerable ranges (Du Preez *et al.* 1997). Metals essential to life processes pose the threat of toxic reactions at high concentrations and deficiency of diseases at low concentrations (Weiss 1978). Furthermore, both essential and non-essential metals are toxic to aquatic life when present at elevated levels that is outside the tolerable ranges of these species.

Any increase in the concentration of bio-available metals in the aquatic ecosystem may lead to an increase in the bioaccumulation of metals in tissues of aquatic species such as fish. As bioaccumulation refers to the uptake and retention of chemicals/pollutants in the body or tissues of an organism, it can only occur if the rate of uptake by the organism exceeds the rate of elimination (Spacie & Hamelink 1993). In fish, metals may enter via the food ingested, non-food particles ingested, intake of water, the gills or the skin (Du Preez 1997). In the aquatic system it is usually difficult to establish whether the metal has entered the fish through dietary routes or through membranes or both. The relative importance of these routes varies but the most significant factor may be the bioavailability of the metal for bioaccumulation, that is the combined uptake from the surrounding water, food ingested as well as from the non-food particles ingested (Du Preez 1997).

The monitoring of the bioaccumulation of metals in aquatic systems can provide useful information (Du Preez 1997; Du Preez *et al.* 1997) which can be used to:

(1) assess the extent of bioaccumulation in a temporal and spatial context where spatial monitoring may provide data that would identify unknown areas that have been contaminated, while at known discharges it will provide some information regarding the area being affected and temporal data will provide information regarding the trend of bioaccumulation, which will in turn be used to identify stability improvement or deterioration,

(2) assess fitness for human or animal consumption. The monitoring concentration levels in fish or other organisms, which are used as food, assists in avoiding consumption of contaminated food. he detected levels can, for example, be judged against standards set for food in general (Mance 1987) and

(3) assessing organisms' health. High concentrations of pollutants can influence the life processes of organisms and ultimately the survival of the species. An excellent example is the effects of ichlorodiphenyl-trichloroethane (DDT) which affects the calcium metabolism of birds resulting in the thinning of eggshells. This makes the egg very fragile and prone to cracking (Fleming *et al.* 1983).

Despite the fact that South Africa has various mining, industrial, domestic and agricultural activities, the possible metal bioaccumulation by fish from these activities has only recently received attention (for example: Bezuidenhout *et al.* 1990; Du Preez & Steyn, 1992; De Wet *et al.* 1994; Grobler *et al.* 1994; Roux *et al.* 1994; Seymore 1994; Steenkamp *et al.* 1993; Van den Heever & Frey 1994; Seymore *et al.* 1995, 1996a,b,; Claassen 1996; Schoonbee *et al.* 1996; Van den Heever & Frey 1996; Van Vuren *et al.* 1994; Du Preez *et al.* 1997). The present study focuses on the bioaccumulation of selected metals in fish from the Olifants River (Mpumalanga) whose catchment is impacted by anthropogenic activities that may result in elevated metals in aquatic biota, especially fish. Fish were selected because studies have shown that they have a tendency to bioaccumulate metal and have been used for the assessment of metal pollution.

9.3.2 *Materials and Methods*

Field Sampling

Fish collected during the period February 1994 to May 1995 (Table 9.4) at selected localities, namely (Fig. 8.5) 7 (Witbank Dam), 11 (Olifants River Lodge), 13 (Middelburg Dam), 14 (Klein Olifants River), 15 (Loskop Dam), 17 (Mamba Weir), 19 (Phalaborwa Barrage) and 20 (Nhlanganini Dam) by means of gill nets (70-120mm stretched mesh size). The captured fish were identified and the mass as well as total lengths recorded (Table 9.4). The fish were then dissected on a polythene dissection board, using clean, stainless steel tools, wearing surgical gloves. The skin, muscle, gill filaments and liver were removed, placed into clean, pre-washed glass bottles and frozen until further analysis in the laboratory.

Laboratory procedures

All glassware was soaked in a 2% Contrad soap solution (Merck chemicals) for 24 hours, rinsed in distilled water, acid-washed in 1M HCl for 24 hours and rinsed again in distilled water (Giesy & Wiener, 1977), prior to use.

Prior to sample preparation, the tissues were thawed and rinsed in distilled water to remove excess mucus coating, or other foreign particles that could have adsorbed metals. Approximately 5 grams of each sample were dried in an oven at 60°C for a period of 48 hours. In order to determine the percentage of moisture of each sample, the wet and dry mass of the samples were recorded. The samples were digested by adding concentrated nitric acid (55%) and perchloric acid (70%) to one gram of dry tissue, in a 2:1 ratio in a 50ml Erlenmeyer flask and this process was performed on a hot plate at 200-250°C for ± four hours until the solutions were clear (Van Loon, 1980). After digestion, each solution was filtered through an acid resistant 0.45 µm filter paper under vacuum. After each sample had been filtered, the filtering system was rinsed with distilled water and each sample was made up to 50ml with distilled water and stored in pre-washed glass bottles, until determination of metal concentrations.

To determine the metal (aluminium, copper, chromium, iron, lead, manganese, nickel, zinc) concentrations in the tissue samples of the fish, a Varian Atomic Absorption

TABLE 9.4: MEAN MASS AND LENGTH OF FISH CAPTURED DURING THE STUDY PERIOD

Survey	Locality	Specie	N M/F	Mass (kg) X±SD	Length (cm) X±SD	Survey	Locality	Specie	N M/F	Mass (kg) X±SD	Length (cm) X±SD
February 1994	7	*C. gariepinus*	7M/3F	1.8±0.9	60.0±10.9	May 1994	7	*C. gariepinus*	*	*	*
		L. umbratus	14M/6F	1.1±0.2	46.0±10.9			*L. umbratus*	7M/13F	1.9±1.0	45.3±1.5
	11	*C. gariepinus*	4M/6F	0.8±0.5	46.0±6.7		11	*C. gariepinus*	9M/11F	2.2±2.4	64.8±4.8
	13	*L. umbratus*	10M/10F	0.8±0.1	42.0±2.0		13	*L. umbratus*	13M/7F	1.0±0.1	46.0±2.0
	14	*C. gariepinus*	7M/13F	1.7±0.4	58.8±4.9		14	*C. gariepinus*	*	*	*
	15	*C. gariepinus*	8M/12F	2.3±0.8	62.5±5.8		15	*C. gariepinus*	7M/6F	1.9±0.4	57.9±11.3
		O. mossambicus	7M/13F	0.8±0.06	34.8±1.6			*O. mossambicus*	12M/8F	0.9±0.3	33.4±3.4
	17	*C. gariepinus*	6M/5F	0.6±0.1	36.1±13.8		17	*C. gariepinus*	12M/6F	0.7±0.4	40.2±15.4
		O. mossambicus	17M/3F	0.2±0.05	20.8±1.8			*O. mossambicus*	15M/5F	0.1±0.04	19.1±1.1
August 1994	7	*L. umbratus*	6M/14F	1.5±0.2	51.0±2.9	November 1994	7	*L. umbratus*	11M/9F	1.5±0.2	49.6±26.6
	11	*C. gariepinus*	2M/4F	1.3±0.3	52.4±6.7		11	*C. gariepinus*	2M/1F	1.4±0.4	57.3±6.0
		L. umbratus	4M/4F	1.4±0.2	51.4±2.6			*L. umbratus*	5M/15F	1.4±0.2	50.8±1.5
	13	*L. umbratus*	15M/5F	1.0±0.1	45.0±2.0		13	*L. umbratus*	12M/8F	1.1±0.2	49.0±3.0
	14	*C. gariepinus*	*	*	*		14	*C. gariepinus*	10M/4F	1.2±0.5	49.8±8.5
		L. umbratus	5M/15F	1.5±0.1	52.8±3.0			*L. umbratus*	3F	1.7±0.2	56.0±3.6
	15	*C. gariepinus*	4M/8F	1.9±0.3	59.8±6.8		15	*C. gariepinus*	4M/1F	2.2±0.8	57.1±9.9
		O. mossambicus	9M/11F	1.0±0.2	35.7±2.3			*O. mossambicus*	5M/15F	0.9±0.1	35.1±1.1
	17	*C. gariepinus*	10M/10F	0.6±0.6	42.2±10.4		17	*C. gariepinus*	8M/11F	0.6±0.1	42.6±2.8
		O. mossambicus	13M/7F	0.3±0.1	23.4±3.5			*O. mossambicus*	12M/8F	0.3±0.1	24.0±3.1
February 1995	7	*L. umbratus*	18M/2F	0.9±0.2	45.0±2.9	May 1995	7	*L. umbratus*	5M15F	1.4±0.2	52.1±2.4
	11	*C. gariepinus*	9M/4F	1.0±0.3	49.2±5.7		11	*C. gariepinus*	13M/7F	1.5±0.7	57.2±8.2
		L. umbratus	6M/14F	1.2±1.2	50.1±2.2			*L. umbratus*	5M/2F	1.6±0.1	53.4±1.5
	13	*L. umbratus*	13M/7F	1.1±0.1	48.0±2.0		13	*L. umbratus*	17M/3F	1.0±0.1	48.0±2.0
	14	*C. gariepinus*	2M/2F	1.4±0.3	53.3±2.5		14	*C. gariepinus*	3F	1.5±0.3	55.3±9.9
		L. umbratus	14M/6F	1.5±0.4	52.0±3.9			*L. umbratus*	5M/2F	1.4±0.3	54.2±1.4
	15	*C. gariepinus*	4M/1F	2.5±0.4	59.8±21.1		15	*C. gariepinus*	*	*	*
		O. mossambicus	14M/6F	0.8±0.2	33.8±2.5			*O. mossambicus*	16M/4F	1.2±0.2	39.2±2.0
	17	*C. gariepinus*	2M/2F	0.4±0.1	38.0±2.8		17	*C. gariepinus*	8M/9F	1.0±1.3	49.7±15.6
		O. mossambicus	16M/4F	0.1±0.06	21.8±2.3			*O. mossambicus*	11M/4F	0.1±0.06	20.5±1.3
							19	*O. mossambicus*	9M/11F	0.3±0.06	24±1.3
							20	*C. gariepinus*	15M/2F	1.4±0.4	57.8±6.9
								O. mossambicus	12M/8F	0.6±0.1	31.7±2.6

* No data available

Spectrophotometer (Spectra AA-10) was used. Analytical standards for the metals were prepared from Holpro stock solutions. To ensure accurate and precise determination of trace elements in freshwater biological samples, a standard tissue sample (IAESA/R1/64) was used.

Metal contamination from the laboratory was avoided and a triplicate acid blank was also analysed.

Statistical procedures

Statistical analyses were performed by using the STATISTICA for Windows and STATSGRAPHICS version 7 programmes. The different statistical analyses were only performed on certain data, as sample sizes did not allow the analyses of all the data for all the cases. The capturing success varied and it was not possible to obtain large numbers (preferably >20 individuals; Seymore, 1994) of each fish species at both localities during a specific survey. The variation in capturing success and the capturing of fish that varied in mass and length, limited the statistical analyses to some extent.

9.3.3 *Results*

Metal bioaccumulation in tissues/organs

The general pattern of copper bioaccumulation in the organs/tissues of the fish (Table 9.5) was liver > gills > skin > muscle. Liver tissue generally contained copper levels significantly higher ($p<0.05$) than the gills, which again had significantly higher levels than the skin and muscle tissues. However, in some instances no significant differences between the gills and liver, the muscle and liver tissue (*Clarias gariepinus* from localities 11 and 14) or the gills and muscle tissue (for example *Labeo umbratus* from Localities 11 and 14) could be detected. Tissue/organ differences in zinc bioaccumulation (Table 9.6) were not able clear to be distinguished. Muscle tissue usually had lower levels of zinc than that of the other tissues/organs sampled. From the data presented, it was, however, not clear which of the tissues would have the highest zinc levels (Table 9.6). Aluminium accumulated mostly in the gills and liver of the fish followed by the muscle and skin tissues (Table 9.7). Comparisons between the different tissue types were predominantly significant ($p<0.05$), except occasionally for muscle and skin tissue. The highest iron concentrations were found in the liver followed by the gills, while lower levels were detected in the skin and muscle tissues (Table 9.8). The iron bioaccumulation therefore resulted in a general order of liver ≈ gill > muscle ≈ skin.

The bioaccumulation pattern of nickel was not clear (Table 9.9). For example, fish from locality 13 did not show significant difference in nickel levels, while fish from the other localities showed that the gills and live tissues were usually significantly ($p<0.05$) higher than the levels in the muscle and skin tissues. Trends in manganese bioaccumulation in the selected tissues were generally similar for all the species at the different localities (Table 9.10) The higher concentrations of manganese were clearly found in the gills, followed by the liver, skin and muscle. These differences were mostly significant

Table 9.5: Copper concentrations (ug/g dry mass) in the tissues/organs of fish from the selected sites in the Olifants River Catchment.

Locality	Specie		Copper Concentration ug/g				Locality	Specie		Copper Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*C. gariepinus*	n	10	10	10	10	7	*C. gariepinus*	n	*	*	*	*
		Range	1-3	2-5	3-15	17-60			Range	*	*	*	*
		X±SD	2±1	3±1	7±4	30±14			X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	20	20	20	20
		Range	3-10	2-6	5-8	112-784			Range	4-13	5-13	3-6	171-715
		X±SD	6±2	4±1	6±1	391±180			X±SD	7±2	8±2	5±1	441±173
11	*C. gariepinus*	n	10	10	10	10	11	*C. gariepinus*	n	20	20	20	20
		Range	3-5	3-5	3-41	37-93			Range	1-3	2-6	4-7	5-70
		X±SD	4±1	4±1	13±11	54±17			X±SD	2±1	2±1	5±1	43±17
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	20	20	20	20
		Range	2-37	2-40	1-39	107-1685			Range	1-8	1-13	2-17	59-820
		X±SD	10±10	14±13	17±11	478±387			X±SD	4±2	4±3	7±3	435±208
14	*C. gariepinus*	n	20	20	20	20	14	*C. gariepinus*	n	*	*	*	*
		Range	4-28	3-32	7-72	22-93			Range	*	*	*	*
		X±SD	14±9	14±10	29±22	42±17			X±SD	*	*	*	*
15	*C. gariepinus*	n	20	20	20	20	15	*C. gariepinus*	n	13	12	12	11
		Range	1-10	1-17	3-12	12-48			Range	1-27	1-3	4-11	12-66
		X±SD	3±1	3±3	6±1	30±10			X±SD	3±7	2±1	7±2	35±16
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	19	20	20	20
		Range	1-18	1-4	2-67	3-312			Range	1-69	1-67	1-86	1-54
		X±SD	4±4	2±1	8±14	62±77			X±SD	8±15	11±19	17±26	10±14
17	*C. gariepinus*	n	10	10	11	10	17	*C. gariepinus*	n	18	18	18	18
		Range	1-3	1-5	1-61	1-42			Range	1-41	1-6	6-18	10-80
		X±SD	2±1	1±1	9±17	16±12			X±SD	5±9	3±1	10±3	49±21
	O. mossambicus	n	20	20	19	15		*O. mossambicus*	n	20	20	20	20
		Range	1-43	1-10	2-160	23-496			Range	1-5	1-2	1-8	8-486
		X±SD	14±11	4±2	40±50	175±128			X±SD	2±1	1±1	4±1	119±150

Table 9.5: (Continued)

Locality	Specie		Copper Concentration ug/g				Locality	Specie		Copper Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994							**SURVEY: NOVEMBER 1994**						
7	*L. umbratus*	n Range X±SD	20 2-8 4±2	20 2-4 3±1	20 5-12 7±2	20 104-552 336±131	7	*L. umbratus*	n Range X±SD	20 21-7 4±1	20 3-10 4±2	20 6-12 8±1	20 179-943 501±249
11	*C. gariepinus*	n Range X±SD	6 2-3 2±1	6 3-5 4±1	6 5-8 6±1	6 45-88 64±15	11	*C. gariepinus*	n Range X±SD	3 2-19 8±1	3 4-5 4±1	3 8-9 8±1	3 23-34 28±5
	L. umbratus	n Range X±SD	8 2-3 2±1	8 4-31 19±9	8 6-21 9±5	8 285-825 568±196		*L. umbratus*	n Range X±SD	20 3-17 6±3	20 5-40 11±8	20 6-26 12±6	20 146-741 503±174
13	*L. umbratus*	n Range X±SD	20 1-4 2±1	20 2-13 4±3	20 5-20 9±3	20 37-916 258±225	13	*L. umbratus*	n Range X±SD	17 2-21 6±5	17 2-16 5±4	17 4-19 8±3	17 46-858 286±232
14	*C. gariepinus*	n Range X±SD	* * *	* * *	* * *	* * *	14	*C. gariepinus*	n Range X±SD	14 3-9 5±2	14 3-14 6±3	14 8-57 22±14	14 11-35 20±7
	L. umbratus	n Range X±SD	20 2-12 4±2	20 3-15 7±4	20 4-14 8±3	20 89-605 286±152		*L. umbratus*	n Range X±SD	3 2-18 8±9	3 4-6 5±1	3 5-7 6±1	3 37-80 57±22
15	*C. gariepinus*	n Range X±SD	12 1-3 2±1	12 2-3 2±1	12 4-10 6±1	12 6-74 29±16	15	*C. gariepinus*	n Range X±SD	5 1-3 2±1	5 1-4 2±1	5 5-8 6±1	5 10-29 21±8
	O. mossambicus	n Range X±SD	20 1-7 3±1	20 1-2 1±1	20 4-9 6±1	20 5-115 37±38		*O. mossambicus*	n Range X±SD	20 1-2 2±1	20 1-2 2±1	20 4-20 6±3	20 4-64 24±19
17	*C. gariepinus*	n Range X±SD	20 1-4 2±1	20 1-3 2±1	19 6-16 9±2	20 12-102 59±25	17	*C. gariepinus*	n Range X±SD	19 1-3 2±1	19 1-5 2±1	19 6-16 11±3	19 8-119 43±26
	O. mossambicus	n Range X±SD	20 2-11 4±2	20 1-5 2±1	20 5-16 8±3	17 10-528 81±135		*O. mossambicus*	n Range X±SD	20 1-4 2±1	20 1-3 2±1	20 4-21 9±3	18 14-339 144±86

Table 9.5: (Continued)

Locality	Specie		Copper Concentration ug/g				Locality	Specie		Copper Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995							**SURVEY: MAY 1995**						
7	*L. umbratus*	n Range X±SD	20 3-14 6±3	20 2-10 4±2	20 2-22 7±4	20 321-968 587±194	7	*L. umbratus*	n Range X±SD	20 3-8 5±1	20 3-5 4±1	20 5-10 7±1	20 245-845 505±171
11	*C. gariepinus*	n Range X±SD	13 2-30 9±10	13 2-15 4±3	13 6-40 11±9	13 3-148 30±38	11	*C. gariepinus*	n Range X±SD	20 1-10 3±2	20 2-17 4±3	20 4-9 6±1	20 23-51 35±9
	L. umbratus	n Range X±SD	20 3-20 7±5	20 3-16 7±4	20 6-13 8±2	20 45-1470 527±425		*L. umbratus*	n Range X±SD	7 3-4 3±1	7 3-7 5±2	7 5-12 8±3	7 29-72 50±16
13	*L. umbratus*	n Range X±SD	20 1-15 4±4	20 2-56 10±13	20 4-35 10±8	20 46-1654 692±555	13	*L. umbratus*	n Range X±SD	19 2-34 7±8	19 2-43 7±10	19 4-15 7±2	19 1-1445 498±448
14	*C. gariepinus*	n Range X±SD	4 3-4 4±1	4 4-7 5±1	4 8-19 13±5	4 17-29 24±5	14	*C. gariepinus*	n Range X±SD	3 2-5 4±1	3 3-4 3±1	3 5-7 6±1	3 33-46 40±6
	L. umbratus	n Range X±SD	20 2-22 5±4	20 3-17 6±4	20 5-33 11±7	20 44-1100 360±374		*L. umbratus*	n Range X±SD	7 2-12 6±4	7 3-10 5±3	7 5-18 7±5	7 86-325 187±89
15	*C. gariepinus*	n Range X±SD	5 1-2 2±1	5 2-7 3±2	5 1-8 6±2	5 10-51 34±16	15	*C. gariepinus*	n Range X±SD	* * *	* * *	* * *	* * *
	O. mossambicus	n Range X±SD	20 2-20 6±4	20 2-32 7±7	20 5-33 12±7	20 15-325 110±90		*O. mossambicus*	n Range X±SD	20 1-3 2±1	20 1-5 1±1	20 2-10 5±1	20 3-165 48±40
17	*C. gariepinus*	n Range X±SD	4 3-6 4±1	4 4-6 5±1	4 6-21 11±7	4 71-106 88±16	17	*C. gariepinus*	n Range X±SD	17 1-7 3±1	17 1-4 2±1	17 7-30 12±5	17 25-135 75±26
	O. mossambicus	n Range X±SD	20 2-16 5±3	20 2-23 5±4	20 3-26 9±4	19 2-407 95±105		*O. mossambicus*	n Range X±SD	13 2-21 9±7	13 1-12 5±2	13 7-45 15±12	13 29-843 277±246
							19	*O. mossambicus*	n Range X±SD	20 1-8 2±1	20 1-2 1±1	20 1-8 3±1	20 8-243 93±73
							20	*C. gariepinus*	n Range X±SD	17 1-3 1±1	17 1-8 1±2	17 3-5 1±1	17 51-135 79±25
								O. mossambicus	n Range X±SD	20 1-3 2±1	20 1-3 1±1	19 2-4 3±1	19 117-989 466±248

Table 9.6: Zinc concentrations (ug/g dry mass) in the tissues/organs of the fish from the selected sites in the Olifants River Catchment.

Locality	Specie		Zinc Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994						
7	*C. gariepinus*	n	10	10	10	10
		Range	31-131	28-118	66-285	25-160
		X±SD	85±31	65±35	143±81	103±52
	L. umbratus	n	20	20	20	20
		Range	38-124	14-86	89-201	65-157
		X±SD	64±20	33±16	138±34	106±31
11	*C. gariepinus*	n	10	10	10	10
		Range	54-131	25-53	39-240	107-197
		X±SD	93±25	33±8	131±52	136±26
13	*L. umbratus*	n	20	20	20	20
		Range	11-132	6-93	2-41	9-155
		X±SD	32±37	29±20	14±11	49±44
14	*C. gariepinus*	n	20	20	20	20
		Range	63-230	18-48	84-175	64-160
		X±SD	127±46	31±8	126±21	99±27
15	*C. gariepinus*	n	20	20	20	20
		Range	75-227	18-42	67-169	71-126
		X±SD	139±36	26±6	116±28	103±15
	O mossambicus	n	20	20	20	20
		Range	46-277	9-27	45-103	28-122
		X±SD	146±61	20±4	79±15	77±25
17	*C. gariepinus*	n	11	10	11	10
		Range	17-157	4-27	7-162	27-105
		X±SD	44±39	8±6	43±42	45±22
	O. mossambicus	n	20	20	19	16
		Range	28-342	2-86	48-625	10-781
		X±SD	129±76	38±16	185±131	172±185

Locality	Specie		Zinc Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: MAY 1994						
7	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20
		Range	45-108	73-189	20-34	82-163
		X±SD	72±15	126±28	26±4	129±25
11	*C. gariepinus*	n	20	20	20	20
		Range	36-175	21-70	57-152	67-193
		X±SD	87±35	31±12	88±22	115±29
13	*L. umbratus*	n	20	20	20	20
		Range	58-119	20-55	63-125	115±361
		X±SD	28±15	31±7	87±16	192±59
14	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
15	*C. gariepinus*	n	13	12	12	12
		Range	22-116	14-42	76-188	81-188
		X±SD	62±29	25±7	112±28	121±34
	O. mossambicus	n	19	20	20	20
		Range	23-164	13-613	16-166	24-225
		X±SD	78±40	105±132	78±43	110±67
17	*C. gariepinus*	n	18	18	18	18
		Range	30-713	17-41	66-195	58-229
		X±SD	115±152	29±6	114±33	138±42
	O. mossambicus	n	20	20	20	20
		Range	10-144	16-33	13-91	21-85
		X±SD	75±39	24±5	55±19	48±17

Table 9.6: (Continued)

Locality	Specie		Zinc Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994						
7	*L. umbratus*	n Range X±SD	20 46-108 77±20	20 20-36 26±5	20 97-163 123±21	20 79-151 116±21
11	*C. gariepinus*	n Range X±SD	6 98-129 111±11	6 25-44 36±7	6 92-113 104±9	6 121-173 144±19
	L. umbratus	n Range X±SD	8 60-127 87±22	8 24-79 36±18	8 114-260 165±45	8 106-184 137±29
13	*L. umbratus*	n Range X±SD	20 72-154 111±24	20 21-59 34±11	20 57-111 78±13	20 76-177 115±25
14	*C. gariepinus*	n Range X±SD	* * *	* * *	* * *	* * *
	L. umbratus	n Range X±SD	20 51-176 94±33	20 19-44 29±7	20 16-376 144±76	20 79-192 127±31
15	*C. gariepinus*	n Range X±SD	12 37-204 82±50	12 26-64 37±11	12 93-151 124±19	12 53-163 102±31
	O. mossambicus	n Range X±SD	20 94-235 149±38	20 19-75 31±11	20 86-141 108±14	20 37-172 69±29
17	*C. gariepinus*	n Range X±SD	20 55-125 87±20	20 25-40 32±4	19 99-277 138±45	20 101-217 150±29
	O. mossambicus	n Range X±SD	20 55-337 133±71	20 16-53 33±12	20 75-163 109±20	20 56-266 97±52

Locality	Specie		Zinc Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: NOVEMBER 1994						
7	*L. umbratus*	n Range X±SD	20 79-88 65±14	20 23-60 35±11	20 107±249 149±34	20 91-221 139±31
11	*C. gariepinus*	n Range X±SD	3 105-171 133±35	3 63-80 73±9	3 183-199 189±9	3 114-136 125±11
	L. umbratus	n Range X±SD	20 35-123 74±24	20 25-67 38±12	20 116-275 179±46	20 84-172 115±28
13	*L. umbratus*	n Range X±SD	17 45-212 115±42	17 28-91 56±22	17 57-140 99±27	17 36-256 137±56
14	*C. gariepinus*	n Range X±SD	14 56-139 92±25	14 37-85 46±139	14 120-328 200±63	14 71-128 98±18
	L. umbratus	n Range X±SD	3 31-52 41±11	3 28-32 30±2	3 148-187 162±21	3 77-98 90±12
15	*C. gariepinus*	n Range X±SD	5 40-130 72±36	5 20-207 72±76	5 95-161 126±25	5 87-125 106±14
	O. mossambicus	n Range X±SD	20 32-170 119±39	20 24-40 29±4	20 77-136 100±15	30 46-514 92±101
17	*C. gariepinus*	n Range X±SD	19 17-221 98±46	19 23-57 33±9	19 126-209 163±25	19 98-226 139±33
	O. mossambicus	n Range X±SD	20 38-130 91±24	20 17-33 25±4	13 66-120 88±17	18 50-281 128±57

Table 9.6: (Continued)

Locality	Specie		Zinc Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	49-135	24-73	93-216	130-265
		X±SD	82±27	39±11	149±31	176±36
11	*C. gariepinus*	n	13	13	13	13
		Range	64-186	30-70	107-278	18-208
		X±SD	126±38	47±11	142±47	110±49
	L. umbratus	n	20	20	20	20
		Range	55-215	18-91	108-271	42-301
		X±SD	110±47	47±22	184±41	90±53
13	*L. umbratus*	n	20	20	20	20
		Range	42-164	20-76	64-180	100-238
		X±SD	87±31	45±16	93±26	158±41
14	*C. gariepinus*	n	4	4	4	4
		Range	44-117	38-74	110-536	59-124
		X±SD	75±31	53±15	257±196	100±28
	L. umbratus	n	20	20	20	20
		Range	48-256	29-88	100-504	66-424
		X±SD	122±54	46±14	175±100	145±77
15	*C. gariepinus*	n	5	5	5	5
		Range	48-206	41-71	18-238	75-137
		X±SD	103±60	56±12	156±110	117±24
	O. mossambicus	n	20	20	20	20
		Range	24-216	25-496	52-126	37-248
		X±SD	137±51	64±102	105±17	84±49
17	*C. gariepinus*	n	4	4	4	3
		Range	37-96	26-55	100-146	171-299
		X±SD	75±26	37±13	127±19	231±64
	O. mossambicus	n	20	20	20	19
		Range	27-116	20-48	17-145	4-252
		X±SD	68±20	28±6	68±30	91±72

Locality	Specie		Zinc Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: MAY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	59-136	17-50	105-206	109-262
		X±SD	92±25	32±8	143±32	160±38
11	*C. gariepinus*	n	20	20	20	20
		Range	59-216	30-80	94-143	114-227
		X±SD	122±44	42±11	117±14	149±28
	L. umbratus	n	7	7	7	7
		Range	57-173	19-62	116-168	81-153
		X±SD	100±42	35±14	139±20	104±24
13	*L. umbratus*	n	19	19	19	19
		Range	47-189	18-68	36-180	113-230
		X±SD	91±34	36±12	105±36	167±36
14	*C. gariepinus*	n	3	3	3	3
		Range	82-152	33-54	98-277	98-159
		X±SD	118±35	44±10	169±95	122±33
	L. umbratus	n	7	7	7	7
		Range	115-227	37-77	118-180	148-245
		X±SD	160±40	52±16	143±21	182±31
15	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	O. mossambicus	n	20	20	20	20
		Range	50-387	25-53	66-131	31-82
		X±SD	167±79	38±7	109±16	54±12
17	*C. gariepinus*	n	17	17	17	17
		Range	57-157	27-73	118-421	70-378
		X±SD	92±33	42±11	207±82	243±76
	O. mossambicus	n	13	13	13	13
		Range	57-188	30-72	89-387	35-275
		X±SD	109±35	44±15	158±82	165±63
19	*O. mossambicus*	n	20	20	20	20
		Range	6-152	13-132	56-392	20-233
		X±SD	86±36	41±24	117±74	79±50
20	*O. mossambicus*	n	17	17	17	17
		Range	32-262	18-110	12-197	120-245
		X±SD	82±63	46±26	103±55	166±35
	O. mossambicus	n	20	20	19	20
		Range	51-137	12-82	48-115	46-213
		X±SD	90±24	28±15	80±18	90±49

Table 9.7: Aluminium concentrations (ug/g dry mass) in the tissues/organs of fish from the selected sites in the Olifants River Catchment.

Locality	Specie		Aluminium Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994						
7	*C. gariepinus*	n	10	10	10	10
		Range	20-55	14-45	33-89	15-45
		X±SD	32±11	25±11	46±20	28±10
	L. umbratus	n	20	20	20	20
		Range	10-59	11-73	12-395	16-67
		X±SD	30±13	28±18	131±122	39±14
11	*C. gariepinus*	n	10	10	10	10
		Range	8-7	10-57	33-435	17-123
		X±SD	33±18	26±16	166±127	53±31
13	*L. umbratus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
14	*C. gariepinus*	n	20	20	20	20
		Range	14-53	11-52	42-178	21-52
		X±SD	35±11	28±11	110±36	46±12
15	*C. gariepinus*	n	20	19	20	20
		Range	6-61	6-82	41-251	22-171
		X±SD	31±15	26±17	116±50	83±44
	O mossambicus	n	20	20	20	20
		Range	2-161	1-71	21-339	19-215
		X±SD	37±37	24±22	158±116	91±53
17	*C. gariepinus*	n	10	10	10	10
		Range	9-70	3-32	25-508	7-58
		X±SD	30±17	14±10	145±161	29±16
	O. mossambicus	n	20	20	19	16
		Range	8-157	1-58	40-752	9-933
		X±SD	64±40	21±14	362±220	249±269

Locality	Specie		Aluminium Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: MAY 1994						
7	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20
		Range	14-46	20-77	6-35	8-39
		X±SD	30±13	48±17	18±8	22±8
11	*C. gariepinus*	n	20	20	20	20
		Range	4-42	4-20	35-175	13-95
		X±SD	13±9	11±5	76±48	33±19
13	*L. umbratus*	n	20	20	20	20
		Range	15-111	12-63	8-68	18-690
		X±SD	33±21	28±11	27±15	128±178
14	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
15	*C. gariepinus*	n	13	13	13	13
		Range	1-47	2-30	34-236	39-171
		X±SD	15±11	14±8	69±54	77±48
	O. mossambicus	n	19	20	20	20
		Range	5-224	8-105	7-248	8-118
		X±SD	58±58	40±31	57±58	47±37
17	*C. gariepinus*	n	18	18	17	17
		Range	9-498	2-121	204-1493	15-776
		X±SD	95±133	45±38	869±479	172±212
	O. mossambicus	n	20	20	20	20
		Range	34-940	15-272	73-1451	21-1136
		X±SD	200±219	62±54	611±335	288±301

Table 9.7: (Continued)

Locality	Specie		Aluminium Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994						
7	*L. umbratus*	n	20	20	20	20
		Range	17-49	19-52	46-167	14-46
		X±SD	33±9	32±9	98±36	32±9
11	*C. gariepinus*	n	6	6	6	6
		Range	16-48	13-49	47-336	21-57
		X±SD	29±12	23±13	147±103	31±14
	L. umbratus	n	8	8	8	8
		Range	9-26	23-66	75-192	12-66
		X±SD	18±8	42±16	111±38	28±20
13	*L. umbratus*	n	20	20	20	20
		Range	57-247	40-227	58-1014	53-325
		X±SD	111±48	106±54	328±228	145±71
14	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20
		Range	8-46	8-42	27-221	8-76
		X±SD	26±11	19±9	74±44	23±16
15	*C. gariepinus*	n	12	12	12	12
		Range	6-47	12-65	33-229	15-100
		X±SD	23±11	28±17	92±53	42±28
	O mossambicus	n	19	15	20	20
		Range	3-34	1-32	2-45	10-118
		X±SD	16±8	9±8	26±11	39±23
17	*C. gariepinus*	n	20	20	18	20
		Range	41-1011	46-469	610-4605	44-4160
		X±SD	155±208	123±119	2146±1094	411±891
	O. mossambicus	n	20	20	20	20
		Range	68-1178	31-219	163-1855	25-2242
		X±SD	218±246	73±49	677±392	333±568

Locality	Specie		Aluminium Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: NOVEMBER 1994						
7	*L. umbratus*	n	20	20	20	20
		Range	17-76	19-85	36-95	10-87
		X±SD	34±15	41±14	60±15	22±7
11	*C. gariepinus*	n	3	3	3	3
		Range	46-62	23-87	184-316	46-62
		X±SD	52±9	54±32	234±72	27±3
	L. umbratus	n	20	20	20	20
		Range	13-52	12-110	45-437	15-116
		X±SD	28±10	34±20	158±99	38±25
13	*L. umbratus*	n	17	17	17	17
		Range	15-214	5-270	31-487	14-605
		X±SD	81±67	60±73	169±113	136±166
14	*C. gariepinus*	n	14	14	14	14
		Range	46-642	13-68	78-192	26-45
		X±SD	209±203	26±14	122±38	35±5
	L. umbratus	n	3	3	3	3
		Range	39-61	25-78	92-145	22-39
		X±SD	53±12	58±30	114±28	32±9
15	*C. gariepinus*	n	5	5	5	5
		Range	41-101	12-675	112-859	36-239
		X±SD	58±25	167±285	291±318	122±93
	O. mossambicus	n	20	20	20	20
		Range	7-95	1-55	171-1992	21-221
		X±SD	32±23	22±18	973±651	78±55
17	*C. gariepinus*	n	19	19	11	19
		Range	41-4915	100-454	407-6384	60-6959
		X±SD	957±1542	251±120	2270±1801	622±1538
	O. mossambicus	n	20	20	16	18
		Range	27-1608	15-153	932-12391	122-12978
		X±SD	386±457	67±36	3782±3464	1239±2956

Table 9.7: (Continued)

Locality	Specie		Aluminium Concentration ug/g				Locality	Specie		Aluminium Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995							**SURVEY: MAY 1995**						
7	*L. umbratus*	n	20	20	20	20	7	*C. gariepinus*	n	20	20	20	20
		Range	16-191	15-94	38-714	21-104			Range	19-90	11-84	89-442	19-56
		X±SD	32±40	38±24	134±163	55±28			X±SD	43±20	38±20	220±115	32±9
11	*C. gariepinus*	n	13	13	13	13	11	*C. gariepinus*	n	20	20	20	20
		Range	18-84	14-78	51-433	17-120			Range	6-38	8-79	24-118	7-47
		X±SD	40±21	41±21	173±113	47±30			X±SD	19±9	23±17	58±26	18±10
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	7	7	7	7
		Range	19-132	13-42	31-456	15-379			Range	18-42	16-27	53-99	19-55
		X±SD	40±21	26±9	113±117	68±86			X±SD	29±9	21±4	79±20	34±15
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	19	19	19	19
		Range	6-106	7-296	13-350	7-312			Range	16-155	8-52	34-1435	12-378
		X±SD	32±28	36±64	94±96	86±106			X±SD	43±30	24±13	271±377	92±114
14	*C. gariepinus*	n	4	4	4	4	14	*C. gariepinus*	n	3	3	3	3
		Range	76-117	84-134	156-652	80-182			Range	51-54	31-50	134-160	22-46
		X±SD	98±21	109±20	371±212	109±49			X±SD	53±2	43±10	150±14	36±2
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	7	7	7	7
		Range	21-122	22-106	63-449	36-175			Range	28-137	17-47	68-259	15-251
		X±SD	61±23	59±25	172±102	79±37			X±SD	57±38	31±11	173±60	72±86
15	*C. gariepinus*	n	5	5	5	5	15	*C. gariepinus*	n	*	*	*	*
		Range	48-310	36-154	52-2210	35-4570			Range	*	*	*	*
		X±SD	140±105	71±47	521±944	956±2019			X±SD	*	*	*	*
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	22-323	15-478	65-340	35-495			Range	20-81	12-58	39-275	17-203
		X±SD	66±64	60±100	178±76	119±98			X±SD	45±15	31±12	113±49	61±42
17	*C. gariepinus*	n	4	4	3	4	17	*C. gariepinus*	n	17	17	14	17
		Range	52-600	27-120	162-469	114-196			Range	23-181	16-166	119-2815	46-291
		X±SD	210±262	54±40	264±176	156±33			X±SD	85±44	54±36	963±788	129±74
	O. mossambicus	n	20	20	20	18		*O. mossambicus*	n	13	13	13	13
		Range	23-464	4-46	248-3663	4-3371			Range	27-531	21-93	349-2582	89-1350
		X±SD	99±104	20±11	1084±923	631±875			X±SD	148±172	43±22	1001±742	312±344
							19	*O. mossambicus*	n	20	20	20	20
									Range	4-357	7-86	38-2200	21-297
									X±SD	67±70	35±20	122±65	114±80
							20	*C. gariepinus*	n	17	17	17	17
									Range	4-27	5-98	12-199	9-85
									X±SD	13±6	22±21	78±45	27±17
								O. mossambicus	n	20	20	19	20
									Range	9-55	2-66	23-110	17-187
									X±SD	26±13	17±13	50±20	52±48

* Data not available

Table 9.8: Iron concentrations (ug/g dry mass) in the tissues/organs of fish from selected sites in the Olifants River Catchment.

Locality	Specie		Iron Concentration ug/g				Locality	Specie		Iron Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*C. gariepinus*	n	10	10	10	10	7	*C. gariepinus*	n	*	*	*	*
		Range	98-192	121-252	156-331	121-757			Range	*	*	*	*
		X±SD	136±39	179±55	255±59	353±288			X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	20	20	20	20
		Range	61-115	64-283	179-591	128-718			Range	25-167	105-365	55-118	191-993
		X±SD	78±16	103±53	339±104	377±172			X±SD	87±28	251±67	87±17	429±214
11	*C. gariepinus*	n	10	10	10	10	11	*C. gariepinus*	n	20	20	20	20
		Range	63-177	58-118	132-1028	710-2426			Range	42-187	59-229	252-673	158-3050
		X±SD	104±40	72±18	466±290	1586±556			X±SD	101±40	149±36	347±96	1514±756
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	20	20	20	20
		Range	25-45	41-54	5-268	40-15150			Range	107-280	101-524	232-941	259-3916
		X±SD	31±5	48±3	54±51	4023±4396			X±SD	200±41	212±85	454±171	1004±834
14	*C. gariepinus*	n	20	20	20	20	14	*C. gariepinus*	n	*	*	*	*
		Range	79-302	79-212	137-530	289-2935			Range	*	*	*	*
		X±SD	147±49	121±38	324±109	1201±757			X±SD	*	*	*	*
15	*C. gariepinus*	n	20	20	20	13	15	*C. gariepinus*	n	13	12	12	8
		Range	102-282	61-657	160-914	2978-20148			Range	56-399	67-473	151-1843	3923-16061
		X±SD	157±47	144±155	354±180	8969±5149			X±SD	175±105	241±149	533±461	9763±4935
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	19	20	20	20
		Range	56-529	29-458	61-950	8-977			Range	34-998	25-1900	28-1404	40-1310
		X±SD	309±135	230±124	496±286	216±218			X±SD	287±302	301±335	284±336	331±333
17	*C. gariepinus*	n	13	10	11	10	17	*C. gariepinus*	n	18	18	18	18
		Range	58-396	43-327	100-996	164-2422			Range	46-2054	58-312	290-2590	378-8554
		X±SD	217±106	132±75	402±300	623±659			X±SD	267±455	162±82	1019±566	2467±2165
	O. mossambicus	n	20	20	19	15		*O. mossambicus*	n	20	20	20	20
		Range	41-4145	55-681	339-5698	82-6688			Range	14-978	36-779	190-1559	82-1588
		X±SD	1429±1101	461±166	2190±1232	2218±1844			X±SD	254±270	184±182	765±359	552±422

Table 9.8: (Continued)

Locality	Specie		Iron Concentration ug/g				Locality	Specie		Iron Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994							**SURVEY: NOVEMBER 1994**						
7	*L. umbratus*	n	20	20	20	20	7	*L. umbratus*	n	20	20	20	20
		Range	70-200	72-177	240-380	254-732			Range	60-262	104-350	294-538	250-946
		X±SD	115±41	124±35	321±46	409±143			X±SD	132±67	175±66	375±66	535±164
11	*C. gariepinus*	n	6	6	6	6	11	*C. gariepinus*	n	3	3	3	3
		Range	92-279	114-207	363-560	765-1372			Range	162-297	154-311	623-853	550-2138
		X±SD	169±80	140±35	447±70	1074±246			X±SD	216±71	255±88	737±115	1531±858
	L. umbratus	n	8	8	8	8		*L. umbratus*	n	20	20	20	20
		Range	75-117	99-186	240-443	772-3634			Range	355-1387	482-1404	589-2765	779-6799
		X±SD	101±13	141±31	329±61	2218±1168			X±SD	620±261	739±275	1234±608	3967±1770
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	17	17	17	17
		Range	72-301	69-255	282-803	3-1073			Range	129-1449	124-799	247-1505	146-1663
		X±SD	133±58	130±56	424±117	592±252			X±SD	479±419	361±238	757±334	754±411
14	*C. gariepinus*	n	*	*	*	*	14	*C. gariepinus*	n	14	14	14	14
		Range	*	*	*	*			Range	179-1969	126-271	542-2839	428-3603
		X±SD	*	*	*	*			X±SD	469±446	184±48	1303±769	1997±200
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	3	3	3	3
		Range	44-224	59-134	197-703	234-766			Range	133-496	173-215	399-462	222-413
		X±SD	82±41	92±25	337±125	398±152			X±SD	280±191	195±21	437±34	348±108
15	*C. gariepinus*	n	12	12	12	12	15	*C. gariepinus*	n	5	5	5	4
		Range	239-1434	158-700	232-1559	1691-18851			Range	142-423	84-415	111-975	10043-16971
		X±SD	501±323	450±130	855±338	8854±6130			X±SD	237±109	226±141	480±313	12888±3288
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	225-420	187-332	544-840	518-2040			Range	77-351	105-377	665-2331	333-5678
		X±SD	297±58	271±42	684±71	951±357			X±SD	201±80	216±94	1181±465	944±1138
17	*C. gariepinus*	n	20	20	19	20	17	*C. gariepinus*	n	12	19	19	19
		Range	72-920	76-480	648-4475	203-3987			Range	91-709	108-15248	463-4339	259-6335
		X±SD	191±182	159±114	2235±1014	1738±1013			X±SD	261±172	986±3454	1939±827	1638±1639
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	15	20	18
		Range	79-1157	60-161	542-2185	162-2319			Range	100-680	83-193	813-3737	266-3346
		X±SD	248±231	84±24	1095±463	719±558			X±SD	273±165	106±228	1900±926	1010±725

Table 9.8: (Continued)

Locality	Specie		Iron Concentration ug/g				Locality	Specie		Iron Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995							**SURVEY: MAY 1995**						
7	*L. umbratus*	n	20	20	20	20	7	*L. umbratus*	n	20	20	20	20
		Range	105-1313	109-720	169-1397	1003-2287			Range	145-297	136-287	298-1380	340-1586
		X±SD	563±447	252±165	721±417	1626±438			X±SD	207±38	194±39	665±342	920±381
11	*C. gariepinus*	n	13	13	13	13	11	*C. gariepinus*	n	20	20	20	20
		Range	94-339	79-426	258-1151	513-1663			Range	81-455	128-708	216-877	334-3389
		X±SD	206±79	214±111	564±254	1073±396			X±SD	238±103	272±131	491±148	1366±959
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	7	7	7	7
		Range	150-1759	132-748	332-1037	340-6902			Range	117-692	135-421	406-3274	446-3459
		X±SD	366±375	257±179	583±192	3461±1944			X±SD	289±210	245±104	1547±1288	2225±1261
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	19	19	19	19
		Range	96-813	166-755	229-1067	328-3567			Range	137-461	172-441	229-1852	50-7665
		X±SD	340±204	327±157	531±260	716±725			X±SD	260±84	266±86	629±421	1989±1837
14	*C. gariepinus*	n	4	4	4	4	14	*C. gariepinus*	n	3	3	3	3
		Range	132-155	171-261	393-1344	653-1096			Range	135-337	145-421	406-667	588-709
		X±SD	140±10	211±41	733±419	892±187			X±SD	266±114	246±157	515±135	646±60
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	7	7	7	7
		Range	85-255	69-257	267-1687	193-691			Range	114-287	141-310	444-991	285-1438
		X±SD	145±43	139±46	607±423	344±131			X±SD	216±65	208±67	595±209	674±400
15	*C. gariepinus*	n	5	5	5	5	15	*O. mossambicus*	n	*	*	*	*
		Range	136-440	181-310	316-4545	5486-16763			Range	*	*	*	*
		X±SD	273±133	233±47	1379±1783	10607±4875			X±SD	*	*	*	*
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	124-483	132-1491	535-2478	594-3186			Range	79-276	70-216	312-741	158-1311
		X±SD	194±76	292±353	792±417	1213±584			X±SD	129±54	127±44	502±126	474±299
17	*C. gariepinus*	n	4	4	4	3	17	*C. gariepinus*	n	17	17	17	16
		Range	195-528	130-239	383-9561	1551-7705			Range	67-320	83-227	379-3048	656-9028
		X±SD	292±154	180±54	2783±4522	3677±3489			X±SD	164±61	133±33	1376±705	2597±2100
	O. mossambicus	n	20	20	20	19		*O. mossambicus*	n	13	13	13	13
		Range	103-1593	83-228	333-2701	78-4158			Range	121-645	87-149	589-2969	364-1177
		X±SD	296±317	123±40	1424±764	1107±1058			X±SD	297±181	110±18	1271±712	695±262
							19	*O. mossambicus*	n	19	20	20	19
									Range	6-288	10-145	132-791	118-648
									X±SD	106±64	51±31	307±153	248±140
							20	*C. gariepinus*	n	17	17	17	16
									Range	46-204	26-348	120-403	3017-19946
									X±SD	77±36	79±71	224±86	12759±5674
								O. mossambicus	n	20	20	19	20
									Range	36-99	22-116	129-296	1143-6185
									X±SD	64±14	45±21	203±50	2361±1187

* Data not available

Table 9.9: Nickel concentrations (ug/g dry mass) in the tissues/organs of the fish from selected sites in the Olifants River Catchment.

Locality	Specie		Nickel Concentration ug/g				Locality	Specie		Nickel Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*C. gariepinus*	n	10	10	10	10	7	*C. gariepinus*	n	*	*	*	*
		Range	10-15	8-18	10-32	11-14			Range	*	*	*	*
		X±SD	13±2	13±3	15±7	13±1			X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	20	20	20	20
		Range	7-14	5-19	3-18	4-17			Range	9-13	10-19	10-21	8-13
		X±SD	9±2	10±4	12±3	10±3			X±SD	11±1	13±2	12±4	11±1
11	*C. gariepinus*	n	10	10	10	10	11	*C. gariepinus*	n	20	20	20	20
		Range	7-17	7-15	8-129	9-27			Range	4-19	6-22	11-30	7-18
		X±SD	11±4	9±2	34±36	19±6			X±SD	12±5	15±5	18±5	12±4
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	20	20	20	20
		Range	6-23	9-27	2-26	10-58			Range	10-33	10-30	17-32	9-30
		X±SD	11±5	14±5	16±5	22±12			X±SD	23±6	22±4	25±4	22±6
14	*C. gariepinus*	n	20	20	20	20	14	*C. gariepinus*	n	*	*	*	*
		Range	9-22	9-27	15-41	8-23			Range	*	*	*	*
		X±SD	15±5	14±5	26±8	16±4			X±SD	*	*	*	*
15	*C. gariepinus*	n	20	20	20	20	15	*C. gariepinus*	n	13	12	12	12
		Range	7-20	6-15	10-22	7-17			Range	5-61	8-58	13-269	12-130
		X±SD	11±3	9±2	12±2	10±2			X±SD	23±18	32±22	71±72	48±38
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	19	20	20	20
		Range	2-71	3-70	7-70	5-89			Range	3-11	4-34	2-14	5-21
		X±SD	41±21	35±20	35±19	33±23			X±SD	6±1	9±6	7±3	9±4
17	*C. gariepinus*	n	11	10	11	10	17	*C. gariepinus*	n	18	18	18	18
		Range	15-55	6-48	8-82	6-55			Range	4-262	5-31	14-158	4-52
		X±SD	30±12	19±11	25±24	26±13			X±SD	27±59	15±9	44±36	27±15
	O. mossambicus	n	20	20	19	16		*O. mossambicus*	n	20	20	20	20
		Range	9-610	8-107	15-721	4-858			Range	2-38	3-25	3-118	4-153
		X±SD	220±164	67±28	269±164	314±243			X±SD	15±13	13±8	33±30	39±43

Table 9.9: (Continued)

Localtiy	Specie		Nickel Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994						
7	*L. umbratus*	n	20	20	20	20
		Range	8-24	10-23	13-36	10-28
		X±SD	16±6	18±5	21±6	16±6
11	*C. gariepinus*	n	6	6	6	6
		Range	6-12	7-10	10-32	7-27
		X±SD	9±3	8±1	18±9	11±8
	L. umbratus	n	8	8	8	8
		Range	5-10	6-14	11-22	6-13
		X±SD	8±2	9±3	15±4	9±2
13	*L. umbratus*	n	20	20	20	20
		Range	6-34	6-33	6-38	7-30
		X±SD	15±9	16±8	16±10	16±8
14	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20
		Range	5-14	6-17	8-24	6-18
		X±SD	9±3	10±3	14±5	10±35
15	*C. gariepinus*	n	12	12	12	12
		Range	21-57	37-50	48-121	24-69
		X±SD	37±11	42±4	66±20	43±14
	O. mossambicus	n	20	20	19	20
		Range	26-47	22-42	42-69	26-74
		X±SD	32±5	32±5	55±6	44±14
17	*C. gariepinus*	n	20	20	19	20
		Range	7-15	7-10	13-91	6-35
		X±SD	9±2	8±1	37±19	17±9
	O. mossambicus	n	20	20	20	20
		Range	8-32	6-9	12-46	8-119
		X±SD	14±7	8±1	23±9	23±29

Locality	Specie		Nickel Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: NOVEMBER 1994						
7	*L. umbratus*	n	20	20	20	20
		Range	5-21	6-34	9-27	4-22
		X±SD	11±5	16±6	18±6	13-5
11	*C. gariepinus*	n	3	3	3	3
		Range	10-11	10-13	26-28	10-11
		X±SD	10±1	12±2	27±1	11±1
	L. umbratus	n	20	20	20	20
		Range	10-38	13-36	15-45	10-37
		X±SD	19±8	23±8	25±9	27±8
13	*L. umbratus*	n	17	17	17	17
		Range	5-82	5-82	14-85	9-79
		X±SD	33±27	33±21	40±23	31±20
14	*C. gariepinus*	n	14	14	14	14
		Range	7-23	6-24	21-182	9-34
		X±SD	14±5	14±6	71±48	17±7
	L. umbratus	n	3	3	3	3
		Range	7-14	9-14	12-14	7-19
		X±SD	10±3	11±2	13±1	14±6
15	*C. gariepinus*	n	5	5	5	5
		Range	4-5	3-7	9-19	5-7
		X±SD	4±1	5±1	12±3	6±1
	O. mossambicus	n	20	20	20	20
		Range	3-36	2-49	10-86	3-75
		X±SD	18±14	22±17	38±26	28±21
17	*C. gariepinus*	n	19	19	19	19
		Range	11-28	1-14	16-85	13-40
		X±SD	16±4	8±6	40±16	22±7
	O. mossambicus	n	20	20	20	18
		Range	17-42	17-18	21-113	17-183
		X±SD	23±7	18±1	48±22	64±49

Table 9.9: (Continued)

Locality	Specie		Nickel Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994						
7	*L. umbratus*	n	20	20	20	20
		Range	12-160	13-115	16-69	17-84
		X±SD	70±57	36±28	28±16	38±21
14	*C. gariepinus*	n	13	13	13	13
		Range	5-40	5-54	11-76	6-50
		X±SD	20±11	23±15	40±23	27±15
	L. umbratus	n	20	20	20	20
		Range	15-86	14-91	16-105	13-103
		X±SD	31±20	31±20	39±24	39±22
13	*L. umbratus*	n	20	20	20	20
		Range	11-132	15-100	17-113	13-85
		X±SD	43±32	46±24	52±32	37±21
14	*C. gariepinus*	n	4	4	4	4
		Range	8-10	10-18	19-50	8-15
		X±SD	9±1	14±4	31±14	11±3
	L. umbratus	n	20	20	20	20
		Range	5-30	5-27	7-35	6-19
		X±SD	11±6	10±5	14±7	10±4
15	*C. gariepinus*	n	5	5	5	5
		Range	4-11	4-14	7-11	2-20
		X±SD	6±2	6±4	9±1	7±7
	O. mossambicus	n	20	20	20	20
		Range	8-15	10-110	15-31	9-25
		X±SD	11±1	18±21	22±3	16±4
17	*C. gariepinus*	n	4	4	4	4
		Range	9-24	13-15	18-55	33-93
		X±SD	15±6	14±1	32±15	66±29
	O. mossambicus	n	20	20	20	19
		Range	9-31	8-24	14-104	9-151
		X±SD	17±6	12+4	35±19	61±44

Locality	Specie		Nickel Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: MAY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	15-32	15-32	18-30	15-38
		X±SD	22±6	21±5	23±4	23±7
14	*C. gariepinus*	n	20	20	20	20
		Range	6-65	9-67	19-111	5-66
		X±SD	30±17	31±18	40±21	34±21
	L. umbratus	n	7	7	7	7
		Range	8-132	9-64	21-106	10-82
		X±SD	42±46	27±25	44±34	48±27
13	*L. umbratus*	n	19	19	19	19
		Range	17-53	21-50	20-39	17-68
		X±SD	34±12	32±10	28±5	38±14
14	*C. gariepinus*	n	3	3	3	3
		Range	8-40	9-43	13-160	14-18
		X±SD	24±16	21±19	32±21	16±2
	L. umbratus	n	7	7	7	7
		Range	13-43	11-49	13-58	8-64
		X±SD	21±12	24±15	34±19	35-22
15	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	O. mossambicus	n	20	20	20	20
		Range	4-13	7-19	10-23	6-23
		X±SD	9±2	11±2	18±3	14±4
17	*C. gariepinus*	n	17	17	17	17
		Range	6-17	9-16	16-95	11-69
		X±SD	11±3	12±1	47±22	31±15
	O. mossambicus	n	13	13	13	20
		Range	13-49	10-15	23-120	28-135
		X±SD	27±13	12±1	45±26	70±35
19	*O. mossambicus*	n	20	20	20	20
		Range	1-15	1-14	8-52	4-135
		X±SD	8±3	5±2	18±10	28±33
20	*C. gariepinus*	n	17	17	17	17
		Range	1-14	3-16	2-16	3-15
		X±SD	4±3	6±4	7±4	7±3
	O. mossambicus	n	20	20	19	20
		Range	2-5	2-10	3-12	3-46
		X±SD	4±1	4±2	7±2	12±9

* Data not available

Table 9.10: Manganese concentrations (ug/g dry mass) in the tissues/organs of fish from the selected sites in the Olifants River Catchment.

Locality	Specie		Manganese Concentration ug/g				Locality	Specie		Manganese Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*C. gariepinus*	n	10	10	10	10	7	*C. gariepinus*	n	*	*	*	*
		Range	3-9	4-9	24-78	3-13			Range	*	*	*	*
		X±SD	5±2	5±2	40±19	7±4			X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	20	20	20	20
		Range	2-6	2-6	77-162	11-30			Range	1-8	2-6	62-148	2-12
		X±SD	3±1	4±1	106±22	17±5			X±SD	4±2	3±1	87±20	7±2
11	*C. gariepinus*	n	10	10	10	10	11	*C. gariepinus*	n	20	20	20	20
		Range	1-24	1-3	6-81	5-10			Range	1-16	1-4	6-49	2-8
		X±SD	4±7	2±1	43±24	7±2			X±SD	3±3	2±1	29±12	4±2
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	20	20	20	20
		Range	2-8	2-8	9-94	4-82			Range	1-7	1-15	33-91	4-24
		X±SD	4±2	5±1	61±19	19±18			X±SD	4±2	4±3	59±14	12±5
14	*C. gariepinus*	n	20	20	20	20	14	*C. gariepinus*	n	*	*	*	*
		Range	2-8	3-6	17-138	7-14			Range	*	*	*	*
		X±SD	4±1	4±1	11±7	10±2			X±SD	*	*	*	*
15	*C. gariepinus*	n	20	20	10	20	15	*C. gariepinus*	n	13	12	12	12
		Range	2-5	1-8	21-106	5-13			Range	1-13	1-12	31-127	6-28
		X±SD	3±1	2±1	63±21	7±1			X±SD	5±3	6±4	68±36	14±7
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	19	20	20	20
		Range	1-18	1-8	38-131	1-104			Range	1-69	1-62	1-63	1-55
		X±SD	5±3	3±2	79±24	42±35			X±SD	21±23	23±23	21±25	16±19
17	*C. gariepinus*	n	11	10	11	10	17	*C. gariepinus*	n	18	18	17	17
		Range	2-8	1-7	5-56	2-9			Range	1-51	1-5	26-102	5-90
		X±SD	5±2	3±1	22±18	5±2			X±SD	6±11	3±1	54±20	15±19
	O. mossambicus	n	20	20	19	16		*O. mossambicus*	n	20	20	20	20
		Range	1-85	1-14	14-129	2-112			Range	1-27	1-11	5-50	3-54
		X±SD	28±21	9±3	62±28	45±32			X±SD	7±5	4±2	30±11	14±11

Table 9.10: (Continued)

Locality	Specie		Manganese Concentration ug/g				Locality	Specie		Manganese Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994							**SURVEY: NOVEMBER 1994**						
7	*L. umbratus*	n	20	20	20	20	7	*L. umbratus*	n	20	20	20	20
		Range	1-4	1-5	59-140	3-9			Range	2-9	2-16	53-105	35-92
		X±SD	2±1	4±1	107±25	6±2			X±SD	5±2	5±3	78±14	56±16
11	*C. gariepinus*	n	6	6	6	6	11	*C. gariepinus*	n	3	3	3	3
		Range	1-5	2-3	15-47	2-6			Range	8-52	5-6	11-87	5-18
		X±SD	3±2	2±1	25±12	3±2			X±SD	28±24	5±1	50±38	10±7
	L. umbratus	n	8	8	8	8		*L. umbratus*	n	20	20	20	20
		Range	2-5	2-5	51-108	3-7			Range	1-13	1-9	63-270	5-53
		X±SD	3±1	3±1	73±19	4±1			X±SD	6±3	5±2	118±50	14±12
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	17	17	17	17
		Range	3-52	2-26	43-101	5-39			Range	6-42	3-24	33-136	5-45
		X±SD	12±12	8±5	68±15	13±10			X±SD	16±11	9±6	72±23	18±12
14	*C. gariepinus*	n	*	*	*	*	14	*C. gariepinus*	n	14	14	14	14
		Range	*	*	*	*			Range	4-23	2-5	29-167	5-19
		X±SD	*	*	*	*			X±SD	9±5	3±1	85±39	10±4
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	3	3	3	5
		Range	1-3	1-5	21-65	3-5			Range	3-14	4-6	48-67	8-10
		X±SD	2±1	2±1	34±12	3±1			X±SD	7±6	6±1	59±10	9±1
15	*C. gariepinus*	n	12	12	12	12	15	*C. gariepinus*	n	5	5	5	5
		Range	5-21	6-17	40-106	8-26			Range	3-8	1-9	44-70	8-10
		X±SD	11±4	9±2	64±18	13±5			X±SD	5±1	4±2	62±10	9±1
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	5-11	4-9	44-105	7-17			Range	2-9	2-14	54-178	7-42
		X±SD	7±1	7±1	72±14	11±2			X±SD	6±2	5±3	91±31	20±10
17	*C. gariepinus*	n	20	20	19	20	17	*C. gariepinus*	n	19	19	19	19
		Range	2-17	1-8	38-102	4-29			Range	1-15	1-6	40-132	5-21
		X±SD	4±3	3±2	69±13	10±6			X±SD	4±3	4±1	73±23	10±3
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	18
		Range	2-90	1-7	3-37	3-50			Range	3-29	3-5	31-83	10-369
		X±SD	9±19	2±1	18±9	13±13			X±SD	9±7	4±1	52±17	54±85

Table 9.10: (Continued)

Locality	Specie		Manganese Concentration ug/g				Locality	Specie		Manganese Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995							**SURVEY: MAY 1995**						
7	*L. umbratus*	n	20	20	20	20	7	*L. umbratus*	n	20	20	20	20
		Range	13-27	4-22	70-192	9-57			Range	4-10	4-9	79-172	7-18
		X±SD	13±9	9±5	134±33	27±17			X±SD	6±2	5±1	109±22	12±3
11	*C. gariepinus*	n	13	13	13	13	11	*C. gariepinus*	n	20	20	20	20
		Range	2-9	1-10	35-133	2-19			Range	2-13	1-26	23-71	5-40
		X±SD	6±2	5±3	80±32	11±4			X±SD	7±3	8±5	40±12	11±7
	L. umbratus	n	20	20	20	19		*L. umbratus*	n	7	7	7	7
		Range	4-66	3-24	99-272	6-44			Range	2-11	2-7	71-93	4-17
		X±SD	13±14	9±6	167±53	23±25			X±SD	6±4	4±2	83±9	12±5
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	19	19	19	19
		Range	3-81	4-20	31-108	5-70			Range	17-53	21-50	20-39	17-68
		X±SD	15±17	9±5	59±19	19±15			X±SD	34±12	32±10	27±5	38±14
14	*C. gariepinus*	n	4	4	4	4	14	*C. gariepinus*	n	3	3	3	3
		Range	1-3	2-6	27-97	2-8			Range	2-8	1-6	13-29	4-5
		X±SD	2±1	4±1	59±29	5±3			X±SD	5±3	3±3	23±8	4±1
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	7	7	7	7
		Range	1-4	1-7	24-184	1-8			Range	3-17	1-6	31-48	4-21
		X±SD	2±1	3±2	60±41	4±2			X±SD	6±5	3±2	39±6	8±6
15	*C. gariepinus*	n	5	5	5	5	15	*C. gariepinus*	n	*	*	*	*
		Range	4-9	5-12	26-69	7-19			Range	*	*	*	*
		X±SD	7±2	7±2	42±15	11±5			X±SD	*	*	*	*
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	2-13	1-78	58-371	8-121			Range	1-5	2-4	32-85	3-19
		X±SD	6±3	7±16	179±69	43±23			X±SD	3±1	2±1	64±15	8±3
17	*C. gariepinus*	n	4	4	4	4	17	*C. gariepinus*	n	17	17	17	17
		Range	1-5	1-2	25-218	1-67			Range	1-7	2-5	43-125	4-18
		X±SD	3±1	1±1	83±89	25±29			X±SD	4±1	3±1	77±22	10±4
	O. mossambicus	n	20	20	20	19		*O. mossambicus*	n	13	13	13	13
		Range	1-10	1-3	12-79	1-130			Range	2-17	2-3	33-97	10-45
		X±SD	4±2	1±1	47±15	29±31			X±SD	6±4	3±1	53±19	26±11
							19	*O. mossambicus*	n	20	20	20	20
									Range	1-44	1-7	13-41	4-41
									X±SD	6±9	2±1	25±7	15±9
							20	*C. gariepinus*	n	17	16	17	17
									Range	1-7	1-5	6-69	2-38
									X±SD	2±1	2±1	48±14	7±8
								O. mossambicus	n	20	20	19	20
									Range	1-3	1-4	1-110	5-147
									X±SD	2±1	1±1	68±25	17±31

* Data not available.

Table 9.11: Lead concentrations (ug/g dry mass) in the tissues/organs of fish from the selected sites in the Olifants River Catchment.

Locality	Specie		Lead Concentration ug/g				Locality	Specie		Lead Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*C. gariepinus*	n	10	10	10	10	7	*C. gariepinus*	n	*	*	*	*
		Range	14-70	9-23	15-49	18-26			Range	*	*	*	*
		X±SD	11±4	14±4	28±11	13±6			X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20		*L. umbratus*	n				
		Range	4-11	4-21	15-23	5-18			Range	2-12	6-17	3-18	2-14
		X±SD	9±2	10±4	19±2	10±3			X±SD	5±3	12±2	7±4	7±3
11	*C. gariepinus*	n	10	10	10	10	11	*C. gariepinus*	n	20	20	20	20
		Range	4-7	4-8	6-91	4-15			Range	1-6	1-9	4-24	1-8
		X±SD	5±1	5±1	27±26	10±4			X±SD	3±2	5±2	9±4	3±2
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	20	20	20	20
		Range	2-10	4-12	1-16	5-28			Range	1-8	1-7	7-11	2-9
		X±SD	4±2	6±2	11±3	10±6			X±SD	4±2	4±2	10±1	5±2
14	*C. gariepinus*	n	20	20	20	20	14	*C. gariepinus*	n	*	*	*	*
		Range	5-10	8-10	10-34	5-11			Range	*	*	*	*
		X±SD	8±2	8±1	19±6	8±2			X±SD	*	*	*	*
15	*C. gariepinus*	n	20	20	20	20	15	*C. gariepinus*	n	13	12	12	12
		Range	2-17	4-14	11-27	6-12			Range	1-3	1-5	9-27	3-8
		X±SD	9±3	9±2	17±4	8±11			X±SD	1±1	2±1	16±4	5±1
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	19	20	20	20
		Range	2-18	3-16	6-20	2-16			Range	1-8	1-8	1-9	1-14
		X±SD	8±4	7±3	13±4	6±3			X±SD	2±2	2±2	4±2	4±3
17	*C. gariepinus*	n	11	16	11	10	17	*C. gariepinus*	n	17	17	18	18
		Range	1-13	1-15	2-22	1-7			Range	1-6	1-8	1-47	1-19
		X±SD	5±3	3±4	8±6	3±2			X±SD	2±2	2±2	16±9	3±4
	O. mossambicus	n	20	20	19	16		*O. mossambicus*	n	20	20	20	20
		Range	9-106	1-15	17-132	3-154			Range	1-5	1-4	2-19	1-13
		X±SD	41±28	9±3	55±29	54±43			X±SD	1±1	1±1	9±4	3±3

Table 9.11: (Continued)

Locality	Specie		Lead Concentration ug/g				Locality	Specie		Lead Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: AUGUST 1994							SURVEY: NOVEMBER 1994						
7	*L. umbratus*	n	20	20	20	20	7	*L. umbratus*	n	20	20	20	20
		Range	5-12	6-12	12-18	6-11			Range	1-7	1-7	8-16	2-6
		X±SD	7±2	7±1	15±2	9±1			X±SD	2±1	4±2	10±3	4±1
11	*C. gariepinus*	n	6	6	6	6	11	*C. gariepinus*	n	3	3	3	3
		Range	3-10	4-6	7-24	4-20			Range	3-4	3-4	19-27	2-5
		X±SD	6±3	5±1	15±7	8±6			X±SD	3±1	3±1	22±4	3±2
	L. umbratus	n	8	8	8	8		*L. umbratus*	n	20	20	20	20
		Range	4-7	5-8	11-21	6-9			Range	1-7	1-9	5-16	2-9
		X±SD	6±1	6±1	15±4	8±1			X±SD	4±1	4±3	11±3	5±2
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	17	17	17	17
		Range	2-16	3-18	9-20	1-16			Range	2-10	1-6	8-18	1-11
		X±SD	7±4	8±4	15±3	8±5			X±SD	5±3	3±1	12±3	5±3
14	*C. gariepinus*	n	*	*	*	*	14	*C. gariepinus*	n	14	14	14	14
		Range	*	*	*	*			Range	15-41	3-11	17-73	4-15
		X±SD	*	*	*	*			X±SD	6±3	6±3	39±18	8±3
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	3	3	3	3
		Range	4-12	6-15	10-27	5-12			Range	2-5	3-7	9-17	3-9
		X±SD	8±3	9±3	16±5	9±3			X±SD	3±1	4±2	10±1	5±4
15	*C. gariepinus*	n	12	12	12	12	15	*C. gariepinus*	n	5	5	5	5
		Range	6-18	9-19	27-59	9-21			Range	6-12	5-12	23-59	11-22
		X±SD	11±3	13±2	34±9	14±4			X±SD	9±1	10±2	35±14	17±3
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	6-13	5-13	18-31	7-17			Range	6-11	6-16	16-37	6-71
		X±SD	9±1	9±2	27±3	11±2			X±SD	8±1	10±2	27±4	14±14
17	*C. gariepinus*	n	20	20	20	20	17	*C. gariepinus*	n	19	19	19	19
		Range	10-38	4-5	9-52	3-18			Range	2-13	9-15	13-52	6-18
		X±SD	17±6	5±1	21±10	8±4			X±SD	5±2	11±1	30±11	11±3
	O. mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	19
		Range	2-40	5-6	9-26	2-57			Range	7-21	8-9	18-87	6-76
		X±SD	11±9	6±1	15±5	10±13			X±SD	11±3	9±1	47±20	25±19

Table 9.11: (Continued)

Locality	Specie		Lead Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	2-12	2-13	18-29	5-20
		X±SD	6±3	8±3	22±3	10±4
11	*C. gariepinus*	n	13	13	13	13
		Range	4-25	3-12	9-47	5-22
		X±SD	9±5	8±3	22±10	11±5
	L. umbratus	n	20	20	20	20
		Range	2-38	2-12	9-24	2-104
		X±SD	8±8	8±3	15±5	12±22
13	*L. umbratus*	n	20	20	20	20
		Range	4-13	5-16	13-26	2-18
		X±SD	8±3	9±3	21±3	10±4
14	*C. gariepinus*	n	4	4	4	4
		Range	6-8	10-16	15-48	8-9
		X±SD	7±1	11±3	31±13	9±1
	L. umbratus	n	20	20	20	20
		Range	4-15	4-12	9-45	4-11
		X±SD	8±3	8±2	15±8	8±2
15	*C. gariepinus*	n	5	5	5	5
		Range	6-11	6-14	15-22	7-27
		X±SD	8±1	10±2	19±2	13±8
	O mossambicus	n	20	20	20	20
		Range	6-17	6-188	16-29	5-17
		X±SD	9±2	14±22	23±3	10±3
17	*C. gariepinus*	n	4	4	4	4
		Range	7-19	11-17	22-35	28-78
		X±SD	12±5	14±2	31±5	51±21
	O. mossambicus	n	20	20	20	19
		Range	8-29	9-23	13-108	12-154
		X±SD	16±6	13±4	42±21	58±44

Locality	Specie		Lead Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: MAY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	2-7	2-18	7-26	1-6
		X±SD	4±1	6±4	12±5	4±1
11	*C. gariepinus*	n	20	20	20	20
		Range	1-10	2-13	4-14	1-9
		X±SD	5±3	6±3	10±3	5±3
	L. umbratus	n	7	7	7	7
		Range	4-38	5-24	5-69	11-45
		X±SD	14±12	12±8	32±37	21±12
13	*L. umbratus*	n	19	19	19	19
		Range	2-15	3-13	10-22	1-26
		X±SD	8±4	9±3	17±3	11±6
14	*C. gariepinus*	n	3	3	3	3
		Range	3-6	2-6	10-20	2-8
		X±SD	5±2	5±2	14±6	5±3
	L. umbratus	n	7	7	7	7
		Range	2-4	1-2	7-12	1-5
		X±SD	3±1	2±1	9±2	3±1
15	*C. gariepinus*	n	+	+	+	+
		Range	+	+	+	+
		X±SD	+	+	+	+
	O. mossambicus	n	20	20	20	19
		Range	2-11	5-15	12-24	4-15
		X±SD	7±2	10±2	20±3	11±3
17	*C. gariepinus*	n	17	17	17	17
		Range	3-13	1-13	16-75	5-33
		X±SD	6±2	7±2	38±16	18±9
	O. mossambicus	n	13	13	13	13
		Range	9-44	6-15	31-131	22-140
		X±SD	25±12	10±2	51±27	62±37
19	*O. mossambicus*	n	20	20	20	20
		Range	1-35	1-33	8-52	1-232
		X±SD	9±7	7±7	18±10	26±50
20	*C. gariepinus*	n	17	17	17	17
		Range	1-18	3-12	12-28	4-12
		X±SD	4±3	6±2	17±3	5±2
	O. mossambicus	n	20	20	19	20
		Range	2-6	1-10	5-20	3-43
		X±SD	4±1	4±2	13±3	12±9

* Data not available

Table 9.12: Chromium concentrations (ug/g dry mass) in the tissues/organs of fish from the selected sites in the Olifants River Catchment.

Locality	Specie		Chromium Concentration ug/g				Locality	Specie		Chromium Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*C. gariepinus*	n	10	10	10	10	7	*C. gariepinus*	n	*	*	*	*
		Range	12-32	11-23	14-23	13-24			Range	*	*	*	*
		X±SD	20±5	18±4	19±3	18±4			X±SD	*	*	*	*
	L. umbratus	n	20	20	20	20		*L. umbratus*	n	20	20	20	20
		Range	10-78	10-38	11-79	10-92			Range	5-21	6-25	7-21	6-19
		X±SD	29±24	14±7	26±21	28±25			X±SD	11±3	12±5	12±4	11±2
11	*C. gariepinus*	n	10	10	10	10	11	*C. gariepinus*	n	20	20	20	20
		Range	12-23	11-19	11-207	17-35			Range	6-16	8-20	10-27	6-62
		X±SD	16±4	14±2	50±59	27±8			X±SD	11±3	14±4	17±5	14±12
13	*L. umbratus*	n	20	20	20	20	13	*L. umbratus*	n	20	20	20	20
		Range	9-23	2-40	2-24	12-59			Range	13-33	14-40	14-37	14-32
		X±SD	14±4	14±13	17±5	27±13			X±SD	25±5	27±6	28±5	25±6
14	*C. gariepinus*	n	20	20	20	20	14	*C. gariepinus*	n	*	*	*	*
		Range	10-30	11-33	17-44	10-30			Range	*	*	*	*
		X±SD	19±7	16±6	28±8	19±5			X±SD	*	*	*	*
15	*C. gariepinus*	n	20	20	20	20	15	*C. gariepinus*	n	13	12	12	12
		Range	6-19	5-11	8-15	4-13			Range	6-119	8-102	12-498	12-199
		X±SD	11±3	9±2	10±2	8±2			X±SD	43±36	54±43	120±139	78±68
	O mossambicus	n	20	20	20	20		*O. mossambicus*	n	20	20	20	20
		Range	4-106	5-107	8-101	5-139			Range	5-15	3-32	2-10	4-41
		X±SD	63±33	51±29	50±29	49±35			X±SD	9±2	7±6	5±2	8±8
17	*C. gariepinus*	n	11	10	11	10	17	*C. gariepinus*	n	18	18	18	18
		Range	11-87	7-66	10-123	9-75			Range	3-448	3-44	10-251	1-75
		X±SD	44±21	26±16	46±34	36±18			X±SD	41±102	19±17	61±65	34±25
	O. mossambicus	n	20	20	19	16		*O. mossambicus*	n	20	19	20	20
		Range	26-844	7-160	10-1023	3-1110			Range	1-77	1-41	1-777	1-245
		X±SD	305±229	89±40	376±227	401±338			X±SD	22±25	18±15	46±52	58±76

Table 9.12: (Continued)

Locality	Specie		Chromium Concentration ug/g				Locality	Specie		Chromium Concentration ug/g			
			Skin	Muscle	Gills	Liver				Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1994							**SURVEY: MAY 1994**						
7	*L. umbratus*	n Range X±SD	20 12-34 20±8	20 12-34 22±7	20 14-32 22±6	20 11-35 20±7	7	*C. gariepinus*	n Range X±SD	20 9-36 19±10	20 11-37 22±7	20 11-57 26±15	20 9-39 20±10
11	*C. gariepinus*	n Range X±SD	6 11-20 14±4	6 10-11 11±1	6 15-44 25±12	6 8-41 16±13	11	*C. gariepinus*	n Range X±SD	3 28-32 30±2	3 24-30 26±3	3 51-59 54±4	3 22-30 27±4
	L. umbratus	n Range X±SD	8 9-16 13±3	8 9-22 13±4	8 13-28 18±6	8 7-14 10±2		*L. umbratus*	n Range X±SD	20 12-54 30±11	20 17-48 28±9	20 17-58 33±11	20 11-53 29±12
13	*L. umbratus*	n Range X±SD	20 11-49 24±12	20 11-52 20±11	20 11-48 24±12	20 12-31 20±8	13	*L. umbratus*	n Range X±SD	17 27-215 79±60	17 20-156 70±40	17 26-183 87±55	17 22-106 58±24
14	*C. gariepinus*	n Range X±SD	* * *	* * *	* * *	* * *	14	*C. gariepinus*	n Range X±SD	14 20-37 27±9	14 14-41 26±10	14 36-374 130±97	14 17-67 33±15
	L. umbratus	n Range X±SD	20 7-23 15±5	20 10-33 16 ±6	20 10-105 24±20	20 11-32 19±7		*L. umbratus*	n Range X±SD	3 14-25 20±5	3 19-26 23±4	3 23-29 26±3	3 15-46 30±9
15	*C. gariepinus*	n Range X±SD	12 36-111 66±20	12 60-92 73±9	12 73-201 107±36	12 30-105 62±21	15	*C. gariepinus*	n Range X±SD	5 9-22 16±4	5 11-26 19±5	5 15-71 31±22	5 22-28 24±2
	O. mossambicus	n Range X±SD	20 49-92 57±10	20 38-75 58±8	20 66-108 91±10	20 41-115 72±21		*O. mossambicus*	n Range X±SD	20 11-73 38±23	20 13-79 43±27	20 23-159 70±42	20 13-142 55±36
17	*C. gariepinus*	n Range X±SD	20 9-20 12±3	20 8-14 11±1	19 17-103 50±21	20 1-50 25±14	17	*C. gariepinus*	n Range X±SD	19 8-22 13±4	19 10-19 11±2	19 17-66 39±13	19 8-32 16±5
	O. mossambicus	n Range X±SD	20 5-27 11±5	20 9-13 11±1	20 16-75 34±17	20 9-183 35±46		*O. mossambicus*	n Range X±SD	20 15-38 21±6	20 14-16 15±1	20 18-88 37±16	19 8-116 38±31

Table 9.12: (Continued)

Locality	Specie		Chromium Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: FEBRUARY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	17-396	18-197	22-184	22-171
		X±SD	124±111	60±54	61±53	66±45
11	*C. gariepinus*	n	13	13	13	13
		Range	12-53	12-68	20-90	13-70
		X±SD	30±13	33±17	56±25	40±15
	L. umbratus	n	20	20	20	20
		Range	18-140	16-163	21-152	18-243
		X±SD	47±34	48±42	47±27	58±48
13	*L. umbratus*	n	20	20	20	20
		Range	19-222	21-168	21-185	19-146
		X±SD	68±61	65±43	69±52	45±34
14	*C. gariepinus*	n	4	4	4	4
		Range	18-21	20-32	45-100	18-26
		X±SD	19±1	28±6	62±13	22±3
	L. umbratus	n	20	20	20	20
		Range	8-56	11-50	14-65	16-34
		X±SD	25±9	22±9	28±13	23±6
15	*C. gariepinus*	n	5	5	5	5
		Range	13-19	11-23	3-19	8-31
		X±SD	15±2	15±4	9±6	14±9
	O. massambicus	n	20	20	20	20
		Range	15-29	19-205	24-51	15-45
		X±SD	21±3	31±40	36±5	28±7
17	*C. gariepinus*	n	4	4	4	4
		Range	12-31	14-17	18-78	41-106
		X±SD	19±8	15±1	38±27	78±30
	O. ossambicus	n	20	20	20	19
		Range	12-42	12-32	17-138	14-201
		X±SD	24±8	17±5	47±25	82±56

Locality	Specie		Chromium Concentration ug/g			
			Skin	Muscle	Gills	Liver
SURVEY: MAY 1995						
7	*L. umbratus*	n	20	20	20	20
		Range	37-76	37-69	39-67	36-86
		X±SD	53±13	49±11	54±9	54±14
11	*C. gariepinus*	n	20	20	20	20
		Range	13-89	6-109	21-164	21-111
		X±SD	56±22	56±28	70±31	59±25
	L. umbratus	n	7	7	7	7
		Range	21-153	22-90	32-118	27-114
		X±SD	59±47	49±28	67±31	68±28
13	*L. umbratus*	n	19	19	19	19
		Range	17-52	22-51	22-41	21-75
		X±SD	32±7	34±9	29±5	37±13
14	*C. gariepinus*	n	3	3	3	3
		Range	32-80	35-87	41-136	47-500
		X±SD	58±24	54±29	86±48	49±2
	L. umbratus	n	7	7	7	7
		Range	28-74	22-70	31-89	18-97
		X±SD	43±16	44±18	48±22	60±29
15	*C. gariepinus*	n	*	*	*	*
		Range	*	*	*	*
		X±SD	*	*	*	*
	O. mossambicus	n	20	20	20	20
		Range	5-24	7-27	9-38	6-29
		X±SD	10±5	13±6	17±8	14±6
	C. gariepinus	n	17	17	17	17
		Range	6-24	7-15	14-82	8-51
		X±SD	10±4	10±2	40±17	25±12
	O. mossambicus	n	13	13	13	13
		Range	12-42	6-14	17-82	12-120
		X±SD	23±10	11±2	37±18	56±31
19	*O. mossambicus*	n	20	20	20	20
		Range	1-5	1-6	1-11	1-28
		X±SD	1±1	1±1	3±3	2±6
20	*C. gariepinus*	n	17	17	17	17
		Range	1-4	1-7	3-7	1-3
		X±SD	1±1	2±1	4±1	1±1
	O. mossambicus	n	20	20	19	20
		Range	2-5	2-8	2-10	2-27
		X±SD	2±1	3±1	4±1	8±6

* Data not available

($p<0.05$), except occasionally between the muscle and skin. The gills of the fish contained the highest level of lead while the liver usually accumulated the second highest level (Table 9.11). Muscle and skin lead levels were usually lower but the order of importance varied between species localities as well as surveys. The bioaccumulation pattern of chromium was not clear, but it seems as if predominantly high concentrations were found in the gills followed by the liver, muscle and skin tissue of the selected species (Table 9.12).

Fish size and sex differences in metal bioaccumulation.

Where possible, only large adult fish were selected for tissue analysis (Table 9.4). This procedure was followed to obtain sufficient tissue mass (±5g wet mass) for analysis. The statistical analysis, therefore, only focused on a significant size range, and did not include all the size classes found in a natural population. Furthermore, in many cases the sample sizes were too small for individual analysis of the selected species at the different localities for each month, and had to be grouped. The results from these analysis showed that general trends were not evident and were dependent on species, size range, metal in question, as well as the locality. This is clearly shown by the data for levels in fish from different localities. The muscle and skin zinc concentrations of fish (*L. umbratus* and *C. gariepinus*) from localities 11 and 14 showed negative correlations with the size of the fish, thus the larger the fish, the lower the zinc concentrations in these tissues/organs. The zinc concentrations in the liver and gills showed a positive correlation, where an increase in one variable is associated with an increase in the other. The copper concentrations in the skin, muscle and liver of these fish also showed some significant negative correlations with the size of the fish. For both *Oreochromis mossambicus* and *C. gariepinus* from Localities 15, 17 and 20 no definite trend for zinc accumulation in different size groups was observed. However, the zinc concentrations in the one-year-old age group of *C. gariepinus* specimens were relatively higher than the rest of the age groups at Locality 15 and lower than the rest at locality 17. There was a slight (not significant) decrease in the levels of copper in the fish (Localities 15 and 17) as size increased but fish from Locality 20 showed the opposite trend. *Labeo umbratus* of a relative narrow size range (Table 9.4) from locality 13 showed no correlation with either tissue, zinc or copper. Although the trends were not the same, similar observations were made for other metals. It is therefore clear that each data set would reveal different relationships. Only a few significant differences in the bioaccumulation of the selected metal in the different tissues/organs of males and females were found. Copper, zinc and iron levels occasionally showed some correlations, while these were the least observed for lead, manganese, aluminium, iron and chromium concentrations. In general, sex seemed to play a lesser role in the levels of these metals in the selected tissue.

Species differences in bioaccumulation

Statistical analysis were only performed on data where species were sampled in sufficient numbers at a specific locality (Localities 11, 14, 15, 17). Zinc and copper bioaccumulation in the tissues/organs of *C. gariepinus* and *L. umbratus* from Localities 11 and 14 varied significantly in most cases. In May 1994 at Locality 11, significant differences in zinc concentrations for these species were found in the skin and gills, with *C. gariepinus* having higher levels of zinc in the skin, while *L. umbratus* showed higher levels in the gills. Significant differences were also found in the muscles and liver tissue, with

higher levels of zinc in both tissues of *C. gariepinus* in November 1994, February 1995, May 1995 at both localities. In May and November 1994 and February and May 1995 the copper liver and muscle concentrations were significantly higher in *L. umbratus* than in *C. gariepinus*. The copper gill concentrations were, however, higher in *C. gariepinus* in November 1994 at Locality 14. At locality 15 the level of copper in the combined organs/tissues of *C. gariepinus* was usually slightly higher than those of *O. mossambicus* for all surveys, except for summer 1995, when *O. mossambicus* accumulated significantly higher ($p<0.05$) levels of copper. The levels of zinc detected in the fish sampled at locality 15 did not indicate a specific trend for bioaccumulation between the two different species. At Locality 17 there was also no trend in copper accumulation between the two species. However, *O. mossambicus* sampled at Locality 17 accumulated significantly higher levels ($p<0.05$) of zinc during summer 1994, but had lower levels for the rest of the surveys. Generally, at both Localities 15 and 17, the highest levels of zinc were usually detected in *C. gariepinus*.

No definite trend could be established concerning the bioaccumulation of aluminium in the different tissues/organs between the species. For instance, the highest aluminium concentrations in the skin were found for *C. gariepinus* in May and November 1994, while *L. umbratus* accumulated more aluminium in the skin in May 1995 at Locality 14. *L. umbratus* also accumulated more aluminium in the muscle tissue in May and November 1994, but *C. gariepinus* showed higher levels of aluminium in the muscle in February 1995 at Locality 11. Higher concentrations of aluminium were found in the gills and liver of *L. umbratus* in May 1995 at Locality 11 whereas the highest concentrations in the gills in November 1994 at Locality 14 were found for *C. gariepinus*. The levels for aluminium detected in the tissues of *O. mossambicus* and *C. gariepinus* from either Locality 15 or 17 did not differ significantly.

In the case of iron, a trend could be established to some degree, as *L. umbratus* accumulated higher concentrations of the metal in the skin and muscle tissues in November 1994 at Locality 11 compared to *C. gariepinus*. Higher concentrations of iron were found in the gills of *C. gariepinus* in May and November 1994, but in May 1995 at Locality 11, the highest concentrations were found for *L. umbratus*. *C. gariepinus* accumulated more iron in the liver in November 1994, but higher concentrations were found in the liver in November 1994 at Locality 11 and February 1995 at Locality 11 for *L. umbratus*. When the iron levels detected in combined organ/tissue for all surveys were grouped and compared between different species, *C. gariepinus* contained higher levels than *O. mossambicus* at both Localities 15 and 17 (significantly higher at Locality 15). At both Localities 15 and 17, *C. gariepinus* also contained higher levels of iron than *O. mossambicus* during all surveys, except one. The only exception was observed during summer 1994 at Locality 15 when the opposite trend was observed.

Labeo umbratus accumulated significantly ($p<0.05$) more nickel in the skin, liver and muscle in November 1994 at Locality 11 than *C. gariepinus*, which accumulated more nickel in the gills in November 1994 at both localities. Nickel concentrations in the combined organs/tissues at localities 15 and 17 indicated that *O. mossambicus* accumulated significantly ($p<0.05$) higher levels of nickel than *C. gariepinus*. Multiple ANOVA of the nickel concentrations in different species, as well as surveys showed that *O. mossambicus* sampled at Locality 15 had accumulated significantly ($p<0.05$) higher levels of nickel during

three surveys i.e. summer and spring 1994 and summer 1995. Levels of nickel detected in *C. gariepinus*, however, were significantly higher during autumn 1994 and slightly higher during winter 1994. ANOVA of the Mn concentrations in the combined organs/tissues indicated that there was no significant ($p<0.05$) differences between the species at Locality 15. Manganese concentrations in the muscle, liver and gills of *L. umbratus* were mostly very high in comparison to those in the tissues/organs of *C. gariepinus* in May 1994 at Locality 11, November 1994 at Locality 14, February 1995 at Locality 11 and May 1995 at Locality 14. In May 1995 at Locality 11, however, the manganese muscle concentrations were higher for *C. gariepinus* than *L. umbratus*. At Locality 17 there was a significant ($p<0.05$) species difference between the manganese concentrations in the combined organs/tissues for all the surveys. *Oreochromis mossambicus* accumulated significantly higher levels of manganese during summer 1994 and 1995 and slightly higher levels during spring 1994 and autumn 1995. Manganese levels in *C. gariepinus* were, however, higher during the other two surveys undertaken.

Lead muscle and liver concentrations were higher in *L. umbratus* in May 1995 at Locality 11 but *C. gariepinus* accumulated more lead in the skin, gills and liver in November 1994 at Localities 11 and 14, February 1995 at Locality 14 and May 1995 at Locality 11, respectively. At Locality 15, the level of lead accumulated in the combined organs/tissues for all surveys indicated that *C. gariepinus* accumulated significantly higher levels of lead than *O. mossambicus*. Surveys examined separately indicated that *C. gariepinus* contained higher levels of lead than *O. mossambicus* during all surveys except summer 1995. The opposite trend was, however, observed at Locality 17, where *O. mossambicus* contained significantly higher levels of lead than *C. gariepinus*. Surveys examined separately indicated that *O. mossambicus* accumulated the highest level of lead during all surveys except winter 1994.

The only significant differences ($p<0.05$) found between *C. gariepinus* and *L. umbratus*, regarding the bioaccumulation of chromium in the different tissues/organs, were in the gills, with the highest concentrations found in *C. gariepinus* in November 1994 at Localities 11 and 14. At both Localities 15 and 17, *O. mossambicus* contained significantly higher chromium levels than *C. gariepinus* in combined organs/tissues for all surveys. Between survey analysis showed that the two species accumulated the highest levels at different times. No definite trend was thus observed.

Temporal variation

The data sets covered too short a period to establish long-term temporal trends. Temporal variation in metal accumulation by the fish occurred, but no overall trend could be established. As expected, seasonal patterns of accumulation varied between localities, and the metal or tissue in question. For instance, significant differences between seasons were found in the skin tissue of fish from Localities 11 and 14 for manganese, copper, and lead in the liver tissue and in the gill tissue for zinc. Strong significant differences were also found for copper and nickel in all the tissue types between autumn 1994 and summer/autumn 1995 and between winter 1994 and autumn 1995. On the other hand,

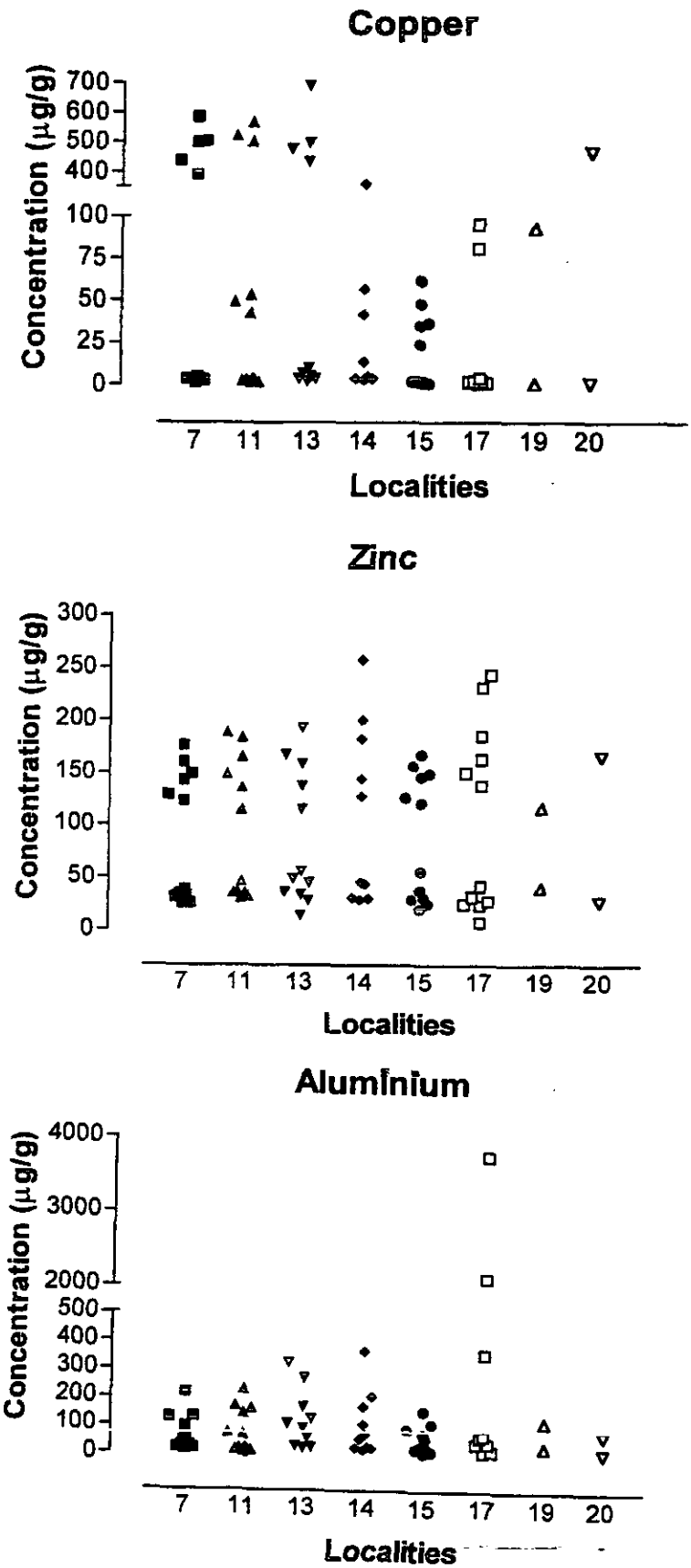

Figure 9.40: Highest and lowest mean copper, zinc and aluminium concentrations detected in the tissues/organs of fish during each survey from the selected localities.

seasonal trends for aluminium and iron accumulated by fish at Localities 15 and 17 as well as between metals. No specific trend could be established for seasonal bioaccumulation of these two metals. From the preceeding, it is therefore evident that seasonal variation was detected in some instances and sometimes not.

Spatial differences

The data from Localities 11 and 14 was statistically compared to give some indication of variation in metal levels in fish from the Klein Olifants River (Locality 11) and Olifants River (Locality 14). The data from Locality 15 (upper-catchment) were statistically compared to that of Locality 17, 19, 20 (lower catchment) to give some indication of differences related to these subcatchments.

From Figure 9.40 the highest mean copper concentration of the tissue in fish from Locality 14 and 15 are generally grouped at lower levels compared to Localities 7, 11 and 13. On two

occasions, fish from Locality 14 had higher mean zinc concentrations compared to Localities 7, 11 and 13 (Figures 9.40).

The copper concentration in the organs/tissues (combined as well as separate) for both species of fish, indicated that fish at Locality 17 accumulated significantly higher ($p<0.05$) levels of copper than the fish at Localities 15 and 19. The combined organ/tissue copper concentrations at Locality 20 (control) usually did not differ significantly ($p<0.05$) from those of other localities, except for *C. gariepinus* at Locality 17, which accumulated significantly higher levels than fish at Locality 20. However, muscle, gill and skin tissues indicated that fish at Locality 20 accumulated significantly lower levels of copper than fish at Localities 15 and 17 (Table 9.5). Liver tissue on the other hand indicated the opposite trends. For both species of fish, liver levels of copper detected at Locality 20 (control) were significantly higher ($p<0.05$) than those at Localities 15, 17 and 19. Zinc levels in combined organs/tissues showed no significant differences (p,0.05) between the fish sampled at different localities over the entire study period. *O. mossambicus* sampled at Locality 15 accumulated higher levels of zinc than those at Locality 17 did, whilst the zinc levels in the fish at Locality 17 and 19 were similar and the fish at Locality 20 had the lowest levels. Zinc levels detected in the combined organs/tissues of *C. gariepinus* did not show variation between the different localities sampled. The zinc content of both the muscle and skin tissues of fish sampled at Locality 15 were usually significantly higher ($p<0.05$) than those at Locality 17 (Table 9.6). Liver zinc concentrations at Locality 17 were, however, significantly higher ($p<0.05$) than those at Locality 15, and the gill zinc content was similar at both Localities 15 and 17. The muscle and gill zinc content of *O. mossambicus* sampled at Locality 19 was the highest of all localities and the skin and liver zinc content at Locality 19 was the lowest of all localities sampled. Generally, the muscle, skin and gill zinc content of both species was the lowest at Locality 20, except for the muscle tissue of *C. gariepinus* sampled at Locality 20 which had the highest zinc level of all localities (significantly higher than Locality 17). Similar to the trend observed in copper accumulation, *C. gariepinus* sampled at Locality 20 had the highest level of liver zinc content (significantly higher than Locality 15). *O mossambicus* at Locality 20 had

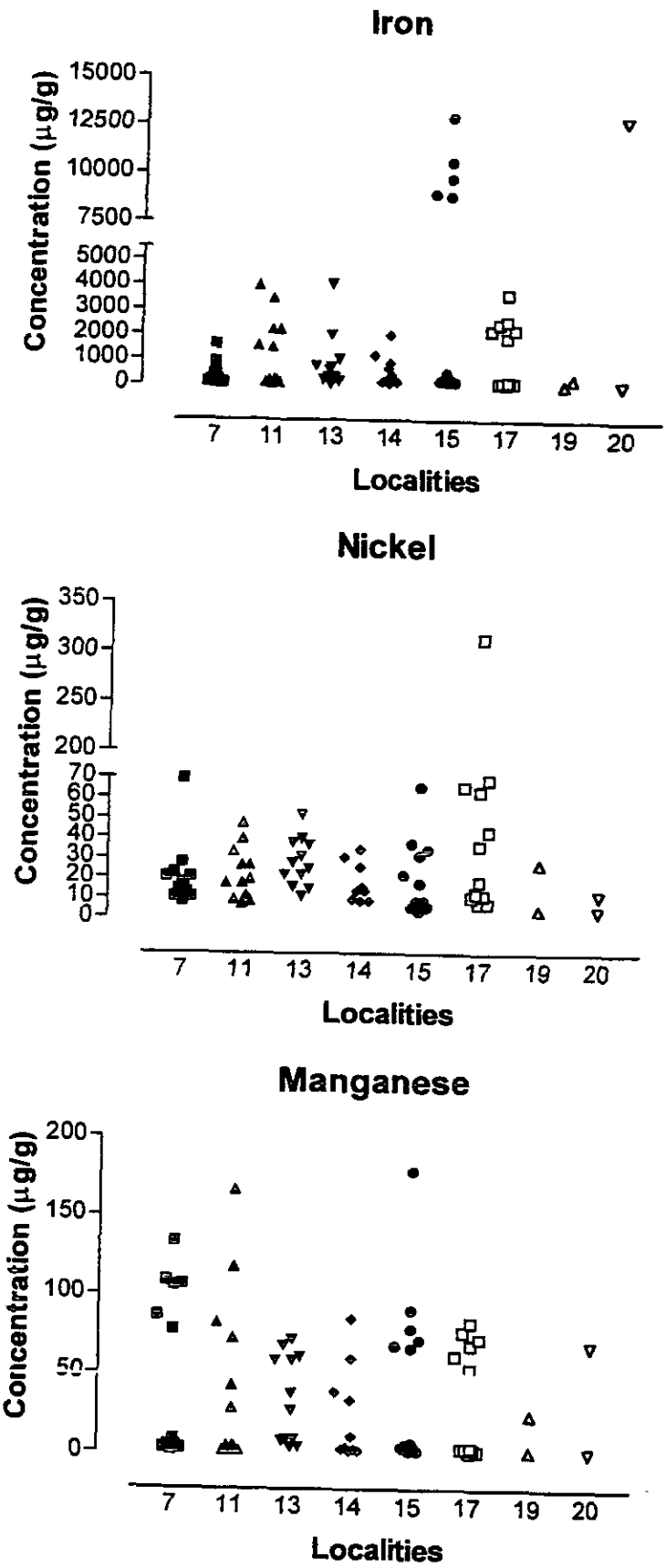

Figure 9.41: Highest and lowest mean iron, nickel and manganese concentrations detected in the tissues/organs of fish from the selected localities during each survey.

zinc levels in their livers slightly lower than those at Locality 17 and slightly higher than those at Locality 15 do.

The highest mean aluminium concentrations were detected in fish from Localities 13, 14 and 17 (Fig. 9.40). In May 1994, aluminium concentrations in the muscle and gills of *L. umbratus* were higher at Locality 14. However, *L. umbratus* from Locality 14 had accumulated high concentrations in the gills (February & May 1995) and in the muscle tissue (February 1995). The skin tissue of *C. gariepinus* captured during May 1995 at Locality 14 also had significantly higher aluminium ($p<0.05$) concentrations compared with concentrations at Locality 14 (Table 9.7). One-way ANOVA indicated that Locality 17 (Mamba Weir, KNP) was the most aluminium-polluted of all the localities where fish were sampled. Locality 19 (Phalaborwa Barrage) had significantly lower ($p<0.05$) levels of aluminium bioaccumulation than Locality 17, and significantly higher ($p<0.05$) levels than Locality 15 (Loskop Dam). In all cases, Locality 20 (Nhlanganini Dam) had significantly lower ($p<0.05$) levels of aluminium bioaccumulation when compared to all other localities.

High mean iron concentrations were detected in fish for Localities 11, 13, 15, 17 as well as from Locality 20 (Fig. 9.41). Fish from Locality 14 showed higher concentrations of the metal, only in May 1994 in the muscle tissue of *L. umbratus* and *C. gariepinus*, while Locality 11 showed higher levels of iron in February and May 1995 in the muscle, liver and skin tissue of *L.* umbratus (Table 9.8). *O. mossambicus* sampled at Locality 17 accumulated significantly higher levels of iron than those sampled at Locality 15. *C. gariepinus*, however, indicated that more accumulation of iron occurred at Locality 15 than 17, although the differences were not significant ($p>0.05$). Muscle, gill and skin tissues of both species at Locality 20 (control) were least impacted by iron. The level of iron detected in the liver of both species, however, was the highest at Locality 20. This caused the level of iron in the combined organs/tissues to be higher at Locality 20 than Locality 19. Fish at Locality 19, however, accumulated significantly lower levels of iron than those sampled at Localities 15 and 17.

The nickel (Fig. 9.41) concentrations were mostly in the same range at Localities 11 and 14. Significant differences between these localities for nickel concentrations were detected during February 1994 for *C. gariepinus* and in February 1995 for *L. umbratus* gill tissue. The levels of nickel detected in *O. mossambicus* at Locality 17 were significantly ($p<0.05$) higher than those at Locality 15. The levels of both Localities 17 and 15 were significantly higher than those at Locality 19, and Locality 20 was significantly ($p<0.05$) lower than all localities. In *C. gariepinus* the nickel concentrations in both the gill and liver tissues were significantly ($p<0.05$) higher at Locality 17 than at Locality 15, but the significantly higher levels of muscle nickel content at Locality 15 caused the combined organs/tissue concentrations to indicate Locality 15 as accumulating slightly higher levels of nickel than Locality 17. Nickel levels in *C. gariepinus* sampled at Locality 20 were also significantly ($p<0.05$) lower than all other localities. High manganese levels (Fig. 9.41) were usually detected in fish tissue from Localities 7, 11 and 15. No significant differences ($p>0.05$) were found for Localities 11 and 24 during May and November 1994 with regard to the manganese concentration in the tissues of the fish. Localities 11 and 14 differed Locality 11 while the concentrations of manganese in the muscle, liver, skin and gills were higher at Locality 11 in February 1995 (Table 9.9). Manganese levels detected at Locality 15 were

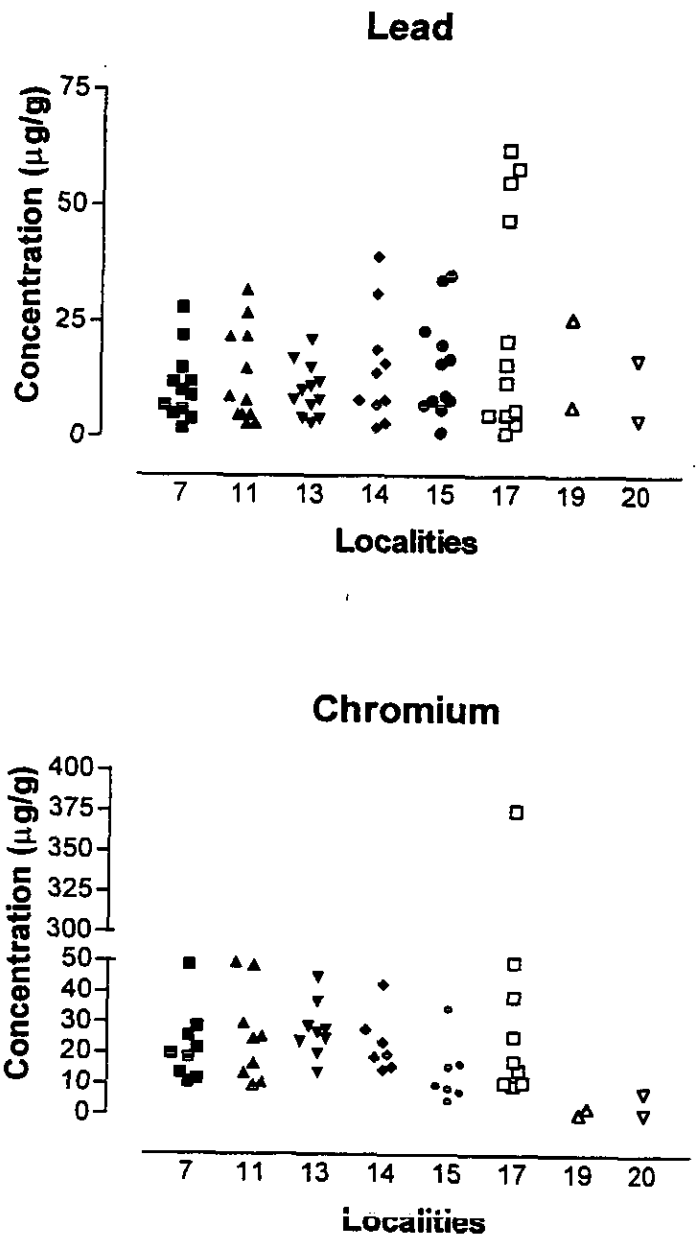

Figure 9.4 2: Highest and lowest mean lead and chromium concentrations detected in the tissues/organs of fish in the selected localities during each survey.

higher than those detected at Locality 17. The difference was significant for the combined tissues of *C. gariepinus*, gill tissue of *O. mossambicus* and muscle tissue of both species. The only exception was the skin tissue of *O. mossambicus* at Locality 17, which contained significantly ($p<0.05$) higher levels of manganese than those sampled at Locality 15. The general picture, however, indicates that fish sampled at Locality 15 were exposed to higher levels of manganese than fish from Locality 17. Manganese levels detected at Locality 19 were always lower than those at Locality 17 (significant for combined organs/tissues and skin tissue separate). The level of manganese detected in the combined organs/tissues of both species at Locality 20 (control) was significantly ($p<0.05$) lower than those at Localities 15 and 17. Different organs/tissues indicated the same trend. Muscle and skin tissues of *O. mossambicus* sampled at Locality 19 accumulated significantly higher manganese levels than those at Locality 20 (Table 9.10).

The highest lead levels were detected in fish from Localities 14, 15 and 17 (Fig. 9.42). No significant differences were found for Localities 11 and 14 during May and November 1994 with regard to lead concentrations in the fish tissues. There were significant locality differences with regards to the lead concentrations in the skin and muscle and the gills of *L. umbratus* in February 1994 with higher values at Locality 14 and in May 1995, where the highest concentrations were found at Locality 11 in *C. gariepinus*. The highest lead concentrations in the liver and gill tissue were found in February and May 1995 at Locality 11 in *C. gariepinus*. The levels of lead detected in combined organs/tissues indicated different spatial bioaccumulation trends between different species. *O. mossambicus* at Locality 17 (Mamba weir, KNP) indicated significantly ($p<0.05$) more lead pollution than at Locality 15 (Loskop Dam). *C. gariepinus* , however, indicated the opposite trend with fish at Locality 15 accumulating significantly higher levels of lead than those at Locality 17 did. Levels of lead detected in *O. mossambicus* sampled at Locality 19 (Phalaborwa Barrage) were significantly lower than those detected at Locality 17. Lead concentrations in *O. mossambicus* were the lowest at Locality 20 (control), significantly lower than all other localities. Levels of lead detected in *C. gariepinus* at Locality 20 were significantly lower than those at Locality 15 and slightly lower ($p>0.05$) than those at Locality 17.

Mean chromium concentrations in fish from most of the localities (Fig. 9.42) showed some high values during some surveys. Significant differences between Localities 11 and 14 in chromium levels were detected in the gills, muscle and liver of *L. umbratus* (February 1995) and skin and liver (August 1994) for *C. gariepinus*. Chromium levels detected in different organs and tissues clearly indicated similar spatial bioaccumulation trends. Concentrations of chromium detected in the combined organs/tissues of both *O. mossambicus* and *C. gariepinus* did not differ significantly between Localities 15 and 17. The levels of chromium detected in the gills of fish sampled at Locality 17 were, however, in both cases significantly higher than those detected at Locality 15. *O. mossambicus* sampled at Locality 19 accumulated significantly lower levels of chromium than those sampled at Locality 17. Chromium levels detected at Locality 20 (control) were significantly lower than those for *O. mossambicus* and *C. gariepinus*, detected at Localities 15 and 17. *O. mossambicus* sampled at Locality 19 accumulated significantly lower levels of chromium than fish sampled at Locality 20.

Metal bioaccumulation in tissues/organs

The majority of studies on metal concentrations in aquatic organisms, emphasise bioaccumulation of several metals in different tissues/organs of different species of fish (Bezuidenhout *et al.* 1990; du Preez & Steyn, 1992; Seymore *et al.* 1995, 1996a,b; Van den Heever & Frey, 1994; Du Preez *et al.* 1997). When fish are exposed to elevated levels of metals in a polluted aquatic ecosystem like the Olifants River (Du Preez & Steyn, 1992; Seymore *et al.* 1995, 1996a,b; Du Preez *et al.* 1997), they tend to take these metals up from their direct environment. It is assumed that most metals are taken up in the ionic form and that this uptake is influenced by various environmental factors such as pH and temperature. The metals enter the body of the fish via the gills and skin, or through the intake of contaminated food or drinking water. Transportation of metals in the fish occurs through the blood where ions are usually bound to proteins. The metals are thus brought into contact with the organs and tissues of the fish and consequently accumulated to a different extent in different organs/tissues. All the metal taken up is not accumulated because fish have the ability to regulate their body metal concentration to a certain extent. Excretion of metals can occur through the gills, bile (via faeces), kidney and skin (Romanenko *et al.*, 1986; Heath, 1987). Different metals are therefore accumulated at different concentrations in the various organs and tissues of fish. This phenomenon was also observed for the selected tissues (skin, muscle, gills and liver) in this study. The differences in the levels of accumulated in the different organs/tissues of a fish can primarily be attributed to the differences in the physiological role of each organ. Regulatory ability, behaviour and feeding habits are other factors that could influence the accumulation differences in the different organs. The metal concentration in the liver (not in direct contact with the metal in the water) which plays a major role in detoxification as well as storage, would therefore differ from the concentrations detected in the gills and skin tissue (in direct contact with the metals in the water) which play a role in the uptake and excretion of the metal. It is therefore important to select more than one tissue when assessing the metal levels in fish. In the present study the selected tissues from a specific fish produced low as well as high values and seem to be good representative organs/tissues to be used in field bioaccumulation monitoring. Muscle tissue should be sampled to test its fitness for human consumption. Skin tissue may be replaced by vertebral which, in general, appear to accumulate higher levels of some metals (Van Vuren *et al.* 1994).

Size, sex and species differences in bioaccumulation.

The relationships between the bioaccumulation of metals and the size of the selected species were not consistent and were either positive or negative with increasing size of the fish. However, in many cases these relationships were not significant. The decrease in metal concentrations with an increase in fish size, can be related to new tissues being incorporated at a greater rate than metals can be actively transported into the tissues to establish a steady state concentration dilution by growth (Cross *et al.* 1973). The usually higher levels of metals in younger fish can be related to the relatively higher oxygen consumption due to increased metabolic processes amongst juvenile fish. Increased metabolism in the younger fish brings them into contact with contaminants to a greater extent than is the case with older fish as more water is flushed over their gill surfaces. The elevated rate of metabolism causes toxins to be taken up and accumulated at a greater rate than in adult fish. Another

hypothesis to explain the phenomenon that younger fish usually contain higher concentrations of contaminants than older specimens of the same species, is that of dilution with growth. This refers to a situation where adult fish incorporate more tissue than juvenile fish at a faster rate than the metals can actively be transported into the tissues. This can cause younger fish to have higher levels of contaminants than older fish exposed to the same environmental conditions (Seenayya & Prahalad 1987; De Wet *et al.* 1994). An increase in metal levels with size can possibly be attributed to the physiological requirements for survival that dictate the maintenance of certain levels of elements. If no relationships are found, the concentration could be due to less rapid growth which allows a steady state of metal uptake and elimination from the tissues as would be the case in adult fish. It should also be noted that fish sampled were generally adults and very few young specimens were included. These results are thus an indication of the variation in metal concentrations between different age groups of post-juvenile fish. Nevertheless, the present data and that of previous studies (De Wet *et al.* 1994) indicate that the size of fish must be considered, especially when dealing with juveniles compared to adult fish. An accurate evaluation would be possible if a definite size range of fish is captured at the same time. This would avoid destructive sampling as performed by De Wet *et al.* (1994) which collected 388 fish to establish the relationship between metals and fish size.

Generally, no significant differences were found between the males and the females with regard to metal bioaccumulation in the difference tissues/organs of the selected fish species. In fish, differences in accumulation by sexes seem to occur only in certain organs (gonads) and during certain stages of their reproductive cycles (e.g. spawning). Zinc, for instance, is necessary for gonadal development of fish. Dietary zinc sources are, however, not adequate during this period and alternative internal resources such as the liver, skin, muscle and vertebrae are then utilised. Furthermore, zinc is also deposited in the gonads during their development and then lost during spawning. On the other hand, males do not require such a high zinc concentration, resulting in a more stable zinc concentration in the body (Seymore *et al.* 1996a). In order to obtain more detailed information with regard to the accumulation of metals in the different sexes, it is suggested that the gonads of both sexes should be examined, together with the tissues such as liver, gills and vertebrae. However, the developmental stage of gonads should also be considered as this may greatly influence the data. For this reason, the use of the gonads as general monitoring tissue, is questionable.

A relationship between metal concentrations in fish and the trophic level of the species studied, is suggested, indicating the importance of the food pathway. Mathis & Cummins (1973) found that significantly higher concentrations of zinc were present in omnivorous fish. Thus, the feeding patterns of *C. gariepinus*, which is omnivorous and catches living prey and eats any organic material, including aquatic weeds, detritus, as well as fish, birds, frogs, small mammals, reptiles, snails, crabs, insects and plant material, are important (Van der Waal, 1972; Pienaar, 1978; Skelton, 1993). *O. mossambicus* feeds primarily on plant material and detritus (Deacon, 1988). Juveniles take many small crustaceans but mainly feed on algae, especially unicellular diatoms. Larger specimens feed on filamentous green algae and adults also ingest aquatic insects, crustaceans, earthworms, small fish as well as bottom sludge rich in organic matter. *Labeo umbratus*, on the other hand, feeds on soft sediment and organic material (Skelton, 1993). Aquatic plants, algae and invertebrates can accumulate metals (Van der Merwe *et al.* (1990); Du Preez *et al.* (1993); Steenkamp *et al.* 1993; 1994a,b,c; Hellawell 1986; De Wet *et al.* 1990) and would therefore be a possible source of metals to their predators, for example, to the species selected in the present study.

Large amounts of silt are also taken in with the food sources by these species, and especially by *L. umbratus*. Silt particles play an important role in the transportation and availability of metals as they are adsorbed onto the silt particles (Von Ayfer 1977; Giesy & Wiener, 1977; Ward *et al.* 1986; Heath, 1987). Elevated metals were also detected in the sediment samples collected during this study. Most metals are furthermore known to become more bioavailable and have increased toxicity with decreasing pH (Shaw & Brown, 1973). The pH in the stomach of *O. mossambicus* decreases significantly (from above 6 to as low as 2.9) after feeding commences (Deacon, 1988). This reduction in pH could thus result in metals becoming more bioavailable from the food sources and silt in the stomach. The metals could therefore be taken up via the intestine, causing increased metal levels in the fish that could result in bioaccumulation. Biomagnification of metals is thus also a possibility in this aquatic ecosystem but an investigation will have to be undertaken to evaluate the metal concentrations and their subsequent release in the different food sources of fish. These factors may well play a definite role in the differences of the metal levels accumulated by the species selected.

Spatial differences in metal bioaccumulation.

Analyses of metal bioaccumulation in the fish collected during this study clearly indicate locality differences (Tables 9.5 to 9.13; Figs. 9.40 to 9.42). Fish from the two sites in the Klein Olifants River (Localities 13 & 14) had similar metal levels, although fish from Locality 13 occasionally had higher copper, iron and nickel levels, while fish from Locality 14 had high zinc, manganese and lead levels. Significant differences between Localities 11 (Olifants River) and 14 (Klein Olifants River) with regard to the bioaccumulation in the fish, were recorded. In general, fish at Locality 11 accumulated more copper, aluminium, iron, nickel, manganese, lead and chromium. No definite trend would be established for the zinc levels. These differences indicate that anthropogenic activities (upstream of Locality 11 (Olifants River) have a higher impact on the fish metal levels than the activities upstream of Locality 14 (Klein Olifants River). Point sources of pollution upstream of Locality 11 may be effluent from the industries or the combined sewage treatment works in the Witbank area. Point as well as diffuse sources from agriculture and especially mines would also contribute to eleviated levels in system: for example, metal levels in the Suurstroom (Locality 8) and Spook Spruit (Locality 10) both of which receive effluent from mines before flowing into the Olifants River before it reaches this locality. At Locality 14 the sewage treatment works upstream of this locality seem to be a major contributor of metals to the system during the drier period and low flows. For example, *L. umbratus* accumulated zinc in the liver at Locality 14 in February and May 1995, which coincides with the higher zinc concentrations in the water at Locality 14 in those months due to the direct input of point source pollution from the combined sewage purification works located upstream from the locality and less rainfall and the low flow of the river, thus concentrating these pollutants. The lower zinc concentration at this locality for *C. gariepinus* can be attributed to the accumulation of zinc by the liver to the maximum concentration and the consequent regulation thereof by the liver. The higher zinc concentration in the liver was also found for *C. gariepinus* in treated sewage water in a study regarding the differences in bioaccumulation of zinc and copper between different tissue types in dam water and treated sewage water (Van den. Heever & Frey, 1994). The copper skin and muscle concentrations were the highest for *C. gariepinus* at Locality 14 in February 1994. In August 1994, the copper liver and muscle concentrations were the highest at Locality 11

and for *L. umbratus* the highest at Locality 14 in May 1995. It must, however, be stressed that depending on biological factors, (e.g. tissue., specie) or environmental factor (e.g. temperature, river flow) fish from Locality 14 would have higher levels than Locality 11.

Analysis of metal bioaccumulation in the fish collected during this study indicates that the fish from Locality 17 (Mamba Weir) had higher copper, aluminium and iron concentrations than fish from Locality 15 (Loskop Dam). For these metals, Locality 17 is therefore more polluted than Locality 15. The zinc levels in the combined tissue of *C. gariepinus* showed no variation between these localities, but those for *O. mossambicus* indicated Locality 15 as being slightly higher ($p>0.05$) than Locality 17. This trend is due to the high muscle and skin zinc levels detected at Locality 15. Liver zinc concentrations of fish at Locality 17were , however, usually significantly higher than those of Locality 15. Manganese concentrations in the fish indicate Locality 15 to be more impacted by manganese than Locality 17. There were no definite indications by the fish tissue levels, as well as by the water concentrations that either both at Locality 15 or 17 were the most polluted by lead or chromium. Gill tissues of both species, however, contained significantly higher chromium levels at Locality 17 than at Locality 15. The levels of chromium detected in the sediment samples collected from Locality 17 also generally contained higher levels of chromium than those from Locality 15. The gills are able to accumulate metals from the sediment and therefore seem to indicate this difference in sediment chromium levels between the two localities. *Oreochromis mossambicus* sampled at Locality 19 (Phalaborwa Barrage) accumulated significantly ($p<0.05$) lower levels of the metals than the fish from Locality 17, which is approximately 15 kilometres downstream. This difference could be ascribed to the influence of the Selati River, a highly polluted tributary of the Olifants River, which converges with the Olifants River before Locality 17. Studies (CSIR 1990; Seymore *et al.* 1994) have shown that the poor quality water of the Selati River can have a negative impact on the Olifants River downstream, especially during low flows.

The control site (Locality 20 - Nhlanganini Dam) should have indicated metal levels in a natural and "unpolluted" system. This was, however, not always the case. Copper levels detected in the water and sediment were relatively high when compared to the other localities. It has to be stressed that these findings were based on the data of a single water and sediment sample. The higher copper levels at Locality 20 could, however, be due to natural causes such as geological impacts and also polluted ground water. Furthermore, the area of this locality is known to be harshly impacted by air pollution from the mining industry in the Phalaborwa area and very high levels of copper depositioning have been recorded in the area (Dr. D. Grobler, pers. comm., 1996). There were, however, some definite differences between Locality 20 and the rest of the sites sampled. The combined organs/tissues of *C. gariepinus* sampled at Locality 20 accumulated the lowest levels of copper, significantly lower than those of Locality 17. Muscle, gill and skin tissues of both species sampled at Locality 20 had significantly lower levels of copper than at Locality 15 and 17, but copper and zinc levels in the liver tissue of fish sampled at Locality 20 were usually significantly higher ($p<0.05$). This could possibly indicate that fish at Localities 15 and 17 have been chronically exposed to these metals and thus have had to regulate their levels to a much greater extent than fish at Locality 20. When fish are exposed to elevated metals, they will strive to detoxify their bodies of the pollutant as effectively as possible. It is possible that they will over-compensate and thus decrease the metal concentration in their liver (detoxifying organ) to a level lower than those usually detected in unpolluted

situations. From the preceding and the fact that data was only once obtained from Locality 20, it is clear that the data from this site must be used with caution.

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10 Experimental

10.1 The effect of selected metals on the haematology, osmoregulation and metabolism of *Oreochromis mossambicus*

A geologist treats all metals as being natural, but to a biologist some are more natural than other is (Coombs, 1980). Under normal circumstances, metals which are mainly beneficial, indeed essential, such as copper and zinc, may become pollutants when present in excess by exhibiting toxic effects on organisms (Davenport, 1985; Tort *et al*, 1987b; Mason, 1997). The distribution of metals, overland and sea, is not uniform and as a consequence living organisms have adapted to these conditions as well as to the availability of certain metals. Human interventions have, however, resulted in a redistribution of metals. With the result that localised areas of high concentrations are found, not surprisingly, closely situated to areas of high industrial activity (Coombs, 1980; Haslam, 1990). Human destructive influence on the aquatic environment is in the form of sublethal pollution, which results in chronic stress conditions, which have a negative effect on aquatic life.

Selye (1950) defined stress as: "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force". However, a better definition was given by Esch and Hazen (1978) as: "the effect of an environmental alteration of force that extends homeostatic or stabilising process beyond their normal limits, at any level of biological organisation". But Wedemeyer and McLeay (1981) suggested that a stress factor, or stressor, is an environmental change that is severe enough to require a "physiological" response on the part of a fish, a population, or an ecosystem. Adaptation to the stressor will occur, if the stress response can re-establish a satisfactory relationship between the changed environment and the fish, population, or ecosystem.

When a mammal is subjected to almost any kind of stress, it exhibits a generalised group of physiological responses (Heath, 1987). Firstly, a rapid elevation in adrenaline and noradrenalin which mobilises muscle glycogen into blood sugar, causes blood pressure to rise, and in general causes the body to undergo the "fright of flight" response. Secondly, a stage of resistance during which adaptation occurs. And thirdly, a stage of exhaustion if adaptation is lost because the stress was too severe or long lasting. This was collectively termed by Selye (1973) as the general adaptation syndrome (GAS). Although it is not universally agreed that GAS occurs in fishes, it is widely accepted that the stress response as a whole is characterised by physiological changes. These physiological changes tend to be similar for stressors as varied as anaesthesia, fright, forced swimming, disease treatments, handling, scale loss, or rapid temperature change (Wedemeyer and McLeay, 1981). Thus, the stress response of fish is analogous in many ways to that occurring in higher vertebrates.

The effects of pollutants on fish are evaluated by acute and chronic toxicity tests. The methods for acute toxicity tests are standardised in many cases and the final evaluation is described in terms of the incipient LC_{50} or lethal threshold concentration (Sprague, 1969; 1970; 1971; 1973; Rand and Petrocelli, 1985; Van der Merwe, 1993; Svobová *et al.*, 1994; Nussey *et al.*, 1996). These tests are based solely on the mortality of fish. However, normal physiological processes are affected long before the death of an organism and because death is too extreme a criterion for determining whether a substance

is harmful or not, scientists had to search for physiological and biochemical indicators of health and sublethal toxicant effects (Zachariassen *et al.*, 1991). It was therefore suggested that haematology, behaviour and biochemical changes, growth rate and oxygen consumption of fish can also be used in determining the toxicity of a pollutant during chronic toxicity tests (Fujiya, 1965; Alderdice, 1967; Sprague, 1971; 1976; Wedemeyer and Yasutake, 1977; Wedemeyer and McLeay, 1981; Nussey, 1994).

A good biological indicator should be able to identify environmental problems before the health of aquatic organisms are seriously compromised or altered (Adams, 1990; Jimenez and Stegman, 1990). During the last few decades haematology, the study of blood and blood forming tissues, has become such a biological indicator (Table 1) for ecologists and physiologists (Smit and Hattingh, 1979; 1980; Wedemeyer and McLeay, 1981; Heath, 1987).

In resent years haematological variables were used more often when clinical diagnosis of fish physiology was applied to determine the effects of sublethal concentrations of pollutants. Haematology can be considered as an essential index to the general health status in a number of fish species. It has been illustrated that the use of haematological variables as indicators of stress, toxic substances as well as metals (Table 2), can provide information of the physiological response fish make to a changing external environment. This is the result of the close association, the circulatory system has with the external environment (Casillas and Smith, 1977). Fish live in closest possible contact with their environment, they are extremely dependent upon it and are affected by changed in it (Blaxhall, 1972). Fish could thus be used as an early "warning system" to indicate the presence of pollutants in natural water.

Fish respond to copper or zinc, show haematological changes in ammonia levels, antibody titters, haematocrit values, haemoglobin levels, glucose concentrations, number of white and red blood cells, plasma salt levels, protein concentrations as well as changes in other constituents concentrations. Sometimes changes seem to be long-lived, whilst other times changes are temporary. Experimental differences in water quality, species, purpose, experimental design and copper or zinc concentrations often determine the outcome of a particular study and accentuate the difficulty of interpreting the data (Sorensen, 1991). Nevertheless, some published information seems especially relevant to an understanding of copper (Table 3) and zinc (Table 4) induced effects on fish haematology.

Table 10.1: Physiological function, interpretation and physiological significance of alterations in clinical variables of fish (Larsson *et al.*, 1985).

		Possible physiological significance	
Clinical variables	**Physiological function**	**Low values**	**High values**
Haematology			
Haematocrit (Hct)	Oxygen carrying capacity of blood	Anaemia; haemodilution due to gill damage or impaired osmoregulation	Polycythaemia due to stress; haemoconcentration due to gill damage
Haemoglobin (Hb)	*Oxygen carrying capacity of blood*	*Anaemia; haemodilution due to gill damage or impaired osmoregulation; nutritional diseases*	Polycythaemia due to stress; haemoconcentration due to gill damage
Red blood cell counts (RBC)	Oxygen carrying capacity of blood	Anaemia; haemodilution due to gill damage or impaired osmoregulation	Polycythaemia due to stress; haemoconcentration due to gill damage
Mean cell haemoglobin concentration (MCHC)	Status of RBC; reflects the supply of constituents for the Hb synthesis	Hypochromic anaemia; haemodilution	Polycythaemia due to stress; haemoconcentration due to gill damage
Mean cell haemoglobin (MCH)	Status of RBC; reflects the supply of constituents for the Hb synthesis	Microlytic, hypochronic anaemia, often associated with iron deficiency	Macrocytosis
Mean corpuscular volume (MCV)	Status of RBC; RBC size; reflects a normal or abnormal cell division during erythropoiesis	Shrunken RBC due to hypoxia, stress or impaired water balance; microlytic anaemia	Swollen RBC due to impaired water balance (osmotic stress); macrolytic anaemia
Erythrocytic ALA-D activity	Hb synthesis	Inhibited erythropoiesis; lead poisoning	Stimulated erythropoiesis
Lymphocytes	Immune defence; antibody production, cell mediated and humoral immunological response	Lymphopaenia due to acute stress or impaired production of new cells in blood forming tissues	Lymphocytosis due to bacterial or virus infections or cell necrosis; acute bleedings
Neutrophilic granulocytes	*Immune defence; first defence barrier against* bacteria; fagocytosis; inflammatory responses	*Neutropaenia due to impaired production of new* cells in blood forming tissues	*Neutrophilia due to bacterial infections,* inflammations or cell and tissue damage; acute stress; severe exercise
Thrombocytes (spindle cells)	Bloodclot formation	Thrombocytopaenia due to chronic stress or certain infections	Thrombocytosis due to acute stress (e.g., asphyxia), acute blood loss, inflammatory conditions

Table 10.1: (continued)

Metabolism			
Liver somatic index (LSI)	Metabolism	Starvation or nutritional imbalance	High metabolic activity; induced mixed function oxidise system; sexual maturation (females)
Liver glycogen	Carbohydrate metabolism	Acute or chronic stress; inanition	Liver damage due to excessive vacuolisation; dietary imbalance
Muscle glycogen	Carbohydrate metabolism	Acute or chronic stress; inanition	Dietary imbalance
Blood glucose	Carbohydrate metabolism	Hypoglycemia due to inanition, chronic stress or low liver glycogen content; impaired kidney function; hormonal imbalance	Hyperglycaemia due to acute stress or anoxia; acute liver damage; hormonal imbalance (e.g., insulin deficiency)
Blood lactate	Carbohydrate metabolism	No recognised significance	Acute or chronic stress; swimming fatigue
Total proteins in blood plasma	Transport of substances otherwise insoluble in blood; colloid osmotic pressure; blood clotting; immune defence (immunoglobulins)	Haemodilution; inanition; nutritional imbalance; kidney damage; certain liver damages; infectious diseases	Haemoconcentration; impaired water balance

Table 10.1: (continued)

Osmotic and ionic regulation			
Muscle water content	Osmotic and ionic regulation	Disturbed osmotic balance	Impaired water balance due to acute stress, kidney damage, increased water permeability of gills and skin
Plasma sodium and chloride	Osmotic and ionic regulation; maintenance of osmotic pressure in body fluids; acid-base balance	Haemodilution due to disturbed osmotic balance; impaired active ion uptake due to damaged gills; impaired ion retention in renal tubules	Haemoconcentration due to disturbed water balance
Plasma potassium	Ion regulation; muscle cell function	Impaired active ion uptake via the gills or the intestine; impaired renal ion retention	Haemolysis; tissue damage
Plasma calcium	Ion regulation; membrane permeability; muscle and nerve cell function; skeletal bone metabolism; blood coagulation	Kidney damage (impaired tubular reabsorption); impaired intestinal uptake	Tissue damage; sexual maturation (females)
Plasma magnesium	Ion regulation; metabolism; muscle cell function	Disturbed kidney function	Renal failure (impaired excretion); sexual maturation; anoxia
Plasma inorganic phosphate	Skeletal bone metabolism; energy metabolism	Disturbed kidney function; nutritional imbalance	Acute stress; disturbed kidney function (phosphate retention)

Table 10.2: Haematological and osmotic changes that occur in fish, after exposure to copper.

Species	Exposure time	Exposure level ($mg.l^{-1}$)	Effects	Reference
Pseudopleuronectus americanus	29 days	0.56-3.20	Cu-induced haemolytic anaemia - low haemoglobin, reduced haematocrit and/or decreased number of erythrocytes.	Baker (1969)
Salvelinus fontinalis	6-21 days	0.0382-0.0692	A moderate potentiation of haemopoiesis. Increases in the number of erythrocytes, haemoglobin, plasma glutamic oxalacetic transminase (PGOT), haematocrit (at 6 days only) and total protein (6-21 days).	McKim *et al.* (1970)
Ictalurus punctatus	46 hours	2.5-5.0	Decreased osmolality, followed by a return to normal levels.	Lewis and Lewis (1971)
Notemigonius crysoleucas	46 hours	2.25-5.0	Ionic content of whole blood is decreased below 230mOsm.	Lewis and Lewis (1971)
Ictalurus nebulosus	6,30 & 600 days	3.4-104.0	Increases in haemoglobin concentration and haematocrit.	Christensen *et al.* (1972)
Trichogaster trichopterus	96 hours	0.009	Antibody production is decreased. Breakdown of antibodies, interference with complement production, alteration of protein synthesis, cytotoxic effects on the haemopoietic centers (e.g., spleen), or hematological changes.	Roals and Perlmutter (1977)
Liza marolepis	96 hours	0.11-1.80	Marked eosinophilia.	Helmy *et al.* (1978)
Salmo trutta	38 weeks	0.29	Elevated serum concentrations, reduced haematocrit and weight loss. Immune response is suppressed, especially the secondary response (immune memory cells are suppressed)	O'Neill (1981)
Cyprinus carpio	38 weeks	0.29	Total suppression of immune response (secondary and plateau peaks) and a significant decrease in the primary response	O'Neill (1981)
Mystus vittatus	4,24,100 & 720 hours	5.0	Fish under considerable stress. Increases in haematocrit, erythrocytes and mean corpuscular volume.	Singh and Singh (1981)
Salmo gairdneri	24 hours	0.301	Acute exposure induces leucopaenia, majority of which is the result of lymphocytopaenia. Chronic exposure induces neutrophilia. Prolonged exposure causes anaemia, either by haemolysis or interference with haemoglobin metabolism, the trend towards decreased erythrocytes with acute exposure is most likely the result of stress-induced haemodilution.	Dick and Dixon (1985)
Salmo gairdneri	12 hours	0.0125-0.050	Loss of Na^+, Cl^- and K^+, and increases in plasma glucose and ammonia. Causes mixed inhibition of Na^+-,K^+-ATPase.	Lauren and McDonald (1985)
	12 hours	0.200		
Clarias lazera (juveniles)	96 hours	3.20	All blood samples showed some haemolysis. Significant decreases in erythrocytes, haematocrit and haemoglobin. The anaemia reported is therefore of the haemolytic type, possibly due to the effect of Cu on membrane ATPase, the glycolytic enzyme in the erythrocytes and glutathione.	El-Domiaty (1987)
	48 hours	2.0	Haemolysis, causing decreases in erythrocytes and haematocrit. Changes suggest a process of erythrocyte swelling as shown by an increase in MCV value. Swelling is a consequences of factors like high PCO_2, high lactate concentration or low PO_2 in blood leading to a low ATP concentration which would increase oxygen affinity of the blood. Decrease in the leucocrit.	Tort *et al.* (1987b)
	48 hours	4.0,6.0,8.0& 16.0	Decreases in haematocrit, haemoglobin and erythrocytes as well as in leucocrit and glucose. These changes strongly suggest haemodilution as a consequence of an increase of water content in blood vessels. Reductions in glucose concentrations.	

Table 10.2: (continued)

Salmo gairdneri	24 hours	0.0065	Decreases in plasma levels of Na^+ and a transient effect on Ca^{2+} homeostasis. Inhibition of Na^+, -K^+-ATPase.	Reid ad McDonald (1985)
Oreochromis mossambicus	4 weeks	0.1 and 0.2	Haemodilution, resulted in swelling of erythrocytes combined with release erythrocytes from the erythropoietic organs. This is a mechanism, which reduces the concentration of an irritating factor in the circulatory system.	Cyriac *et al.* (1989)
Barbus Puntius) conchonius	8 weeks	0.190	Prolonged exposure resulted in a consistent polycythaemia accompanied by a marked decrease in the haemoglobin and haematocrit. Haemolytic anaemia. Cu intoxication caused an increase in erythrocytes with a concomitant reduction in mean cell haemoglobin concentration, defective erythropoiesis would seem to be the most logical explanation.	Gill *et al.* (1991)
Clarias gariepinus	96 hours	0.05 & 0.085	Hyperglycemia. Increases in leucocytes. Haemodilution caused by anaemia. Histological damage to gills, liver and kidneys. Damage includes vacuolation and disintegration of renal tubule epithelium, dilatation of glomeruli, and internal haemorrhage.	Van Vuren *et al.* (1994)
Oreochromis mossambicus	96 hours 96 hours	0.16 0.40	Increases in leucocytes and erythrocytes, acidity, Mean corpuscular volume, haemoglobin and haematocrit. Haemodilution. Decreases in erythrocytes through haemolysis and fish become anaemic, decreases in the haematocrit, haemoglobin and mean corpuscular volume. Increases in leucocytes.	Nussey *et al.* (1995b)

Table 10.3: Haematological and osmotic changes that occur in fish, after exposure to zinc.

Species	Exposure time	Exposure level ($mg.l^{-1}$)	Effects	Reference
Salmo gairdneri	7 days	40.0	Arterial oxygen tension as well as the pH of arterial blood decreased precipitously. Decreases were also noted in oxygen uptake and heart rate. Changes in blood osmotic concentration and in blood levels Na^+, K^+, Ca^{2+}, Mg^{2+} were slight	Skidmore (1970)
Ictalurus punctatus	46 hours	8.0,12.0 & 30.0	Reduction in blood serum osmolarity, which can result in mortality. Osmotic drop is principally related to damage to the head gill area of the fish.	Lewis and Lewis (1971)
Notemigonius cryscleucas	46 hours	8.0,12.0 & 30.0	Reduction in blood serum osmolarity, which can result in mortality. Osmotic drop is principally related to damage to the head gill area of the fish.	Lewis and Lewis (1971)
Salmo gairdneri	21 hours	1.43	pH of arterial blood decreases, an indication of lactic acid and carbon dioxide accumulation.	Burton *et al.* (1972)
Fundulus heteroclitus	2 weeks	10.0	Increases in delta-aminolevulinate dehydrase activity (ALA-D) after chronic exposures.	Jackim (1973)
Salmo gairdneri	48 hours	0.02,0.15,0.69, 1.37 & 1.51	Decreases are noted in the rate of arterial oxygen tension (arterial hypoxia) and pH of arterial blood as well as oxygen uptake and heart rate. Zinc also causes some reduction in the amount of oxygen available to fish tissue which causes tissue hypoxia.	Sellers *et al.* (1975)
Salmo gairdneri	12 hours	40.0	Increases in respiratory frequency and decreases in heart frequency.	Hughes and Adeney (1977)
Colisa fasciatus	90 hours	100.0	Decreases in erythrocytes (erythrocytopaenia), haematocrit and leucocytes (leucopaenia).	Mishra and Srivastava (1979)
Salmo trutta	38 weeks	1.06	Suppression of the immune response.	O'Neill (1981)
Cyprinus carpio	38 weeks	1.06	Suppressed primary response and elevated antibody titters.	O'Neill (1981)
Salmo gairdneri	24 hours	40..0	Haemoglobin of the blood in the dorsal aorta is deoxygenated, probably due to the cytological damage to the gills. Haemolysis occurred in the blood vessels	Kodoma *et al.* (1982)
Mystus vittatus	4,24,100 & 720 hours	60.0	Fish exposed to Zn were under considerable stress and this was reflected by the changes marked in the blood variables especially when put to lethal doses. Increases in haematocrit, red blood cells and mean corpuscular volume.	Singh and Singh (1982)
Salmo gairdneri	96 hours	1.5	Hypoxia caused acidosis. Arterial oxygen tension decreased precipitously. The ensuing hyperaemia necessitated a dependence upon lycolysis as demonstrated by the rise in blood lactate concentration. Changes in other blood variables were generally consistent with acidosis. The maintenance of haematocrit, despite the decrease in erythrocytes, was due to erythrocyte swelling as shown by the decrease in mean cell haemoglobin concentration. Haemoconcentration. Decreases in Cl^- and pH.	Spry and Wood (1984)
Scyliorhinus canicula	24 hours	80.0	Increases in leucocyte and leucocrit as well as erythrocytes. Decreases in haemoglobin, mean cell haemoglobin, mean cell haemoglobin concentration and glucose concentration.	Torres *et al.* (1986)
Tilapia zilli	96 hours	22.0	Significant increases were observed in liver and serum proteins, serum alkaline phosphatase (ALP), erythrocytes, haematocrit and haemoglobin concentrations. Zn exposure reduced liver and serum acid phosphatase (ACP) as well as liver alkaline phosphatase (ALP).	Hilmy *et al.* (1987)
Clarias lazera	96 hours	32.0	Zn exposure reduced liver and serum acid phosphatases (ALP) as well as liver and serum proteins, serum alkaline phosphatase (ALP), erythrocytes, haematocrit and haemoglobin concentrations.	Hilmy *et al.* (1987)

Table 10.4: Effects of aluminium and low pH on the behaviour, reproduction, physiology and haematology of different fish species

Fish	Concentration & pH	Effect	Reference
Salvelinus fontinalis	0 µg/l pH 4 - 4.5	Death due to electrolyte loss	Wood, Playle, Simons, Goss & McDonald, 1988
	0 µg/l pH 4.9 & 5.5	Reduced growth	Siddens, Seim, Curtis & Chapman, 1986
	0 µg/l pH 5.2	Increase in plasma cortisol Increase in blood glucose Proliferation and hypertrophy of chloride cells Lifting of epithelium away from basal lamina Influx of WBC in lymphatic spaces of 2E lamellae Vacuolation and degeneration of pavement epithelial cells	Meuller, Sanchez, Bergman, McDonald, Rhem & Wood, 1991
	0 µg/l pH 6.1	Hypoxia	Wood, Playle, Simons, Goss & McDonald, 1988
	47 µg/l + pH 4.97	Decreased sodium and osmolality Decrease in feeding behaviour Decrease in growth Abnormal vitellogenesis	Mount, Hockett & Gern, 1988
	75 - 150 µg/l + pH 5.2	Damaged gills (lesions extensive and severe) Proliferation of mucus cells throughout gill filament and secondary lamellae Increase in chloride cells Hyperplastic primary filament and secondary lamellae	Meuller, Sanchez, Bergman, McDonald, Rhem & Wood, 1991
	228 µg/l + pH 4.4	Hypertophy of chloride cells Hyperplasia of chloride cells Hypertrophy of pavement epithelial cells Vacuolation & degeneration of chloride and outer epithelial cells	Ingersoll, 1990
	239µg/l + pH 4.4	*Reduction in feeding* Loss of equilibrium Increased activity Excessive mucus secretion Changes in skin pigmentation Reduced growth	Ingersoll, 1990
	333 µg/l + pH 4.8	Increase in ventilation Decrease in mean arterial HbO_2 Increase in $PaCO_2$	Walker, Wood & Bergman, 1991
	333 µg/l + pH 5.2	Acute hyperventilation	Walker *et al.*, 1991

Table 10.4: (Continued)

Fish	Concentration and pH	Effects	Reference
Salmo gairdneri	0 µg/l pH 4.0	Reduction in oxygen transport Loss of sodium & chloride ions	Neville, 1985
	0 µg/l pH 4 - 4.5	Increase in heart rate Increase in mean arterial blood pressure Increase in haematocrit Plasma acidosis Reduction in plasma ions Redistribution of body water Haemoconcentration causes large increase in blood viscosity	Milligan & Wood, 1982
	0 µg/l pH 4.5	Continued coughing indicates harmful irritant	Neville, 1985
	1.6 µM + pH 6.1	Aluminium gradually detected as an irritant	Neville, 1985
	2.8 µM + pH 4	Toxicity of acid reduced rather than increased as aluminium accumulated on gill tissue	Neville, 1985
	2.8 µM + pH 4.5	Severe tissue damage Major disturbances in oxygen uptake efficiency and ionoregulation	Neville, 1985
	2.8 µM + pH 5	Impaired oxygen uptake Increased ventilation rate Slight loss of electrolytes due to increased permeability of gill epithelium	Neville, 1985
	2.8 µM + pH 6.1	Impaired oxygen uptake despite tremendous increase in ventilation effort	Neville, 1985
	2.8 µM + pH 6.5	Gradually developing, mild, ventilatory response	Neville, 1985
	33.3 µM + pH 5	Severe electrolyte loss	Neville, 1985

Table 10.4: (continued)

Fish	Concentration & pH	Effects	References
Oncorhynchus mykiss	0 µg/l + pH 4.7	Increase in blood glucose Decrease of chloride ions Decrease of osmolality	Brown, MacLatchy, Hara & Eales, 1990
	60 µg/l +pH 5.0	Increase in haematocrit Decrease in plasma sodium concentration Increase in plasma cortisol Severe blood acidosis Decrease in blood PO_2 Increase in epinephrine & norepinephrine Elevated levels of glucose	Witters, Puymbroeck & Vanderborght, 1991
	27 µg/l +pH 5.2	Severe decline in chloride ions Increase in protein concentration Increase in haematocrit	Reid, McDonald & Rhem, 1991
Morone saxatilis	0 µg/l + pH 5 & 5.5	100 % mortality within 7 days	Buckler, Mehrle, Cleveland, & Dwyer, 1987
	100 µg/l + pH 6.5	97 % mortality at day 7 Extensive loss of sodium and potassium ions Plasma acidosis Erythrocyte swelling Increase in haematocrit Decrease in capacity for O_2 transport	Buckler *et al.*, 1987
Salmo salar	235 µg/l + pH 5.0	Died within period of 80 hours Mortality increased with increasing temperature Higher ventilation frequency Loss of plasma chloride and sodium Increase in haematocrit concentration (1, 6 & 10 EC) At 6 EC, the mean plasma osmolality increased	Poléo & Muniz, 1993

10.2 Materials and methods.

10.2.1 Choice of tests organism.

Thousands of chemical substances are used for mining, industrial, horticultural, forestry, domestic and agricultural activities and their number increase annually (Davenport, 1985; Hellawell, 1986; Abel, 1989; Lloyd, 1992). To determine the ecological status of a stream or river, it is important to consider an effective and reliable test organism. According to Hellawell (1986) almost any species can be used as a test organism, but since our knowledge of the autecology of the majority of species is minimal, and even if it was not the case, our resources are limited, we must select those organisms which are potentially most useful for the particular problem in hand. In selecting test organisms for environmental protection the following attributes may be particularly desirable (USEPA, 1976; 1979; Rand and Petrocelli,1985; Hellawell, 1986; Lamberson and Swartz, 1988):

- a test organism must be part of a food chain which can influence people or any other important species;
- during toxicology tests it is preferable to use indigenous species of the ecosystem that may receive impact (these organisms are acclimatised to specific condition where information on pollutants of a certain area must be obtained;
- species representing a broad range of sensitivities should be used whenever possible, since sensitivities vary among species;
- a test organism must readily be identified, because taxonomic uncertainties can confuse data interpretation;
- a test organism must be tested effortlessly, without using expensive equipment and must not be labour-intensive;
- a sufficient number of test organisms of the same size and age must be available for tests;
- test organisms must also be available throughout the year and not be too expensive;
- test organisms should be widely available and species with a cosmopolitan distribution should be considered;
- abundant autecological data must be available on a test organism, so that the data from a test may be more easily interpreted;
- species that are of commercial, ecological, economic or recreation importance should be included;
- species should be amenable to routine maintenance in the laboratory and they should be easily cultured in the laboratory so that chronic toxicity can be conducted;
- there must be a degree of repeatability, when a test organism is chosen;
- in a biological community a test organism must show a low variability on genetic and niche level; and
- an area's ecological balance cannot be disturbed when the test organism is removed from its natural habitat.

Fish are a measure of environmental health, because everything that happens on the landscape goes into rivers. For many years fish have been valued as excellent indicators of water quality (Solbé, 1979; Hellawell, 1986; Mason, 1991). Fish have also been the most popular test organism because they are presumed to be the best understood organisms in the aquatic environment and they are perceived as most valuable by many researchers (Buikema *et al.*, 1982; Haslam, 1990; Water Research Commission, 1994).

The Mozambique tilapia, *Oreochromis mossambicus* (Figure 1), was chosen as test organism for the experimental part of this project because of its wide distribution in river systems throughout South Africa (Skelton, 1993).

10.2.2. Obtaining, transportation, general holding system and laboratory conditions

Oreochromis mossambicus specimens were obtained from the University of Zululand, KwaZulu-Natal, South Africa. Fish were transported to the aquarium (laboratory) at Rand Afrikaans University (RAU), in a 1 000-litre plastic transport tank filled with borehole water. The water in this tank was aerated with compressed air. Mortalities during transport did not exceed 0.3%.

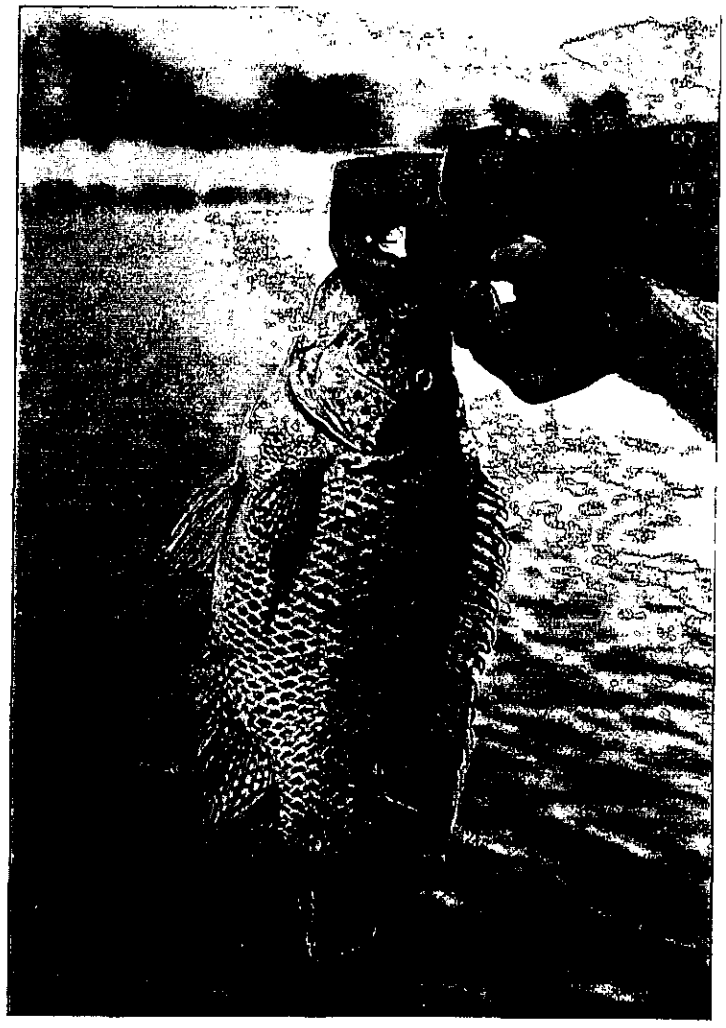

Figure: 10.1 The Mozambique tilapia, *Oreochromis mossambicus*.

At the aquarium the fish were transferred to a recirculating system. Which consisted of two, 1 000 litre plastic reservoirs and a biological filter (Figure 2), filled with borehole water. The physico-chemical characteristics of the borehole water are compiled in Table 5. The water in these reservoirs was oxygenated by means of compressed air and air-stones, and a third thereof was replaced weekly. The recirculating system comprised of PVC fittings to discount any toxic effects copper, galvanised plumbing or brass fitting may have (Nussey *et al.*, 1995b). On arrival fish underwent a weeklong daily infection treatment with coarse salt. Fish were not fed for 72 hours after arrival, to minimise stress-induced mortalities (Carmical *et al.*, 1984). Healthy fish were allowed to acclimatise at a temperature of 23±1°C, in the recirculating system, for three months and fed daily on commercial trout pellets (protein = 39.9%; lipid = 5.3%; ash = 9.6%; carbohydrate = 45.2%; energy = 22.8kJ/g). Temperature was controlled by a thermostat and photoperiod was regulated with an electric timer to produce 12:12 hour day:night, because day-length can influence metabolism and behaviour of fish (Grobler *et al.*, 1989).

Table 10.5: Physico-chemical characteristics of borehole water, as determined by the Institute for Water Quality Studies (IQWS).

VARIABLE	VALUE
PH	7.20
Temperature (°C)	23±1
Ammonium (NH_4, $mg.l^{-1}$)	0.04
Nitrogen as $NO_3 + NO_2$ ($mg.l^{-1}$)	1.96
Fluoride (F^-, $mg.l^{-1}$)	0.50
Total alkalinity as $CaCO_3$ ($mg.l^{-1}$)	49.00
Sodium (Na^+, $mg.l^{-1}$)	4.00
Magnesium (Mg^{2+}, $mg.l^{-1}$)	8.00
Silicon (Si, $mg.l^{-1}$)	10.10
Phosphate (PO_4, $mg.l^{-1}$)	0.023
Sulphate (SO_4, $mg.l^{-1}$)	17.00
Bicarbonate (HCO_3, $mg.l^{-1}$)	63.00
Chloride (Cl^-, $mg.l^{-1}$)	6.00
Potassium (K^+, $mg.l^{-1}$)	1.90
Calcium (Ca^{2+}, $mg.l^{-1}$)	12.00
Conductivity (Ec, Ms/m)	15.60
Total hardness as $CaCO_3$ ($mg.l^{-1}$)	79.00
Total dissolved solids (TDS, $mg.l^{-1}$)	117.00
,Sodium absorption rate (SAR)	0.30

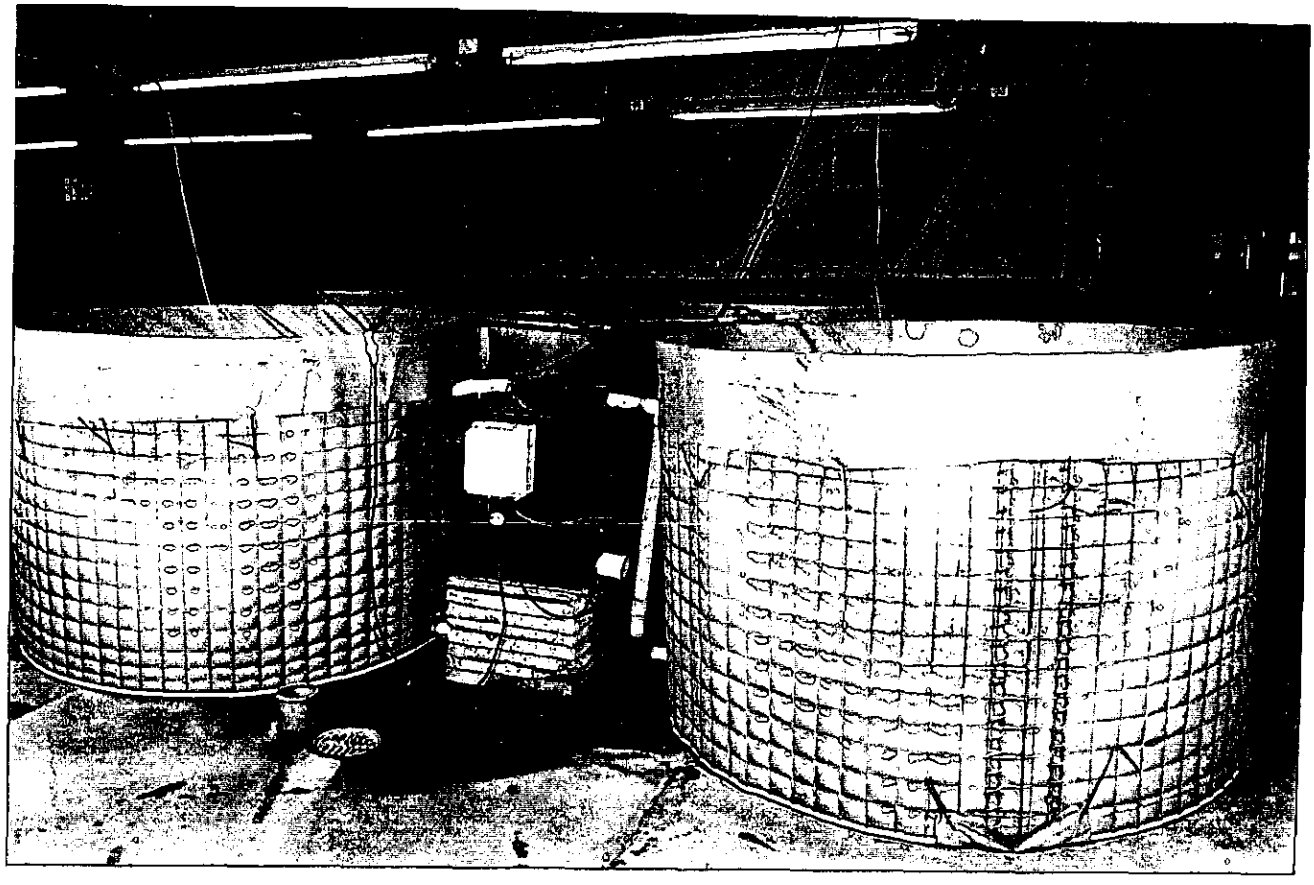

FIGURE 10.2: General holding system (Recirculating system).

After the initial acclimatisation period (three months) the fish were transferred from the general holding system (Figure 2) to the tanks of an experimental flow-through system (Figure 3). Buikema *et al.* (1982) and Rand and Petrocelli (1985) discussed the advantages and disadvantages of such a system.

During this study five flow-through systems (Figure 3, 4 and 5) were used. The design of the exposure system was based on the systems used previously and modified to allow for a constant water replacement rate in the exposure tanks (Coetzee, 1996; Barnhoorn, 1997). Each system (Figure 3) consisted of four, 100 litre experimental glass tanks (1A-!D). Water was pumped by means of a submergible electric pump (2) from the supply tank (1a). The supply tank (1a) was filled with borehole water during the acclimatisation period of fish in the experimental tanks (one-week). At this time the supply tank (1a) also acted as a biological filter (3), filled with crushed concrete stones, which removed excretory and solid products from the recirculating water. During exposures the supply tank (1a) was removed and was replaced by a 1 000-litre glass reservoir (1b), containing the test solution (toxicant, i.e., copper chloride of zinc chloride). When conducting acidic pH exposures the pH meter and pump (10) as well as the H_2SO_4 reservoir (11) were utilised. The supply pipe (5) supplied water to the experimental tanks (1A-1D) from the supply tank (1a) or 1 000 litre reservoir (1b). Water flowed out of the experimental tanks (1A-1D) through the outlet pipe (7), which extended from the bottom of the tank to the desired water level. A wider screening pipe (8), the same height as the tank, was placed over the outlet pipe (7). The screening pipe (8) prevented fish from getting stuck and thereby clogging the outlet pipe (7). The screening pipe (8) had indentations at the base, which caused a mild sucking action. This action transported water, waste and excretion products via the outlet pipe (7) to a collective drainage pipe (9). The drainage pipe (9) circulated water back to the supply tank (1a) during acclimatisation, whilst water containing the toxicant was transported to a drain during exposures.

10.2.3 Experimental procedure.

10.2.3.1 Controls.

Controls are an important part of any toxicity test because in the study of the effects of metals on fish, a baseline of values must be stipulated. A control test should be run for every toxicity test, because without the knowledge of what is normal, it is difficult to differentiate between the normal and pathological state (Sprague, 1973; Smit *et al.*, 1979; Heath, 1987). Controls during this study were performed after the initial acclimation period of three months, by transferring specimens into a flow-through system, where they were held at 23±1°C for another two weeks, where after blood samples were taken. These fish were treated in the same way as experimental organisms, but the water they were kept in was borehole water - with no metal added to it. The control fish were fed every second day during the first week and feeding was suspended 48 hours before the actual test period commenced.

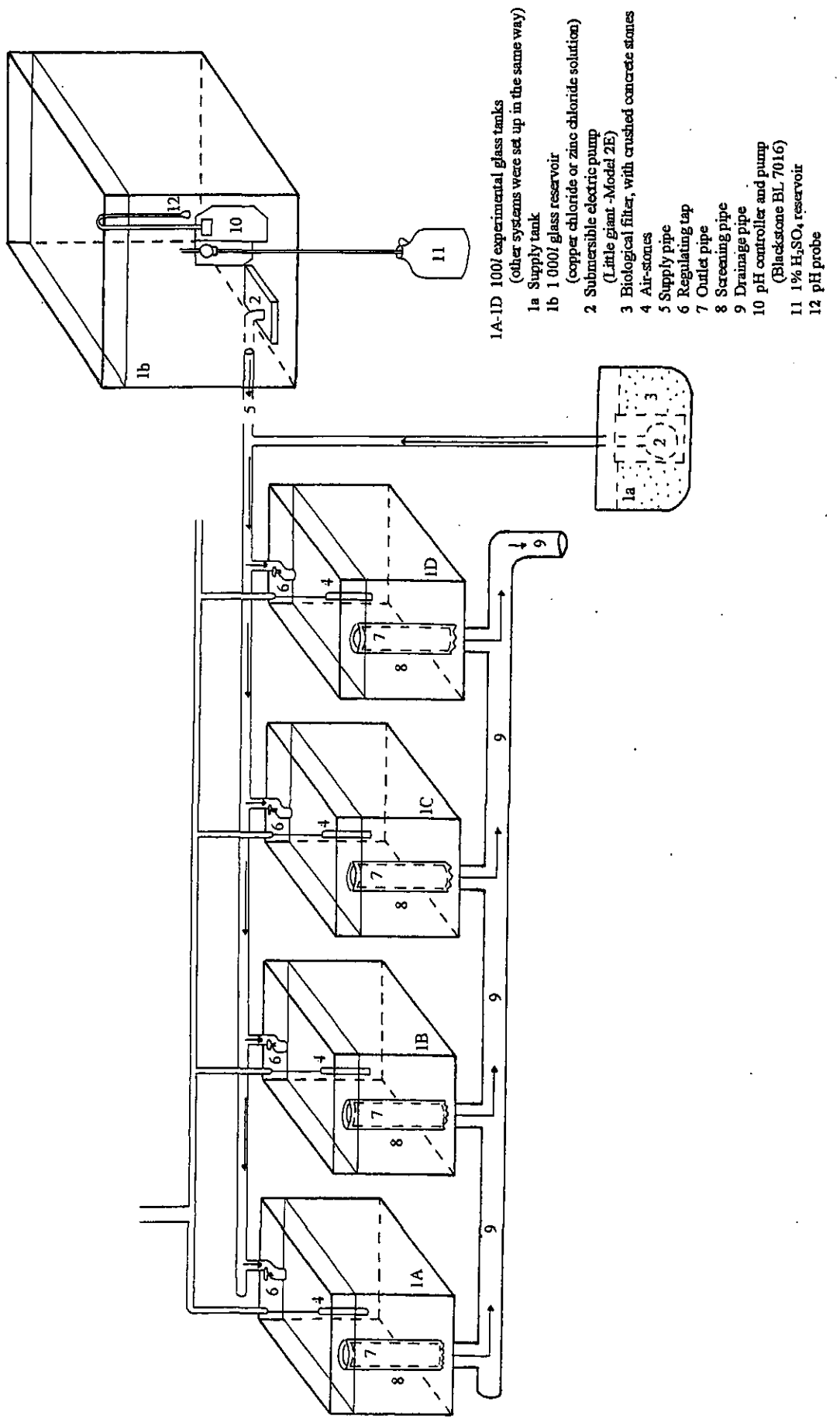

Figure 10.3: Diagram of the experimental flow-through system.

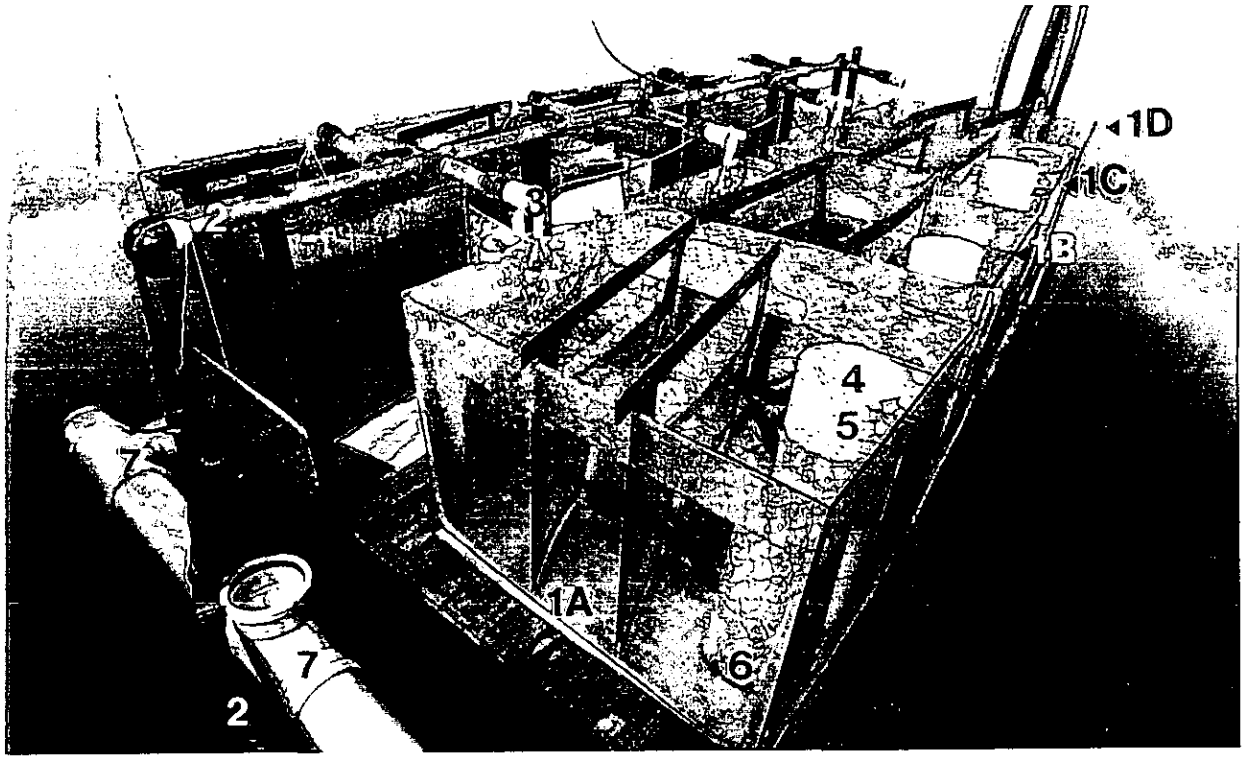

Figure 10.4: Exposure tanks in experimental flow-through system

10.2.3.2 Exposure of test organisms.

After a one week acclimation period *O. mossambicus* was exposed to different copper (Tables 10.6a and 10.6b), zinc (Tables 10.7a and 10.7b), aluminium (Table 10.8) and manganese (Table 10.9) concentrations, at the desired water pH, respectively. Short-term (96 hours) exposure experiments were conducted for each of the metals mentioned. Concentrations which were chosen represented the seasonal metal concentrations in the water of the Upper Catchment of the Olifants River, as well as minimum and maximum guideline values suggested by Kempster et al., 1982).

Metal concentrations were added directly to the glass tanks containing the fish, after which a continuous supply of the specified concentrations was maintained by pumping the test solution from the 1 000 litre glass reservoir. The flow rate was regulated to supply the 96 hour exposure period. The reservoirs were filled after 48 hours to provide enough test solution. During the acidic exposures, the pH (5.2) was maintained using a **Blackstone BL 7016 pH controller and pump**. This pump system consists of a pH meter connected to a pump, supplying a 1% H_2SO_4 solution to the 1 000 litre reservoir tank until the desired pH was reached (Figure 5).

Copper was administered as copper chloride ($CuCl_2.2H_2O$, MW = 170.48),zinc chloride ($ZnCl_2$, MW = 136.28), aluminium as aluminium chloride ($AlCl_3.6H_2O$, MW = 241.45) and

A

B

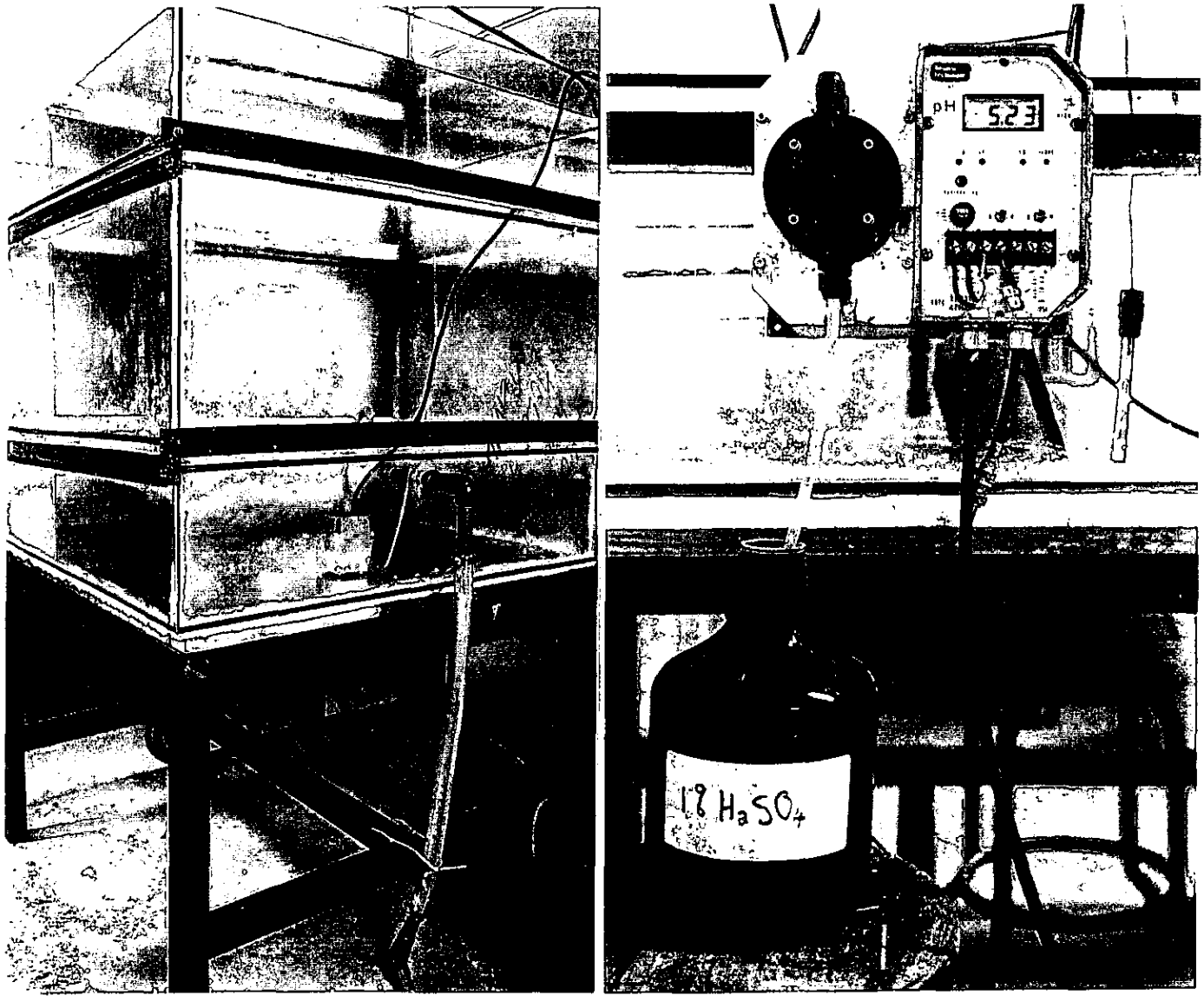

Figure 10.5: A; Glass reservoir (1000*l*) B; pH-pump

manganese as manganese chloride ($MnCl_2.4H_2O$), supplied by SAARCHEM. Metal salts were supplied in a powered form and therefore prior to addition it had to be dissolved in the borehole water, of the experimental tanks, to which the fish were acclimatised. Water samples (50m*l*) were also taken to determine the actual copper (Cu), zinc (Zn), aluminium (Al) and manganese (Mn) concentrations in the water. In the laboratory these samples were acidified, using 5m*l* 70% concentrated perchloric acid and 10m*l* 55% concentrated nitric acid. This mixture was then concentrated on a hot plate to 25m*l* and then made up to 50m*l* with distilled water. Thereafter, the total copper, zinc, aluminium and manganese concentrations (Table10. 6a, 10.6b, 10.7a, 10.7b, 10.8 and 10.9) were determined using a Varian atomic absorption spectrophotometer - SPECTRA AA10). Analytical standards for copper, zinc, aluminium and manganese were prepared from HOLPRO stock solutions (Van Loon, 1980), and the concentrations were calculated as follows:

$$\text{Cu or Zn concentration } (mg.l^{-1}) = \frac{\text{AAS reading } mg.l^{-1}}{\text{Initial volume (50m}l\text{)}} \times \text{Final volume (50m}l\text{)}$$

10.2.3.3 Blood sampling.

After the exposure and recovering periods the fish were removed individually from the experimental tanks, by means of a handnet to subject them to minimum stress (Gey van Pittius *et al.*, 1992; Wepener *et al.*, 1992a; 1992b; Van Vuren *et al.*, 1994; Nussey *et al.*, 1995a; 1995b; 1995c). The skin area where blood was drawn was wiped clean by using a paper towel, to prevent contamination with mucus and water (Blaxhall and Daisley, 1973). Blood samples were immediately collected from the caudal aorta with a 1m*l* heparinised (an anticoagulant - 5 000 U/m*l*) plastic syringe and a 26 G needle, after each fish was placed on its side, its eyes covered to minimise struggling (Figure 6) (Nussey *et al.*, 1995b). Minimal suction power was exerted on the syringe to prevent haemolysis (Klontz and Smith, 1968; Wepener *et al.*, 1992a; 1992b). Blood was transferred from the syringe to a 3m*l* sterile blood tube. After blood samples were extracted, the mass of each fish was determined by means of a Sartorius-universal balance and their lengths were determined from a measuring board (Table 8a and 8b).

10.2.3.4 Measurement of haematological and osmotic variables.

The variables chosen are only acceptable as indicators if (Sibergeld, 1972):

- they show a significant change when the fish is exposed to the toxicant;
- they are directly affected by the toxicant to which the fish is exposed;
- effects on the variable due to conditions of measurement (handling and capture) are separable from the effects due to toxicant exposure; and
- the baseline measurement for control conditions can be identified and replicated for the species.

Table 6a: Sublethal copper concentrations administered to the water during short-term exposures at neutral pH, and the concentrations determined by atomic absorption spectrophotometry

Mean copper concentrations in the Upper Catchment of the Olifants River (February 1994 to May 1995)								Guideline values Kempster *et al.*, 1982					
		Summer 0.0191 ± 0.02		Autumn 0.0124 ± 0.01		Winter 0.0439 ± 0.12		Summer 0.0264 ± 0.01		Minimum value		Maximum value	
Exposure group	*Control*	*Exposure 1*	*Recovering 1*	*Exposure 2*	*Recovering 2*	*Exposure 3*	*Recovering 3*	*Exposure 4*	*Recovering 4*	*Exposure 5*	*Recovering 5*	*Exposure 6*	*Recovering 6*
Exposure time (hours)	96	96	96	96	96	96	96	96	96	96	96	96	96
[$CuCl_2.2H_2O$] mg l^{-1}	-	0.0512	-	0.0333	-	0.1178	-	0.0708	-	0.0134	-	0.5366	-
[Cu] mgl^{-1}	-	0.0191	-	0.0124	-	0.0439	-	0.0264	-	0.0050	-	0.200	-
[Cu] measured in water (mg l^{-1})													
Mean ± SD	0.002 ± 0.002	0.021 ± 0.05	0.011 ± 0.04	0.015 ± 0.03	0.067 ± 0.02	0.047 ± 0.05	0.013 ± 0.01	0.032 ± 0.02	0.018 ± 0.02	0.005 ± 0.03	0.003 ± 0.0004	0.167 ± 0.004	0.163 ± 0.004
Min/max	0.000-0.003	0.011-0.034	0.008-0.019	0.043-0.050	0.046-0.095	0.016-0.105	0.009-0.026	0.016-0.040	0.009-0.034	0.003-0.045	0.002-0.010	0.159-0.173	0.158-0.167

Table 6b: Sublethal copper concentrations administered to the water during short-term exposures at an acidic pH (5.2), and the concentrations determined by atomic absorption spectrophotometry

Mean copper concentrations in the Upper Catchment of the Olifants River (February 1994 to May 1995)									
		Summer 0.0191 ± 0.02		Autumn 0.0124 ± 0.01		Winter 0.0439 ± 0.12		Summer 0.0264 ± 0.01	
Exposure group	Control	Exposure 7	Recovering 7	Exposure 8	Recovering 8	Exposure 9	Recovering 9	Exposure 10	Recovering 10
Exposure time (hours)	96	96	96	96	96	96	96	96	96
[$CuCl_2.2H_2O$] mg l^{-1}	-	0.0512	-	0.0333	-	0.1178	-	0.0708	-
[Cu] mg l^{-1}	-	0.0191	-	0.0124	-	0.0439	-	0.0264	-
[Cu] measured in water (mgl^{-1})									
Mean ± SD	0.002 ± 0.002	0.024 ± 0.03	0.016 ± 0.03	0.021 ± 0.03	0.031 ± 0.04	0.043 ± 0.01	0.011 ± 0.01	0.029 ± 0.03	0.021 ± 0.02
Min/max	0.001-0.004	0.010-0.044	0.012-0.022	0.014-0.024	0.026-0.037	0.033-0.060	0.004-0.025	0.005-0.070	0.005-0.053

$CuCl_2.2H_2O$ not added to control and recovering groups

Table 7a: Sublethal zinc concentrations administered to the water during short-term exposures at a neutral pH, and the concentrations determined by atomic absorption spectrophotometry

Mean zinc concentrations in the Upper Catchment of the Olifants River (February 1994 to May 1995)								Guideline values Kempster *et al.*, 1982					
		Summer 0.2099 ± 0.34		Autumn 0.2535 ± 0.69		Winter 0.3674 ± 1.19		Summer 0..8391 ± 1.86		Minimum value 0.03		Maximum value 0.10	
Exposure group	Control	Exposure 11	Recovering 11	Exposure 12	Recovering 12	Exposure 13	Recovering 13	Exposure 14	Recovering 14	Exposure 15	Recovering 15	Exposure 16	Recovering 16
Exposure time (hours)	96	96	96	96	96	96	96	96	96	96	96	96	96
$[ZnCl_2]$ mg l^{-1}	-	0.4375	-	0.5284	-	0.7658	-	1.7490	-	0.0805	-	0.2683	-
[Zn] mg l^{-1}	-	0.2099	-	0.2535	-	0.3674	-	0.8391	-	0.03	-	0.10	-
[Zn] measured in water (mg l^{-1})													
Mean ± SD	0.008 ± 0.001	0.209 ± 0.29	0.059 ± 0.05	0.261 ± 0.43	0.237 ± 0.06	0.351 ± 0.43	0.148 ± 0.22	0.840 ± 0.38	0.746 ± 0.62	0.032 ± 0.16	0.009 ± 0.02	0.109 ± 0.02	0.068 ± 0.02
Min/max	0.000-0.011	0.205-1.031	0.039-0.096	0.226-1.368	0.218-0.345	0.316-1.458	0.097-0.492	0.143-1.219	0.079-1.611	0.013-0.050	0.007-0.013	0.090-0.142	0.045-0.106

Table 7b: Sublethal zinc concentrations administered to the water during short-term exposures at an acidic pH (5.2), and the concentrations determined by atomic absorption spectrophotometry

Mean zinc concentrations in the Upper Catchment of the Olifants River (February 1994 to May 1995)									
		Summer 0.2099 ± 0.34		Autumn 0.2535 ± 0.69		Winter 0.3674 ± 1.19		Summer 0..8391 ± 1.86	
Exposure group	Control	Exposure 17	Recovering 17	Exposure 18	Recovering 18	Exposure 19	Recovering 19	Exposure 20	Recovering 20
Exposure time (hours)	96	96	96	96	96	96	96	96	96
$[CuCl_2.2H_2O]$ mg l^{-1}	-	0.4375	-	05284	-	0.7658	-	1.749	-
[Cu] mg l^{-1}	-	0.2099	-	0.2535	-	0.3674	-	0.8391	-
[Cu] measured in water (mg l^{-1})									
Mean ± SD	0.011 ± 0.02	0.021 ± 0.01	0.236 ± 0.15	0.261 ± 0.33	0.099 ± 0.074	0.373 ± 0.041	0.085 ± 0.03	0.846 ± 0.06	0.226 ± 0.09
Min/max	0.000-0.032	0.191-0.228	0.084-0.344	0.024-1.115	0.050-0.248	0.338-0.461	0.055-0.136	0.776-0.959	0.131-0.39

$ZnCl_2$ not added to control and recovering groups

Table 10.8: Sublethal aluminium concentrations administered to the water during exposures and concentrations determined through atomic absorption spectrophotometry

Exposure groups	Exposure time	$AlCl_3.6H_2O$ ($mg.l^{-1}$)	[Al] ($mg.l^{-1}$)	[Al] measured in the water
Control	2 weeks	*	*	**
PH 5.2	96 hours	*	*	**
Short-term (A) Mean ±SD Min/max	96 hours	0.54	0.06	 0.058±0.001 0.056 - 0.061
Short-term (B) Mean ± SD Min/max	96 hours	8.95	1.00	 0.97±0.007 0.96 - 1.05
Short-term (C) Mean ± SD Min/max	96 hours	13.42	1.50	 1.48±0.005 1.45 - 1.55
Short-term (D) Mean ± SD Min/max	96 hours	17.90	2.00	 1.94±0.007 1.93 - 2.07

* $AlCl_3.6H_2O$ was not added to the controls and pH 5.2 exposures

** Concentrations are not available

Table 10.9: The sublethal concentrations of manganese administered to the water during exposures

Exposure groups	Control	SLC 10	SLC 15	SLC 20	Control groups	SLC 10	SLC 15	SLC 20
Exposure time	96 hours	96 hours	96 hours	96 hours	28 days	28 days	28 days	28 days
[Mn] mg l^{-1}	0	172.3	259	345	0	172.3	259	345
$MnCl_2$ Applied Mg l^{-1}	0	594	990	1190	0	594	990	1190
Mn in water AAS mgl^{-1}	0	109.2	171.6	196.2	0	136.8	183.7	232.1

SLC = sublethal concentration; AAS = atomic absorption spectrophotometer

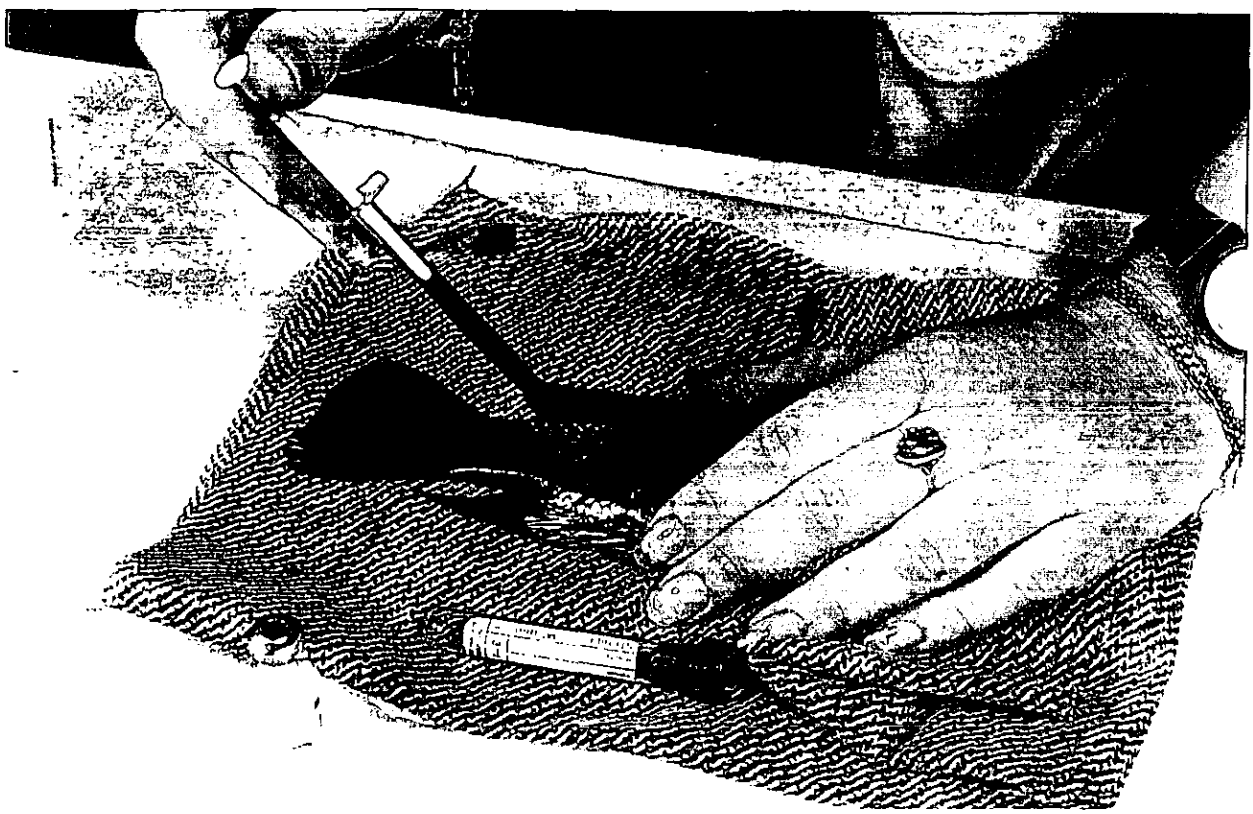

Figure 10.6: Blood being drawn from the caudal aorta of *Oreochromis mossambicus*, with a 1m/ plastic syringe. The fish is placed in a horizontal position on the work surface, and its eyes are covered.

Table 10.10: The haematological and osmotic variables determined in this study.

	Variable measured	Abbreviation	Means of measurement
1	White blood cells	WBC	Sysmex CC-120 microcell counter
2	Red blood cells	RBC	Sysmex CC-120 microcell counter
3	Haemoglobin	Hb	Sysmex CC-120 microcell counter
4	Haematocrit	Hct	Sysmex CC-120 microcell counter
5	Mean corpuscular volume	MCV	Sysmex CC-120 microcell counter
6	Mean cell haemoglobin	MCH	Calculated in picogram/cell = Hb/RBC x 10 (Dacie and Lewis, 1963)
7	Mean cell haemoglobin concentration	MCHC	Hb in 100*ml* blood/Hct x 100 (Dacie and Lewis, 1963)
8	Concentration of hydrogen ions (gram molecules per litre)	PH	Radiometer ABL 30 acid-base analyser
9	Plasma sodium concentration	[Na]	Microlyte 493 Selective ion analyser
10	Plasma potassium concentration	[K]	Microlyte 493 Selective ion analyser
11	Plasma calcium concentration	[Ca]	Microlyte 493 Selective ion analyser
12	Plasma chloride concentration	[Cl}	Microlyte 493 Selective ion analyser
13	Osmolality	Osmo	Osmomat 030 Cryoscopic osmometer
14	Blood oxygen tension	PO_2	Radiometer ABL 30 acid-base analyser

10.2.3.5 Data processing.

Data was processed on a computer utilizing a STATGRAPHICS statistical program.Independent Student's t-tests were performed to prove probability hypotheses and mean values were accepted as being statistically significant if $0.005 < P < 0.05$ (*) and $P < 0.05$ (**). All graphs were prepared from the Microsoft Office Professional computer program.

10.3. Results.

Differences in haematological variable values were measured against control values, determined under controlled laboratory conditions.

After sublethal copper and zinc exposures at a neutral as well as acidic pH , there were increases and/or decreases in the values of the haematological variables measured.). Exposure and recovering experiments resulted in insignificant and significant changes. The first four exposures and exposures11-14 respectively, contained copper and zinc concentrations representing the water content of copper in the upper catchment of the Olifants River from February 1994 to May 1995. Exposures 5, 6, 15 and16 represents the guideline copper and zinc concentrations, respectively as suggested by Kempster *et al.*, 1982,

while exposures seven to 10 and 17-20 were repititions of 1 -4 at pH 5.2. The level of sigficance where significant changes in variable values were obtained were calculated and are given (Tables 10.11 to 10.14) Only significant changes , i.e. either increases or decreases or both, are presented in this section.

10.3.1. Copper.

The number of red (RBC) and white (WBC) blood cells showed a variation of responses from the control values after the different exposure experiments. Significant increases in RBC were recorded after exposure 6 ($P<0.05$) as well as recovering 5 and 6 ($P<0.005$) (Table 10.11). At pH 5.2 exposure 7 and 10 resulted in slight decreases, whilst recovering 7 ($P<0.005$) and recovering 10 ($P<0.05$) showed significant decreases. Exposure 8 as well as recovering 8 ($P<0.05$) resulted in significant decreases. The recovering after 9 ($P<0.05$) caused a significant decrease in the RBC (Table 10.12).

Significant increases in WBC were prominent after recovering 5 ($P<0.05$) and 6 ($P<0.005$) At the acidic pH the copper exposures resulted in more significant differences in the WBC, from the control values After exposure and recovering 7 & 8 ($P<0.005$) significant decreases were recorded. To the contrary significant increases were noted after recovering 9 ($P<0.05$) and 10 ($P<0.005$) (Table 10.11)

The only significant decreases in haemoglobin concentration (Hb) was reported after recovering 4 ($P<0.05$). The "guideline" exposure 5 ($P<0.05$) and 6 ($P<0.005$) caused significant increases, significant increases were also caused after recovering 5 and 6 ($P<0.005$) (Table 10.11). Significant decreases were found after acidic exposure 8 ($P<0.005$) and recovering 7, 10 ($P<0.005$) and 9 ($P<0.05$) (Table 10.12).

After the sublethal copper exposures at the neutral pH, the haematocrit (Hct), showed a significant decrease after recovering 4 ($P<0.005$). "Guideline" exposures 5 ($P<0.05$) and 6 ($P<0.005$) showed significant increases (Table 10.11). The Hct at the acidic pH also showed significant decreases after recovering 7, 8 ($P<0.005$) and 10 ($P<0.05$). (Table 10.12).

The mean corpuscular volume (MCV) showed significant decreases.after recovering 2 and 3 ($P<0.05$) Exposure 4 ($P<0.05$) and recovering 4 ($P<0.005$) resulted in significant decreases. The only significant decrease was recorded after recovering 8 ($P<0.005$) after exposure under acidic conditions (Table 10.12).

After exposure 1 and 2 ($P<0.05$) as well as recovering 1 ($P<0.005$), at the neutral pH, there were significant increases in the mean cell haemoglobin (MCH). (Table 10.11).

Table 10.11: Mean (±S_d) haematological and osmoregulation values of *Oreochromis mossambicus* after exposure to copper, at a neutral pH.

		Mean copper concentrations (mg.l^{-1}) in the Upper Catchment of the Olifants River determined during the study period, February 1994 to May 1995 (Barnhoorn, 1996; Coetzee, 1996; Kotze, 1997; Nussey, 1998).								Guideline values (Kempster *et al.*, 1982)			
		Summer (0.0191 ± 0.02 mg.l^{-1})		Autumn (0.0124 ± 0.01 mg.l^{-1})		Winter (0.0439 ± 0.12 mg.l^{-1})		Spring (0.0264 ± 0.01 mg.l^{-1})		Minimum value (0.005 mg.l^{-1})		Maximum value (0.20 mg.l^{-1})	
Exposure group	Control	Exposure 1 (0.0191)	Recovering 1	Exposure 2 (0.0124)	Recovering 2	Exposure 3 (0.0439)	Recovering 3	Exposure 4 (0.0264)	Recovering 4	Exposure 5 (0.005)	Recovering 5	Exposure 6 (0.20)	Recovering 6
Number of fish (n)	120	10	10	10	10	10	10	10	10	10	10	10	10
WBC (X10^3.mm^{-3})													
Mean ± S_d	97.73±28.67	88.94±33.67	87.54±29.21	100.72±27.34	90.32±12.07	105.14±34.32	116.49±34.02	126.73±42.29	95.17±27.44	113.36±22.38	137.34±28.47	109.85±43.88	154.22±28.76
min/max	45.70-132.50	48.20-138.60	45.40-128.90	51.10-134.30	70.80-109.80	76.60-19.80	69.40-176.70	67.70-184.20	47.60-139.40	85.80-161.10	106.10-173.80	43.70-187.20	110.50-199.90
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05	⇔	<0.005
RBC (X10^6.mm^{-3})													
Mean ± S_d	1.55±0.22	1.45±0.31	1.43±0.29	1.55±0.19	1.59±0.15	1.73±0.41	1.73±0.49	1.62±0.39	1.39±0.13	1.69±0.20	1.92±0.22	1.86±0.34	2.00±0.30
min/max	1.16-1.87	1.04-1.88	0.96-1.80	1.21-1.79	1.31-1.86	1.45-2.80	1.14-2.67	1.07-2.15	1.14-1.56	1.43-2.27	1.63-2.27	1.36-2.46	1.73-2.65
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.005	<0.05	<0.005
Hb (g.dl^{-1})													
Mean ± S_d	7.44±1.18	8.43±1.46	7.99±2.00	8.09±1.06	7.91±0.96	8.89±2.11	9.18±2.67	8.45±1.99	6.45±0.74	8.73±1.11	9.64±1.48	9.83±1.65	10.42±1.79
min/max	4.90-8.80	5.60-10.30	5.50-11.20	5.80-9.80	6.50-9.70	7.30-14.40	5.50-14.40	5.70-12.20	5.20-7.60	7.60-11.40	7.70-12.80	7.40-13.40	8.90-14.10
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05	<0.05	<0.005	<0.005	<0.005
Hct (%)													
Mean ± S_d	28.42±6.29	25.41±6.88	25.97±4.86	25.55±4.85	25.19±3.03	30.70±8.84	27.34±8.89	24.07±6.74	20.55±4.20	33.10±6.72	34.73±6.78	34.85±8.98	38.88±7.73
min/max	19.10-40.60	14.70-37.40	15.40-33.40	17.40-31.50	19.30-28.10	23.00-48.90	17.10-46.90	13.60-32.80	15.30-30.70	22.70-42.80	23.30-47.20	21.00-52.30	26.00-56.90
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.005	⇔	<0.05	⇔	<0.005
MCV (µm^3)													
Mean ± S_d	183.80±29.14	174.30±21.50	178.00±22.16	164.10±24.13	158.60±11.73	175.90±24.74	159.10±15.38	145.90±25.15	146.90±19.47	190.80±34.48	180.50±28.98	185.80±18.78	195.30±28.13
min/max	144.0-228.0	134.0-209.0	153.0-213.0	138.0-211.0	141.0-180.0	152.0-233.0	140.0-178.0	121.0-201.0	131.0-197.0	148.0-249.0	139.0-224.0	145.0-212.0	144.0-219.0
P	-	⇔	⇔	⇔	<0.05	⇔	<0.05	<0.05	<0.005	⇔	⇔	⇔	⇔
MCH (pg/cell)													
Mean ± S_d	48.17±4.43	59.27±10.71	55.54±5.64	53.23±6.02	49.88±5.82	51.42±3.12	53.43±6.80	51.85±7.14	46.56±4.93	51.96±3.88	50.16±4.75	53.55±4.75	52.21±3.52
min/max	39.84-54.00	44.97-82.69	46.51-63.58	46.47-62.70	43.71-64.44	45.51-55.56	43.00-65.79	43.56-66.67	40.69-55.74	47.34-60.65	43.41-57.40	43.41-57.40	46.39-57.32
P	-	<0.05	<0.05	<0.05	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05
MCHC (%)													
Mean ± S_d	26.86±5.12	34.38±6.46	31.15±6.67	32.50±6.36	31.86±6.00	29.68±4.76	34.06±4.58	36.64±9.27	32.01±4.37	27.12±4.90	28.50±5.74	29.27±6.44	27.34±5.33
min/max	18.72-34.60	26.47-47.25	20.36-37.82	25.08-42.98	24.73-47.09	20.45-34.92	28.65-43.86	21.65-52.94	24.76-38.42	20.30-34.80	21.37-36.80	22.93-45.24	23.37-37.31
P	-	<0.05	⇔	<0.05	⇔	⇔	<0.005	<0.05	<0.05	⇔	⇔	⇔	⇔

Table 10.11: (continued)

PO_2 (mmHg) Mean ± S_d Min/max P	62.38±37.31 21.80-131.50 ⇔	63.17±30.34 17.60-114.70 ⇔	60.76±33.15 19.50-124.10 ⇔	80.81±39.31 36.10-125.50 ⇔	100.14±43.94 21.40-158.50 ⇔	60.07±37.30 17.90-133.40 ⇔	65.98±39.30 28.10-123.20 ⇔	43.27±22.70 16.00-85.70 ⇔	49.79±18.13 19.50-76.30 ⇔	61.47±24.51 35.10-125.80 ⇔	62.91±46.39 26.00-181.50 ⇔	65.75±34.00 9.90-122.70 ⇔	66.49±37.54 11.10-134.90 ⇔
[Na] (mmol/l) Mean ± S_d min/max P	163.60±10.91 138.0-182.0 -	143.40±5.17 131.0-150.0 <0.005	133.00±10.31 108.0-144.0 <0.005	136.50±4.25 129.0-143.0 <0.005	146.80±14.34 138.0-187.0 <0.05	138.40±6.24 127.0-149.0 <0.005	141.50±18.49 108.0-181.0 <0.005	123.80±22.18 93.0-159.0 <0.005	131.00±11.94 113.0-151.0 <0.005	152.60±4.77 145.0-157.0 <0.05	149.20±6.70 138.0-159.0 <0.005	141.20±10.64 121.0-157.0 <0.005	140.10±11.00 123.0-155.0 <0.005
[K] (mmol/l) Mean ± S_d Min/max P	5.93±1.84 2.80-910 -	6.79±2.71 3.00-10.10 ⇔	7.19±3.08 3.50-12.50 ⇔	7.40±3.73 3.00-12.90 ⇔	9.76±2.37 6.10-14.60 <0.005	11.37±3.60 5.80-17.20 <0.005	7.65±3.12 4.10-15.01 ⇔	11.17±4.38 3.70-15.01 <0.005	10.31±4.39 4.10-15.01 <0.05	5.06±2.28 2.50-9.50 ⇔	5.65±1.94 2.70-9.00 ⇔	10.90±4.58 5.00-15.01 <0.05	5.30±2.63 1.80-9.70
[Ca] (mmol/l) Mean ± S_d Min/max P	0.90±0.31 0.40-1.59 -	0.92±0.37 0.10-2.11 ⇔	0.81±0.51 0.15-2.25 ⇔	0.70±0.36 0.19-1.65 ⇔	0.85±0.25 0.24-2.05 ⇔	0.64±0.36 0.16-1.40 ⇔	0.78±0.23 0.26-1.07 ⇔	0.66±0.28 0.20-1.13 ⇔	0.77±0.13 0.61-1.02 ⇔	0.86±0.16 0.62-1.10 ⇔	0.78±0.17 0.42-1.11 ⇔	0.69±0.25 0.31-1.17 ⇔	0.76±0.23 0.43-1.14 ⇔
[Cl] (mmol/l) Mean ± S_d Min/max P	133.60±12.08 107.0-145.0 -	137.40±9.40 120.0-148.0 ⇔	129.70±12.13 111.0-146.0 ⇔	135.40±6.52 127.0-146.0 ⇔	134.70±10.02 115.0-148.0 ⇔	127.10±7.02 108.0-135.0 ⇔	126.20±15.65 104.0-158.0 ⇔	116.10±19.31 86.0-146.0 <0.05	122.70±11.01 107.0-138.0 <0.05	139.30±9.60 124.0-150.0 ⇔	136.10±7.08 126.0-146.0 ⇔	129.80±11.91 109.0-148.0 ⇔	125.90±10.13 113.0-142.0 ⇔
Osmo (osmol/kg) Mean ± S_d Min/max P	0.30±0.01 0.27-0.31 -	0.32±0.01 0.30-0.33 <0.005	0.29±0.02 0.24-0.33 ⇔	0.31±0.01 0.30-0.32 <0.005	0.31±0.01 0.29-0.33 ⇔	0.29±0.01 0.28-0.30 <0.05	0.29±0.03 0.25-0.36 ⇔	0.27±0.02 0.23-0.30 <0.005	0.28±0.01 0.25-0.30 <0.05	0.31±0.01 0.29-0.32 <0.05	0.29±0.01 0.26-0.31 ⇔	0.31±0.01 0.29-0.33 ⇔	0.29±0.02 0.26-0.31 ⇔

⇔ = P is not significant ($P > 0.05$).

WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin concentration; Hct = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; pH = blood ph value; PO_2 = Blood oxygen tention; [Na] = plasma sodium concentration; [K] = plasma potassium concentration; [Ca] = plasma calcium concentration; [Cl] = plasma chloride concentration; Osmo = total osmolality.

Table 10.12: Mean (± S_d) haematological and osmoregulation values of *Oreochromis mossambicus* after exposure to copper, at an acidic pH.

Exposure group	Acid control	Mean copper concentrations ($mg.l^{-1}$) in the Upper Catchment of the Olifants River determined during the study period, February 1994 to May 1995 (Barnhoorn, 1996; Coetzee, 1996; Kotze, 1997; Nussey, 1998).							
		Summer (0.0191 ± 0.02 $mg.l^{-1}$)		Autumn (0.0124 ± 0.01 $mg.l^{-1}$)		Winter (0.0439 ± 0.12 $mg.l^{-1}$)		Spring (0.0264 ± 0.01 $mg.l^{-1}$)	
		Exposure 7 (0.0191)	Recovering 7	Exposure 8 (0.0124)	Recovering 8	Exposure 9 (0.0439)	Recovering 9	Exposure 10 (0.0264)	Recovering 10
Number of fish (n)	80	10	10	10	10	10	10	10	10
WBC ($X10^3.mm^{-3}$)									
Mean ± S_d	154.85±17.01	122.33±22.73	97.73±22.88	122.64±25.08	115.71±13.88	154.19±33.75	111.78±39.22	131.08±31.58	120.55±21.12
min/max	129.90-177.30	95.70-173.70	65.40-135.40	94.30-175.50	93.30-132.20	106.10-196.70	59.30-165.30	56.10-172.10	103.10-173.20
P	-	<0.005	<0.005	<0.005	<0.005	⇔	<0.05	•	<0.005
RBC ($X10^6.mm^{-3}$)									
Mean ± S_d	1.93±0.19	1.75±0.22	1.55±0.26	1.69±0.21	1.65±0.26	2.10±0.48	1.59±0.35	1.84±0.24	1.63±0.31
min/max	1.59-2.17	1.53-2.19	1.06-1.97	1.46-2.17	1.20-1.91	1.40-2.98	1.13-1.99	1.47-2.11	1.01-2.02
P	-	⇔	<0.005	<0.05	<0.05	⇔	<0.05	⇔	<0.05
Hb ($g.dl^{-1}$)									
Mean ± S_d	9.86±1.46	8.78±1.00	7.42±1.22	8.06±1.19	8.55±1.44	9.66±2.45	8.02±1.51	9.75±1.78	7.60±0.96
min/max	7.10-12.20	7.40-10.80	5.70-9.50	6.40-10.80	6.10-11.10	5.40-14.40	5.70-9.70	6.50-12.60	6.10-8.80
P	-	⇔	<0.005	<0.05	⇔	⇔	<0.05	⇔	<0.005
Hct (%)									
Mean ± S_d	33.01±2.69	31.69±7.17	26.41±5.45	29.10±5.81	24.58±4.97	34.50±8.04	30.46±9.94	29.79±4.45	27.39±7.63
min/max	29.70-37.00	25.40-48.50	17.00-34.50	21.04-38.00	15.00-30.40	21.00-47.10	19.20-46.30	19.70-35.00	14.10-40.90
P	-	⇔	<0.005	⇔	<0.005	⇔	⇔	⇔	<0.05
MCV (μm^3)									
Mean ± S_d	171.90±19.07	179.70±21.97	169.50±13.91	171.80±21.32	148.00±11.26	164.30±13.40	190.30±34.69	163.50±27.21	167.30±33.40
min/max	141.0-201.1	160.0-234.0	156.0-205.0	134.0-199.0	121.0-163.0	148.0-188.0	148.0-244.0	127.0-219.0	140.0-246.0
P	-	⇔	⇔	⇔	<0.005	⇔	⇔	⇔	⇔
MCH (pg/cell)									
Mean ± S_d	51.51±9.66	50.33±5.30	48.39±7.36	47.77±3.06	52.25±7.40	45.83±4.52	50.98±2.34	53.36±9.07	48.19±10.99
min/max	43.78-69.32	44.85-58.82	40.12-66.04	43.64-52.29	40.33-66.67	37.86-52.07	47.45-54.31	41.94-71.15	36.97-70.30
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔
MCHC (%)									
Mean ± S_d	29.98±4.72	28.43±5.07	28.72±5.29	28.10±3.17	35.46±5.55	28.11±3.13	27.72±5.81	33.10±6.13	30.05±10.36
min/max	22.12-37.54	19.59-35.83	23.08-41.18	23.16-32.93	28.40-45.71	24.56-33.56	20.09-35.42	24.46-42.21	20.12-54.35
P	-	⇔	⇔	⇔	<0.05	⇔	⇔	⇔	⇔

Table 10.12: (continued)

pH Mean ± S_d min/max P	7.22±0.08 7.08-7.37 -	7.30±0.08 7.23-7.44 ⇔	7.32±0.08 7.19-7.45 <0.05	7.18±0.05 7.12-7.29 ⇔	7.27±0.06 7.19-7.36 ⇔	7.17±0.07 7.06-7.26 ⇔	7.24±0.09 7.12-7.36 ⇔	7.08±0.15 6.87-7.35 <0.05	7.29±0.08 7.17-7.42 ⇔
PO_2 (mmHg) Mean ± S_d min/max P	58.62±23.80 20.90-86.40 -	49.92±20.42 26.50-98.50 ⇔	73.36±41.98 21.60-163.50 ⇔	94.99±54.69 32.70-180.10 ⇔	89.88±33.61 35.50-138.00 <0.05	55.65±28.13 17.70-119.70 ⇔	76.22±40.72 32.10-153.50 ⇔	72.22±37.40 35.10-130.50 ⇔	56.32±33.21 18.90-121.60 ⇔
[Na] (mmol.l^{-1}) Mean ± S_d min/max P	134.20±10.33 118.0-151.0 -	146.10±8.65 133.0-159.0 <0.005	156.10±6.52 150.0-168.0 <0.005	135.60±13.65 110.0-153.0 ⇔	146.30±5.96 136.0-155.0 <0.005	143.10±11.64 121.0-158.0 ⇔	140.30±15.14 116.0-165.0 ⇔	133.80±10.97 111.0-146.0 ⇔	140.70±11.52 122.0-153.0 ⇔
[K] (mmol.l^{-1}) Mean ± S_d min/max P	6.74±1.40 4.40-8.20 -	6.68±2.23 3.50-9.80 ⇔	4.50±0.96 2.60-5.70 <0.005	9.53±4.76 5.00-15.01 ⇔	9.05±4.18 5.90-15.01 ⇔	10.22±3.42 6.50-15.01 <0.05	9.44±5.01 3.90-15.01 ⇔	11.60±4.43 6.00-15.01 <0.005	5.40±1.73 3.50-8.60 ⇔
[Ca] (mmol.l^{-1}) Mean ± S_d min/max P	0.75±0.13 0.54-0.99 -	0.76±0.13 0.48-0.90 ⇔	0.69±0.16 0.49-0.95 ⇔	0.69±0.14 0.39-0.86 ⇔	0.66±0.14 0.39-0.84 ⇔	0.81±0.18 0.60-1.15 ⇔	0.61±0.18 0.31-0.86 ⇔	0.64±0.11 0.41-0.81 ⇔	0.70±0.20 0.45-1.03 ⇔
[Cl] (mmol.l^{-1}) Mean ± S_d min/max P	113.60±15.73 88.0-133.0 -	130.20±10.66 118.0-147.0 <0.05	140.10±5.04 133.0-148.0 <0.005	120.90±8.60 109.0-139.0 ⇔	124.20±7.47 112.0-137.0 ⇔	126.40±14.30 95.0-142.0 ⇔	118.40±15.49 91.0-137.0 ⇔	119.50±11.68 97.0-133.0 ⇔	121.40±20.27 90.0-149.0 ⇔
Osmo (osmol.kg^{-1}) Mean ± S_d min/max P	0.28±0.01 0.27-0.30 -	0.30±0.01 0.29-0.31 <0.005	0.30±0.01 0.29-0.31 <0.005	0.28±0.01 0.26-0.29 ⇔	0.28±0.001 0.27-0.029 ⇔	0.29±0.01 0.27-0.30 ⇔	0.28±0.02 0.25-0.31 ⇔	0.28±0.01 0.25-0.29 ⇔	0.29±0.01 0.26-0.31 ⇔

⇔ = P is not significant (P > 0.05).

WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin concentration; Hct = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; pH = blood pH value; PO_2 = blood oxygen tention; [Na] = plasma sodium concentration; [K] = plasma potassium concentration; [Ca] = plasma calcium concentration; [Cl] = plasma chloride concentration; Osmo = total osmolality.

After all the exposures and recoverings, at the neutral pH, there were increases in the mean cell haemoglobin concentration (MCHC). After exposures 1, 2 and 4 ($P<0.05$) as well as recovering 3 ($P<0.005$) and 4 ($P<0.05$) increases were significant (Table 10.9). At the acidic pH, recovering 8 ($P<0.05$) caused a significant increase in MCHC (Table 10.12).

Copper concentrations at the neutral pH caused decreases in blood pH values. After exposure and recovering 1, 4 and 6 ($P<0.05$) decreases were significant. Significant decreases were also recorded after exposure 3 ($P<0.005$) and 5 ($P<0.05$) as well as after recovering 2 ($P<0.005$) and 3 ($P<0.05$). (Table 10.11). The blood pH after the exposures at the acidic pH showed a significant increase after exposure 10 ($P<0.05$), and a significant decrease after recovering 7 ($P<0.05$). (Table 10.12).

After exposure 1, 2, 3, 4, 6 ($P<0.005$) and 5 ($P<0.05$) as well as recovering 1, 3, 4, 5, 6 ($P<0.005$) and 2 ($P<0.05$), at the neutral pH, copper caused significant decreases in the plasma sodium concentration ([Na]) (Table 10.11). At the acidic pH there were significant increases after exposure 7 ($P<0.05$) as well as after recovering 7 and 8 ($P<0.005$)(Table 10.12).

Copper caused significant increases at the neutral pH in the plasma potassium concentration ([K]), after exposure 3, 4 ($P<0.005$) and 6 ($P<0.05$) as well as after recovering 2 ($P<0.005$) and 4 ($P<0.05$) (Table 10.11). At pH 5.2, copper exposure 9 ($P<0.05$) and 10 ($P<0.005$) caused significant increases, whilst recovering 7 ($P<0.005$) resulted in a significant decrease(Table 10.12).

The acidic pH and exposure 7 ($P<0.05$) as well as recovering 7 ($P<0.005$) resulted in significant increases (Table 10.12).

At the neutral pH the total osmolality (Osmo) increased significantly after exposure 1, 2 ($P<0.005$) and 5 ($P<0.05$). Significant decreases were recorded after exposure 3 ($P<0.05$) and 4 ($P<0.005$) as well as after recovering 4 ($P<0.05$) (Table 10.11). Significant increases were recorded at the acidic pH, after exposure and recovering 7 ($P<0.005$) (Table 10.12).

The PO_2 at the acidic pH showed a significant increase after recovering 8 (Table 10.12).

10.3.2 Zinc

At the acidic pH, zinc caused significant decreases in WBC after all exposures 17 and 20 ($P<0.05$), 18 and 19 ($P<0.005$) as well as after all recoverings: 17 and 18 ($P<0.05$), 19 and 20 ($P<0.005$) (Table 10.12).

The RBC, after zinc exposures at a neutral pH resulted in a significant increase after recovering 14 ($P<0.05$).A significant decrease in RBC was reported after the 'guideline exposure 15($P<0.05$) (Table 10.11).

At the acidic pH and zinc, a significant decrease after exposure 17 and 19 ($P<0.05$) as well as after recovering 17, 18 ($P<0.05$) and 19($P<0.005$) were observed (Table 10.12).

The only significant difference reported for the Hct at the neutral pH, was a significant decrease after the minimum "guideline" exposure 15 ($P<0.05$). (Table 10.13). At pH 5.2,

the only exposure to show a significant decrease was exposure 20 ($P<0.05$), whilst recovering 17 and 18 ($P<0.05$) also showed significant decreases (Table 10.14).

A significant decrease in MCV were found after, exposure 16 ($P<0.05$) (Table 10.13). After exposure 17 ($P<0.05$), at the acidic pH, there was a significant increase in MCV, whilst after recovering 18 ($P<0.05$) there was a significant decrease (Table 10.14).

Zinc concentrations at the neutral pH caused significant increases in MCH after exposure 12 ($P<0.05$) and 15 ($P<0.005$ (Table 10.13).

At a neutral pH, zinc caused significant increases after both "guideline" exposures (15 and 16, $P<0.05$) in the MCHC (Table 10.13). The acidic pH and zinc concentrations caused significant decreases in MCHC, after exposure 17 ($P<0.05$) and recovering 19 ($P<0.05$) (Table 10.14).

Throughout the zinc exposures and recoverings at a neutral pH, blood pH values showed decreases. These decreaeses were significant after exposures 11, 12, 13, 15 ($P<0.005$) and 14 ($P<0.05$) as well as after recovering 11, 15, 16 ($P<0.005$) and 13, 14 ($P<0.05$) (Table 10.13). In contrast to these decreases, increases were recorded in blood pH values, at the acidic pH. after exposures and recoverings 18, 19 and 20 ($P<0.05$) (Table 10.14).

There were significant decreases in [Na] after all exposures (11, 12, 15, 16 - $P<0.005$ and 13, 14 - $P<0.05$) as well as all recoverings (11, 14, 15,16 - $P<0.05$ and 13, 14 - $P<0.005$), at the neutral pH. (Table 10.13). There were significant increases in [Na] at the acidic pH, after exposure 17, 19 ($P<0.005$), 20 ($P<0.05$) as well as after recoverings 17 ($P<0.05$), 18, 19, 20 ($P<0.005$) (Table 10.14).

The only significant difference for [K] at the neutral pH, was a decrease recorded after recovering 11 ($P<0.05$ (Table 10.13). After exposure and recovering 18 ($P<0.05$) at an acidic pH, there was a significant increase in [K]. (Table 10.14).

There was a significant decrease in [Ca] after exposure 18 ($P<0.05$) (Table 10.14).

[Cl] recorded at the neutral pH and zinc, showed a significant increase after exposure 14 ($P<0.05$) (Table 10.13). There were significant differences in [Cl] at the acidic pH, after all exposures (17 - $P<0.005$ and 18, 19, 20 - $P<0.05$) as well as after all recoverings (17, 20 - $P<0.005$ and 18, 19 - $P<0.05$) (Table 10.14).

At pH 5.2, there were significant increases in osmolality after exposure 17, 18, 19 and 20 ($P<0.05$) as well as after recovering 17 and 19 ($P<0.005$). After recovering 20 only a slight increases was recorded (Table 10.14).

At the acidic pH, there was a significant increases in PO_2 after recovering 20 (Table 10.14)

Table 10.13: Mean ($\pm S_d$) haematological and osmoregulation values of *Oreochromis mossambicus* after exposure to zinc, at a neutral pH.

Exposure group	Control	Mean zinc concentrations ($mg.l^{-1}$) in the Upper Catchment of the Olifants River determined during the study period, February 1994 to May 1995 (Barnhoorn, 1996; Coetzee, 1996; Kotze, 1997; Nussey, 1998).								Guideline values (Kempster *et al.*, 1982)			
		Summer (0.2099 ± 0.34 $mg.l^{-1}$)		Autumn (0.2535 ± 0.69 $mg.l^{-1}$)		Winter (0.3674 ± 1.19 $mg.l^{-1}$)		Spring (0.8391± 1.86 $mg.l^{-1}$)		Minimum value (0.03 $mg.l^{-1}$)		Maximum value (0.10 $mg.l^{-1}$)	
		Exposure 11 (0.2099)	Recovering 11	Exposure 12 (0.2535)	Recovering 12	Exposure 13 (0.3674)	Recovering 13	Exposure 14 (0.8391)	Recovering 14	Exposure 15 (0.03)	Recovering 15	Exposure 16 (0.10)	Recovering 16
Number of fish (n)	120	10	10	10	10	10	10	10	10	10	10	10	10
WBC ($X10^3.mm^{-3}$)													
Mean ± S_d	97.73±28.67	88.11±24.43	104.18±16.53	108.21±33.09	89.58±27.62	89.82±21.34	105.77±30.73	111.27±33.63	117.31±37.50	88.91±19.72	113.03±15.00	101.05±17.85	92.38±20.38
min/max	45.70-132.50	50.50-132.60	75.40-129.50	65.40-164.40	48.80-126.90	69.00-128.60	68.90-165.70	69.70-168.10	61.90-176.00	51.70-113.80	58.80-137.00	67.50-119.60	65.70-114.50
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔
RBC ($X10^3.mm^{-3}$)													
Mean ± S_d	1.55±0.22	1.36±0.20	1.56±0.14	1.66±0.35	1.55±0.37	1.66±0.30	1.59±0.36	1.60±0.23	1.90±0.42	1.25±0.24	1.56±0.11	1.52±0.24	1.53±0.23
min/max	1.16-1.87	1.03-1.72	1.41-1.81	1.12-2.13	1.03-2.17	1.35-2.42	1.12-2.10	1.33-1.93	1.35-2.54	0.88-1.59	1.41-1.77	1.15-1.93	1.19-1.86
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05	<0.05	⇔	⇔	⇔
Hb ($g.dl^{-1}$)													
Mean ± S_d	7.44±1.18	6.79±1.17	8.16±1.19	8.83±1.95	8.00±1.55	6.67±1.38	8.03±1.86	7.49±1.21	8.61±1.97	7.94±1.22	8.06±0.97	7.61±1.01	8.12±1.76
min/max	4.90-8.80	5.40-8.60	6.80-10.10	6.60-12.20	5.30-10.50	6.10-11.20	5.20-10.70	5.80-10.00	5.30-11.40	5.90-9.80	6.50-9.40	5.90-9.30	5.60-11.80
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔
Hct (%)													
Mean ± S_d	28.42±6.29	24.25±5.04	28.67±4.13	30.63±9.85	29.24±9.37	27.30±6.26	28.99±5.10	27.56±5.52	31.87±10.32	21.92±9.50	25.83±4.94	22.89±5.62	28.13±6.80
min/max	19.10-40.60	14.5-32.3	20.40-33.70	15.40-46.30	16.60-46.70	19.60-46.70	22.60-37.40	19.80-35.20	15.40-50.30	11.90-33.40	18.00-30.70	14.00-33.20	20.10-41.30
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05	⇔	⇔	⇔
MCV (μm^3)													
Mean ± S_d	183.80±29.14	180.90±19.54	183.50±23.08	182.40±34.85	187.10±32.12	164.40±16.09	184.60±22.53	172.10±23.82	165.10±27.07	173.20±27.44	167.00±27.35	154.70±18.45	183.50±28.36
min/max	144.0-228.0	149.0-215.0	145.0-218.0	137.0-222.0	149.0-237.0	139.0-196.0	154.0-218.0	145.0-219.0	103.0-209.0	136.0-223.0	127.0-202.0	122.0-176.0	134.0-222.0
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05	⇔
MCH (pg/cell)													
Mean ± S_d	48.16±4.43	49.94±4.65	52.35±7.64	53.42±5.60	53.15±12.02	46.47±3.68	50.46±4.50	47.07±4.38	45.49±5.62	64.81±11.09	51.69±6.09	50.20±2.23	53.01±5.81
min/max	39.84-54.00	42.19-59.44	44.16-70.14	46.15-60.82	46.09-86.41	42.77-54.07	42.86-56.64	51.10-54.74	35.33-55.11	52.48-84.09	40.96-63.09	47.02-54.92	46.78-64.13
P	-	⇔	⇔	<0.05	⇔	⇔	⇔	⇔	⇔	<0.005	⇔	⇔	⇔
MCHC (%)													
Mean ± S_d	26.86±5.12	28.50±4.22	28.89±5.11	30.52±7.61	29.46±9.96	28.35±3.30	27.61±3.62	27.57±3.34	27.91±3.47	38.84±11.81	32.77±7.32	32.95±5.30	29.42±5.16
min/max	18.72-34.60	21.26-32.95	22.15-36.46	21.55-42.86	19.34-53.61	22.56-32.89	23.01-33.62	22.09-31.86	22.66-34.42	24.75-62.18	23.45-43.33	28.01-42.14	22.52-37.81
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔	<0.05	⇔	<0.05	⇔

Table 10.13: (continued)

PH Mean ± S_d min/max P	7.43±0.06 7.37-7.56 -	7.31±0.08 7.16-7.43 <0.005	7.26±0.16 7.05-7.51 <0.005	7.31±0.10 7.14-7.44 <0.005	7.38±0.16 7.08-7.55 ⇔	7.30±0.09 7.12-7.39 <0.005	7.34±0.08 7.23-7.49 <0.05	7.33±0.07 7.24-7.43 <0.05	7.30±0.11 7.15-7.45 <0.05	7.33±0.05 7.21-7.38 <0.005	7.31±0.08 7.13-7.40 <0.005	7.35±0.15 7.12-7.59 ⇔	7.27±0.07 7.20-7.39 <0.005
PO_2 (mmHg) Mean ± S_d min/max P	62.38±37.31 21.80-131.50 -	46.95±26.84 13.31-104.10 ⇔	77.03±50.25 15.00-141.50 ⇔	83.73±34.81 42.50-132.10 ⇔	49.17±30.51 19.20-123.20 ⇔	52.58±34.96 14.70-122.80 ⇔	68.28±38.65 30.00-126.10 ⇔	42.65±18.58 23.60-84.40 ⇔	77.54±34.06 34.40-124.90 ⇔	69.67±33.16 35.00-150.90 ⇔	51.37±20.70 27.20-82.30 ⇔	61.84±52.08 15.20-183.60 ⇔	63.46±29.57 19.40-126.70 ⇔
[Na] (mmol.l^{-1}) Mean ± S_d min/max P	163.60±10.9 1 138.0-182.0 -	148.30±6.17 136.0-158.0 <0.005	152.60±6.38 144.0-164.0 <0.05	142.00±10.1 5 121.0-153.0 <0.005	150.70±4.40 143.0-157.0 <0.005	152.50±4.06 144.0-158.0 <0.05	148.90±8.41 138.0-166.0 <0.005	154.90±3.07 150.0-159.0 <0.05	152.30±9.04 134.0-161.0 <0.05	150.00±5.87 140.0-160.0 <0.005	149.30±10.13 124.0-159.0 <0.05	150.70±5.36 137.0-156.0 <0.005	151.30±6.50 139.0-162.0 <0.05
[K] (mmol.l^{-1}) Mean ± S_d min/max P	5.93±1.84 2.80-9.10 -	4.94±1.67 2.70-8.60 ⇔	4.02±1.74 2.00-7.70 <0.05	6.80±1.97 4.50-9.50	6.09±1.75 4.00-9.80 ⇔	5.47±1.57 3.50-8.00 ⇔	8.52±4.03 2.00-15.01 ⇔	5.18±0.90 3.50-6.90 ⇔	6.77±3.46 2.90-15.01 ⇔	6.05±3.32 2.70-15.01 ⇔	5.02±2.31 2.40-9.30 ⇔	5.88±2.25 3.40-9.60 ⇔	5.87±3.70 2.20-15.01 ⇔
[Ca] (mmol.l^{-1}) Mean ± S_d min/max P	0.90±0.31 0.40-1.59 -	0.92±0.16 0.71-1.16 ⇔	0.90±0.17 0.67-1.16 ⇔	0.67±0.18 0.33-0.90 ⇔	0.85±0.16 0.56-1.09 ⇔	0.87±0.13 0.70-1.05 ⇔	0.85±0.20 0.53-1.26 ⇔	0.79±0.10 0.61-0.96 ⇔	0.86±0.22 0.41-1.10 ⇔	0.79±0.11 0.65-1.02 ⇔	0.79±0.23 0.25-1.03 ⇔	0.84±0.17 0.47-1.13 ⇔	0.84±0.18 0.63-1.18 ⇔
[Cl] (mmol.l^{-1}) Mean ± S_d min/max P	133.60±12.0 8 107.0-145.0 -	134.20±2.78 131.0-138.0 ⇔	131.80±7.50 117.0-146.0 ⇔	126.10±10.7 9 107.0-142.0 ⇔	137.00±5.37 126.0-143.0 ⇔	140.40±6.50 125.0-147.0 ⇔	138.30±8.35 123.0-149.0 ⇔	141.80±1.87 139.0-145.0 <0.05	139.40±7.24 122.0-149.0 ⇔	135.80±8.78 120.0-145.0 ⇔	137.50±11.60 106.0-146.0 ⇔	136.90±7.08 126.0-147.0 ⇔	139.30±5.60 130.0-146.0 ⇔
Osmo (osmol.kg^{-1}) Mean ± S_d min/max P	0.30±0.01 0.27-0.31 -	0.31±0.01 0.29-0.32 ⇔	0.30±0.01 0.29-0.32 ⇔	0.28±0.02 0.23-0.31 ⇔	0.30±0.01 0.29-0.31 ⇔	0.29±0.01 0.28-0.30 ⇔	0.30±0.01 0.28-0.32 ⇔	0.30±0.01 0.30-0.31 ⇔	0.30±0.01 0.28-0.32 ⇔	0.30±0.01 0.29-0.31 ⇔	0.30±0.02 0.26-0.32 ⇔	0.30±0.01 0.28-0.31 ⇔	0.30±0.01 0.29-0.31 ⇔

⇔ = P is not significant (P>0.05).

WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin concentration; Hct = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; pH = blood pH value; PO_2 = blood oxygen tention; [Na] = plasma sodium concentration; [K] = plasma potassium concentration; [Ca] = plasma calcium concentration; [Cl] = plasma chloride concentration; Osmo = total osmolality.

Table 10.14: Mean ($\pm S_d$) haematological and osmoregulation values of *Oreochromis mossambicus* after exposure to zinc, at an acidic pH.

Exposure group	Acid control	Mean zinc concentrations (mg l^{-1}) in the Upper Catchment of the Olifants River determined during the study period, February 1994 to May 1995 (Bamhoorn, 1996; Coetzee, 1996; Kotze, 1997; Nussey, 1998).							
		Summer (0.2099 ± 0.34 mg l^{-1})		Autumn (0.2535 ± 0.69 mg l^{-1})		Winter (0.3674 ± 1.19 mg l^{-1})		Spring (0.8391± 1.86 mg l^{-1})	
		Exposure 17 (0.2099)	Recovering 17	Exposure 18 (0.2535)	Recovering 18	Exposure 19 (0.3674)	Recovering 19	Exposure 20 (0.8391)	Recovering 20
Number of fish (n)	80	10	10	10	10	10	10	10	10
WBC ($X10^3.mm^{-3}$)									
Mean ± S_d	154.85±17.01	135.66±20.08	129.95±18.47	129.36±15.91	116.10±39.64	114.83±27.56	110.55±28.91	126.86±22.82	120.62±26.94
min/max	129.90-177.30	96.40-174.50	101.60-160.50	97.70-151.70	45.40-167.20	73.00-171.70	71.30-165.60	84.20-160.00	64.30-153.90
P	-	<0.05	<0.05	<0.005	<0.05	<0.005	<0.005	<0.05	<0.005
RBC ($X10^6.mm^{-3}$)									
Mean ± S_d	1.93±0.19	1.80±0.20	1.78±0.20	1.97±0.39	1.61±0.47	1.72±0.30	1.73±0.30	1.81±0.24	1.79±0.24
min/max	1.59-2.17	1.43-2.17	1.54-2.15	1.31-2.38	0.98-2.27	1.36-2.36	1.28-2.25	1.43-2.17	1.47-2.18
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔
Hb ($g.dl^{-1}$)									
Mean ± S_d	9.86±1.46	8.38±1.15	8.42±0.99	9.10±1.18	7.42±2.44	8.37±1.41	7.83±1.36	9.17±1.01	8.82±1.29
min/max	7.10-12.20	6.40-10.30	7.00-10.00	7.30-10.60	4.10-11.40	7.00-11.80	5.60-10.20	7.40-10.20	6.40-10.80
P	-	<0.05	<0.05	⇔	<0.05	<0.05	<0.005	⇔	⇔
Hct (%)									
Mean ± S_d	33.01±2.69	33.79±3.40	28.94±3.25	33.33±9.02	25.17±8.83	29.95±7.21	31.53±6.86	28.77±4.32	29.06±6.24
min/max	29.70-37.00	27.70-39.40	24.10-36.00	19.90-44.40	14.20-39.10	19.60-42.10	21.90-45.00	21.70-36.40	19.00-37.00
P	-	⇔	<0.05	⇔	<0.05	⇔	⇔	<0.05	⇔
MCV (μm^3)									
Mean ± S_d	171.90±19.07	188.40±11.44	160.00±12.35	168.50±23.02	154.20±12.80	173.10±17.12	180.60±14.32	158.80±8.09	160.60±16.98
min/max	141.0-201.0	171.0-206.0	145.0-182.0	136.0-200.0	136.0-172.0	144.0-200.0	164.0-200.0	142.0-168.0	128.0-186.0
P	-	<0.05	⇔	⇔	<0.05	⇔	⇔	⇔	⇔
MCH (pg/cell)									
Mean ± S_d	51.51±9.66	46.45±3.22	48.52±5.86	47.03±5.01	45.78±3.82	48.94±3.17	46.26±4.89	51.00±4.63	49.32±4.10
min/max	43.78-69.32	39.89-50.30	41.92-58.33	42.02-58.78	39.05-50.22	42.86-53.85	40.82-57.72	44.24-58.33	43.54-57.67
P	-	⇔	⇔	⇔	⇔	⇔	⇔	⇔	⇔
MCHC (%)									
Mean ± S_d	29.98±4.72	24.80±2.42	29.25±3.25	28.80±6.06	29.76±3.02	29.57±4.80	25.15±2.79	32.20±3.65	30.91±4.12
min/max	22.12-37.54	21.19-27.76	23.06-33.33	23.20-40.53	24.93-34.51	22.58-38.57	21.31-29.72	26.37-37.50	26.33-39.33
P	-	<0.05	⇔	⇔	⇔	⇔	<0.05	⇔	⇔

Table 10.14: (continued)

PH Mean ± S_d min/max P	7.22±0.08 7.08-7.37 -	7.29±0.07 7.19-7.43 ⇔	7.29±0.13 7.05-7.44 ⇔	7.34±0.09 7.22-7.49 <0.05	7.33±0.08 7.19-7.45 <0.05	7.31±0.08 7.16-7.44 <0.05	7.32±0.08 7.22-7.45 <0.05	7.30±0.07 7.19-7.41 <0.05	7.34±0.10 7.22-7.50 <0.05
PO_2 (mmHg) Mean ± S_d min/max P	58.62±23.80 20.90-86.40 -	68.48±28.51 38.20-121.30 ⇔	65.40±27.17 32.10-122.40 ⇔	72.36±49.91 22.90-170.90 ⇔	77.55±37.93 26.00-128.10 ⇔	76.20±37.12 39.20-139.20 ⇔	59.18±32.71 27.40-131.30 ⇔	78.15±37.56 30.10-123.40 ⇔	113.26±48.38 35.20-181.70 <0.005
[Na] (mmol.l^{-1}) Mean ± S_d min/max P	134.20±10.33 118.0-151.0 -	161.40±13.79 138.0-188.0 <0.005	148.20±6.75 136.0-159.0 <0.05	141.90±7.06 132.0-153.0 ⇔	172.10±12.07 154.0-191.0 <0.005	151.10±6.47 143.0-159.0 <0.005	151.80±6.34 143.0-160.0 <0.005	147.20±12.63 125.0-161.0 <0.05	150.70±10.58 128.0-163.0 <0.005
[K] (mmol.l^{-1}) Mean ± S_d min/max P	6.74±1.40 4.40-8.20 -	8.1 ±4.11 3.40-15.01 ⇔	9.51±4.87 4.10-15.01 ⇔	10.05±4.46 4.40-15.01 <0.05	10.70±3.82 6.50-15.01 <0.05	7.63±3.01 5.00-15.01 ⇔	5.24±2.18 2.90-9.70 ⇔	8.17±4.02 4.00-15.01 ⇔	8.18±4.03 3.80-15.01 ⇔
[Ca] (mmol.l^{-1}) Mean ± S_d min/max P	0.75±0.13 0.54-0.99 -	0.76±0.17 0.53-0.99 ⇔	0.75±0.14 0.51-0.98 ⇔	0.62±0.12 0.40-0.78 <0.05	0.68±0.16 0.38-0.90 ⇔	0.65±0.09 0.51-0.80 ⇔	0.75±0.16 0.58-1.01 ⇔	0.70±0.32 0.36-0.75 ⇔	0.62±0.24 0.32-1.08 ⇔
[Cl] (mmol.l^{-1}) Mean ± S_d min/max P	113.60±15.73 88.0-133.0 -	133.80±7.86 115.0-144.0 <0.005	133.50±8.46 123.0-146.0 <0.005	127.30±10.35 114.0-143.0 <0.05	126.50±8.81 114.0-139.0 <0.05	131.20±11.11 108.0-145.0 <0.05	138.80±6.23 130.0-153.0 <0.005	133.20±13.54 108.0-150.0 <0.05	130.00±8.86 115.0-145.0 <0.05
Osmo (osmol.kg^{-1}) Mean ± S_d min/max P	0.28±0.01 0.27-0.30 -	0.30±0.01 0.28-0.31 <0.05	0.30±0.01 0.28-0.31 <0.005	0.29±0.01 0.28-0.30 <0.05	0.28±0.01 0.27-0.29 ⇔	0.29±0.01 0.27-0.31 <0.05	0.30±0.01 0.28-0.31 <0.005	0.30±0.01 0.27-0.31 <0.05	0.29±0.01 0.26-0.31 ⇔

⇔ = P is not significant (P > 0.05).

WBC = white blood cells; RBC = red blood cells; Hb = haemoglobin concentration; Hct = haematocrit; MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean cell haemoglobin concentration; pH = blood pH value; PO_2 = blood oxygen tention; [Na] = plasma sodium concentration; [K] = plasma potassium concentration; [Ca] = plasma calcium concentration; [Cl] = plasma chloride concentration;Osmo=totalosmolality.

10.3.3 Aluminium

After exposure to pH 5.2 and combinations of low pH and different aluminium concentrations, the red blood cell count and the mean haemoglobin concentrations showed insignificant changes, compared to both the control group (A) and the pH 5.2 group (B). The haematocrit and mean corpuscular volume were significantly higher after exposure to 1.5 $mg.l^{-1}$ aluminium at pH 5.2, compared to the control group (A), while the mean corpuscular volume also showed a significant increase after exposure to 1 $mg.l^{-1}$ aluminium at pH 5.2 (Table 10.15). The haematocrit also showed a significant higher value after exposure to 1.5 $mg.l^{-1}$ aluminium at pH 5.2 compared to the pH 5.2 group (B).

Compared to the control group (A), a significant increase ($P<0.05$) in the mean corpuscular volume occurred after exposure to 1.5 $mg.l^{-1}$ aluminium at pH 5.2; a significant decrease occurred after exposure to 1 $mg.l^{-1}$ aluminium at pH 5.2 and a slight decrease occurred after exposure to pH 5.2; 0.06 & 2 $mg.l^{-1}$ aluminium at pH 5.2. Compared to the pH 5.2 exposure group (B), the addition of 1.5 $mg.l^{-1}$ aluminium, caused a significant increase in the mean corpuscular volume, while an insignificant decrease occurred after addition of 0.06, 1 & 2 $mg.l^{-1}$ aluminium (Table 10.15).

After exposure to 0.06 $mg.l^{-1}$, 1 $mg.l^{-1}$ & 1.5 $mg.l^{-1}$ aluminium at pH 5.2, there was a marked increase in the white blood cell count, with the highest value found at 1.5 $mg.l^{-1}$ aluminium at pH 5.2. There was, however, no significant change in the white blood cell count after exposure to 2 $mg.l^{-1}$ aluminium at pH 5.2.

The osmolality showed a significant decrease after exposure to 1 $mg.l^{-1}$, 1.5 $mg.l^{-1}$ & 2 $mg.l^{-2}$ aluminium at pH 5.2 (Table 10.15). The only significant decrease in osmolality, compared to the values obtained after exposure to pH 5.2 (B), was after addition of 2 $mg.l^{-1}$ aluminium. The plasma potassium concentration increased significantly after exposure to 1$mg.l^{-1}$ aluminium at pH 5.2 and after exposure to 2 $mg.l^{-1}$ aluminium at pH 5.2, the concentration was significantly higher than the value obtained after exposure to pH 5.2 (B) (Table10.15). On the other hand, the plasma sodium concentration was significantly lower than the concentrations for the control group (A), after exposure to 1 $mg.l^{-1}$ and 2 $mg.l^{-1}$ aluminium at pH 5.2 (Table 10.15). The mean plasma chloride concentration was significantly lower than the values obtained for the control group (A) as well as the pH 5.2 group (B) after addition of 1 $mg.l^{-1}$ aluminium, but increased again with higher concentrations of aluminium (Table 10.15). The plasma calcium concentration increased significantly after exposure to pH 5.2 and combinations of 0.06 $mg.l^{-1}$ & 1 $mg.l^{-1}$ aluminium at pH 5.2, but a significant decrease was found after exposure to 2 $mg.l^{-1}$ aluminium at pH 5.2 (Table 10.15).

The difference in concentrations and activities of variables involved in the metabolism of *O. mossambicus* were predominantly significant. The glucose concentration increased significantly after exposure to pH 5.2 and 0.06 $mg.l^{-1}$ & 1.5 $mg.l^{-1}$ aluminium at pH 5.2, but compared to the pH 5.2 (B) group, the glucose concentration decreased significantly with the addition of 0.06 $mg.l^{-1}$, 1.5 $mg.l^{-1}$ & 2 $mg.l^{-1}$ aluminium (Table 10.15). Exposure to 1 $mg.l^{-1}$, 1.5 $mg.l^{-1}$ & 2 $mg.l^{-1}$ aluminium at pH 5.2, caused a significant decrease in the pyruvate kinase activity (Table 10.15).

Table 10.15: Mean ($\pm S_D$) haematological, osmoregulatory and carbohydrate metabolism values for *oreochromis mossambicus* after exposure to pH 5.2 and 0.06 mg/l, 1 mg/l, 1.5 mg/l & 2 mg/l aluminium at pH 5.2

Exposure group	Control	pH 5.2	pH 5.2 + 0.06 mg/l	PH 5.2 + 1 mg/l	pH 5.2 + 1.5 mg/l	pH 5.2 + 2 mg/l
Exposure period	96 hours	96 hours	96 hours	96 hours	96 hours	96 hours
Number of fish	32	16	16	16	16	16
RBC ($x10^6$)mm^{-3}						
Mean ± S_d	2.548±0.77	2.80±1.06	2.71±0.98	2.83±0.64	3.07±0.74	2.37±0.58
Min/max	1.35 - 3.75	1.32 - 4.64	1.11 - 4.39	2.01 - 4.3	1.72 - 3.97	1.29 - 3.87
Hb (g/dl)						
Mean ± S_d	16.36±3.35	15.26±3.32	16.81±2.94	18.31±4.8	16.81±3.18	14.26±3.27
Min/max	9 - 21.4	9.9 - 20.4	12.24 - 24.2	9.6 - 25.5	9.9 - 20.3	5.2 - 17.2
Hct (%)						
Mean ± S_d	43.92±14.37	51.43±13.26	48.26±12.75	46.26±13.36	67.56±18.75	46.14±13.73
Min/max	30.15 - 85.3	27.7 - 75.15	27 - 69	26.9 - 75.5	37.2 - 93.3	22.1 - 70.3
MCV (μm^3)						
Mean ± S_d	190.59±25.1	187.28±25.4	181.19±22.24	174.88±14.3	215.81±12.92	187.19±25.17
Min/max	144 - 249.5	147.5 - 239	143.5 - 220.5	152 - 195	194 - 236	133 - 214
WBC ($x\ 10^3$)mm^{-3})						
Mean ± S_d	148±45.51	181.43±59.8	185.04±9.26	182.65±37.5	228.97±67.0	156.10±33.62
Min/max	69.4 - 205.4	14.96 - 90.9	102.5 - 242.5	121.7 - 244.4	115.3 - 367.45	83.75 - 208.55
Osmol (Osmol/kg)						
Mean ± S_d	0.30±0.30	0.29±0.23	0.30±0.01	0.28±0.02	0.28±0.06	0.27±0.02
Min/max	0.28 - 0.41	0.26 - 0.33	0.25 - 0.32	0.23 - 0.30	0.08 - 0.31	0.20 - 0.30
[K] (mmol/l)						
Mean ± S_d	4.25±2.31	3.31±1.09	3.43±1.30	8.06±2.10	3.97±1.27	4.64±1.86
Min/max	1.90 - 8.70	1.80 - 5.10	1.20 - 6.00	4.90 - 13.40	1.54 - 5.90	1.80 - 10.20
[Na] (mmol/l)						
Mean ± S_d	159.31±4.22	158.13±4.80	157.13±11.92	151.88±10.30	99.13±5.29	89.38±8.65
Min/max	150.00 - 167	149.00 - 167.00	119.00 - 172.00	126.00 - 164.00	84.00 - 105.00	64.00 - 100.00

Table 10.15: (continued)

Exposure group	Control	pH 5.2	pH 5.2 + 0.06 mg/l	pH 5.2 + 1 mg/l	pH 5.2 + 1.5 mg/l	pH 5.2 + 2 mg/l
Exposure period	96 hours	96 hours	96 hours	96 hours	96 hours	96 hours
Number of fish	32	16	16	16	16	16
[Cl] (mmol/l) Mean ∀ S_d Min/max	134.69∀11.47 110.00 - 154.00	14.25∀17.89 91.00 - 166.00	127.31∀18.70 88.00 - 163.00	116.60∀19.80 70.00 - 138.00	133.94∀29.92 65.00 - 181.00	127.63∀25.58 78.00 - 191.00
[Ca] (mg %) Mean ∀ S_d Min/max	5.58∀0.87 3.80 - 6.63	10.03∀2.38 6.37 - 15.47	6.85∀2.03 4.04 - 13.29	8.61∀1.77 6.82 - 12.77	4.28∀1.01 3.35 - 6.53	3.88∀0.83 2.39 - 5.12
Glu (mg/100ml) Mean ∀ S_d Min/max	219.91∀43.31 179.6 - 338.79	397.84∀133.5 213.8 - 838.79	257.70∀61.35 182.76 - 375	462.7∀228 239.5 - 980	288.27∀55.87 220.56 - 392.45	230.57∀52.15 175 - 388.1
Lac (mg/100ml) Mean ∀ S_d Min/max	7.44∀3.42 1.38 - 15.32	15.43∀4.66 9.13 - 29.17	13.75∀8.34 6.78 - 35.5	7.58∀4.76 1.79 - 19.3	11.74∀6.57 2.07 - 23.88	14.47∀5.96 2.76 - 23.99
PK (mU/ml) Mean ∀ S_d Min/max	53.64∀7.77 37.21 - 65.52	59.05∀18.82 42.26 - 121.8	50.05∀12.48 34.22 - 69.02	51.14∀35.1 11.28 - 102	39.90∀16.37 17.97 - 71.13	33.87∀18.74 3.06 - 3.06
G-6-P DH (mU/ml) Mean ∀ S_d Min/max	0.62∀0.43 0 - 0.9	1.35∀0.73 0.9 - 2.7	1.13∀0.70 0 - 2.7	7.03∀7.10 0.9 - 22.5	1.63∀1.19 0 - 3.6	2.36∀2.13 0 - 8.1
CHE (U / l) Mean ∀ S_d Min/max	54.25∀32.86 23.46 - 117.3	29.33∀10.49 23.46 - 46.92	48.39∀34.76 23.46 - 140.76	51.76∀32.4 23.46 - 124	21.99∀13.46 0 - 46.92	8.80∀11.73 0 - 23.46

The lactate concentration was significantly higher than the control group values (A), after exposure to pH 5.2, 0.06 $mg.l^{-1}$, 1.5 $mg.l^{-1}$ & 2 $mg.l^{-1}$ aluminium at pH 5.2, but showed a significant decrease if compared to the pH 5.2 group (B) with the addition of 1 $mg.l^{-1}$ aluminium (Table 10.15). The glucose-6-phosphate dehydrogenase activity increased significantly after all the exposures had been performed, with very high values after exposure to 1 $mg.l^{-1}$ aluminium at pH 5.2. The choline esterase activity decreased significantly after exposure to pH 5.2 and 1.5 $mg.l^{-1}$ & 2 $mg.l^{-1}$ aluminium at pH 5.2 (Table 10.15).

10.3.4 Manganese

The differences in the values of haematological, ionic- and osmoregulation and metabolic variables of exposed fish were measured against the control values as determined under controlled laboratory conditions.

The number of white blood cells decreased significantly ($p < 0.05$) after the 0.345 g l^{-1} acute exposure and increased significantly ($p < 0.005$) after the 0.259 g l^{-1} chronic exposure. The number of red blood cells showed a significant decrease ($p < 0.05$) after the 0.345 g l^{-1} acute exposure and a significant increase ($p < 0.05$) after the 0.259 g l^{-1} chronic exposure (Table 10.16).

The haemoglobin concentration decreased significantly ($p < 0.005$) after the 0.345 g l^{-1} acute exposure and resulted in a significant increase ($p < 0.005$) after the 0.259 g l^{-1} chronic exposure (Table 10.16).

The haematocrit decreased significantly ($p < 0.05$) after the 0.345 g l^{-1} acute exposure and increased significantly ($P < 0.05$) after the 0.259 g l^{-1} chronic exposure (Table 10.16).

The mean corpuscle volume showed no significant change after the three acute exposures, but showed a significant decrease ($p < 0.005$) after the 0.259 g l^{-1} chronic exposure (Table 10.16).

After the 0.1732 g l^{-1} acute exposure the plasma sodium concentration ($[Na^+]$) decreased significantly ($p < 0.005$) but after the 0.1732 g l^{-1} chronic exposure, a significant increase ($p < 0.05$) was detected (Table 10.16).

The plasma potassium concentration ($[K^+]$) increased significantly ($p < 0.05$) after the 0. 1732 g l^{-1} acute exposure, followed by a significant decrease ($p < 0.05$) after the 0.259 g 1 and 0.345 g 1 acute exposures. After the 0.1732 g l^{-1} chronic exposure, the plasma potassium concentration also showed a significant decrease ($p < 0.005$), and a significant increase ($p < 0.005$) after the 0.259 g l^{-1} and 0.345 g l^{-1} chronic exposures respectively (Table 10.16).

The plasma calcium concentration ($[Ca^{2+}]$) increased significantly ($p < 0.05$) after the 0.1 732 g/l^{-1} acute exposure, but resulted in a significant decrease ($P < 0.005$) after the 0.259 g l^{-1} and 0.345 g/l^{-1} acute exposures respectively. The chronic exposures showed only significant increases ($p < 0.005$) after the 0.259 g/l^{-1} and 0.345 g/l^{-1} exposures (Table 10.16).

After the 0.1732 g l^{-1} acute and chronic exposures, significant increases ($p < 0.05$) in the plasma chloride concentrations ([Cl-]) were detected (Table 10.16).

Although there were no significant changes in the osmolarity after the acute manganese exposures, there was a significant decrease ($p < 0.05$) in the osmolarity after the 0.345 g l^{-1} exposure (Table 10.16)

The protein concentration showed a significant increase ($P < 0.005$) after the 0.1732g l^{-1} and 0.345 g/l acute exposures and a significant decrease ($p < 0.005$) after the 0.259 g l^{-1} acute exposure. The chronic exposures also resulted in significant decreases ($p < 0.005$)

Table 10.16: Mean haematological, osmoregulation and metabolism values of *Oreochromis mossambicus* after exposure to manganese.

Exposure Groups	Control	Slc 10	Slc 15	Slc 20	Control	Slc 10	Slc 15	Slc 20
Exposure Times	96 hours	96 hours	96 hours	96 hours	28 days	28 days	28 days	28 days
N	10	10	10	10	10	10	10	10
WBC(10^3/mm^{-3})								
Mean ± S_d	144 ± 42	161 ± 30	179 ± 40	98 ± 46	109 ± 25	126 ± 52	178 ± 66	130 ± 84
min/max	69-187	119-203	111-232	60-219	71-157	41-232	76-282	29-237
P	*	*	*	P < 0.05	*	*	P < 0.05	*
RBC(10^6/mm^{-3})								
Mean ± S_d	2 ± 1	2 ± 1	3 ± 1	2 ± 0.2	2 ± 0.3	2 ± 1	3 ± 1	2 ± 1
min/max	1-4	2-3	1-4	1-2	2-3	1-4	1-4	1-4
P	*	*	*	P < 0.05	*	*	P < 0.05	*
Hb(g/dl)								
Mean ± S_d	17 ± 3	17 ± 2	18 ± 2	9 ± 1	9 ± 1	14 ± 3	10 ± 24	9 ± 5
min/max	11-21	14-22	16-20	7-10	6-10	4-17	6-20	4-18
P	*	*	*	P < 0.005	*	*	P < 0.005	*
Hct(%)								
Mean ± S_d	45 ± 16	48 ± 9.7	49 ± 7	30 ± 4	36 ± 10	41 ± 15	38 ± 11	38 ± 19
min/max	32-86	37-69	38-63	24-36	24-62	20-69	23-57	18-70
P	*	*	*	P < 0.05	*	*	P < 0.05	*
MCV(μm^3)								
Mean ± S_d	191 ± 30	198 ± 13	171 ± 16	177 ± 19	171 ± 20	177 ± 17	148 ± 11	169 ± 21
min/max	144-250	174-220	153-206	144-203	147-201	153-199	131-170	149-214
P	*	*	*	*	*	*	P < 0.005	*
[Na^+](mmol/l)								
Mean ± S_d	159 ± 5	141 ± 11	152 ± 5	159 ± 5	145 ± 21	166 ± 12	131 ± 9	143 ± 5
min/max	150-166	121-164	144-161	148-165	101-170	144-183	113-141	137-153
P	*	P < 0.005	*	*	*	P < 0.05	*	*
[Cl^-](mmol/l)								
Mean ± S_d	129 ± 8	141 ± 12	139 ± 15	136 ± 16	177 ± 32	155 ± 25	143 ± 23	148 ± 20
min/max	110-141	115-156	118-164	113-156	111-204	137-206	121-183	96-164
P	*	P < 0.05	*	*	*	*	*	*
[K^+](mmol/l)								
Mean ±S_d	5 ± 3	10 ± 6	3 ± 1	3 ± 1	3 ± 1	2 ± 0.3	6 ± 1	7 ± 2
min/max	2-9	7-26	2-5	2-5	2-5	2-3	5-8	5-10
P	*	P < 0.05	P < 0.05	P < 0.05	*	P < 0.005	P < 0.005	P < 0.005
[Ca^{2+}](mg %)								
Mean ± S_d	5 ± 2	6 ± 1	1 ± 0.2	1 ± 0.1	2 ± 1	2 ± 1	7 ± 1	9 ± 2
min/max	1-7	5-8	0.4-1	1-1	1-4	1-6	5-8	7-12
P	*	P < 0.05	P < 0.005	P < 0.005	*	*	P < 0.005	P < 0.005
OSMO(osmol/kg)								
Mean ± S_d	0.3 ± 0.04	0.3 ± 0.02	0.3 ± 0.01	0.3 ± 0.1	0.3 ± 0.04	0.3 ± 0.03	0.3 ± 0.01	0.3 ± 0.01
min/max	0.3-0.4	0.3-0.3	0.3-0.3	0.3-0.3	0.3-0.5	0.3-0.4	0.3-0.3	0.29-0.3
P	*	*	*	*	*	*	*	P < 0.05

Table 10.16: (continued)

PROT(g/100ml)								
Mean ± S_d	7 ± 0.6	8 ± 1	4 ± 0.2	9 ± 1	7 ± 1	25 ± 2	6 ± 1	5 ± 1
min/max	6-8	6-9	4-5	7-11	6-8	22-27	3-6	4-6
P	*	P < 0.005	P < 0.005	P < 0.005	*	P < 0.05	P < 0.005	P < 0.005
AChE(U/l)								
Mean ± S_d	49 ± 32	21 ± 17	164 ± 132	61 ± 5	58 ± 7	31 ± 1	54 ± 1	63 ± 1
min/max	234-117	0-47	0-422	0-164	0-73	0-78	0-195	0-67
P	*	P < 0.05	*	*	*	*	*	*
GLUC(mg/100ml)								
Mean ± S_d	215 ± 49	292 ± 60	371 ± 56	314 ± 85	106 ± 13	100 ± 15	122 ± 12	121 ± 27
min/max	179-338	207-406	273-464	221-493	94-135	80-122	103-145	83.3-168
P	*	P < 0.005	P < 0.005	P < 0.005	*	*	P < 0.005	*
LACT(mg/100ml)								
Mean ± S_d	8 ± 3	3 ± 2	5 ± 3	18 ± 9	4 ± 0.3	7 ± 0.4	3 ± 0.2	7 ± 1
min/max	5-15	0-7	1-9	9-35	0-9	0.1-14	0-6	0.1-2
P	*	P < 0.005	P < 0.005	*	*	*	*	*
G6P-DH(mU/ml)								
Mean ± S_d	6 ± 7		2 ± 2	6 ± 2	4 ± 3	2 ± 1	11 ± 1	5 ± 4
min/max	1-23	N/A	0-5	2-8	1-10	1-4	2-24	0-10
P	*		*	*	*	*	P < 0.05	*
Pk(mU/ml)								
Mean ± S_d	55 ± 8	83 ± 48	62 ± 45	26 ± 19	9 ± 8	11 ± 1	48 ± 1	8 ± 0.1
min/max	43-66	16-152	25-178	4-54	0-26	10-24	0-325	0-27
P	*	*	*	P < 0.005	*	*	*	*

* = P is not significant (P > 0.05)	[Na^+] = plasma sodium concentration	GLUC = plasma glucose concentration
Slc = sublethal concentration	[Cl^-] = plasma chloride concentration	LACT = plasma lactate concentration
WBC = white blood cell count	[K^+] = plasma potassium concentration	G6P-DH = plasa glucose-6phosphate
RBC = red blood cell count	[Ca^{2+}] = plasma calcium concentration	dehydrogenase
Hb = haemoglobin	OSMO = Osmolarity	Pk = plasma pyruvate kinase
Hct = haematocrit	PROT = plasma protein concentration	
MCV = mean corpuscle volume	AChE = plasma acetylcholine	

after the 0.259 g l^{-1} and 0.345 g l^{-1} exposures as well as a significant increase ($p < 0.05$) after the 0. 1732 g l^{-1} chronic exposure (Table 10.16).

After the 0.1732 g l^{-1} acute exposure, there was a significant decrease ($p < 0.05$) in the plasma acetylcholine esterase concentration but no significant changes after the three chronic exposures (Table 10.16).

The plasma glucose concentration increased significantly ($p < 0.005$) after the three acute exposures, and increased significantly ($p < 0.005$) after the 0.259 g l^{-1} chronic exposure (Table 10.16).

There were significant decreases ($p < 0.005$) in the lactate concentration in the plasma after the 0.1732 g l^{-1} and 0.259 g l^{-1} acute exposures, but no significant changes after the three chronic exposures (Table 10.16).

Although there were no significant changes in the glucose-6-phosphate dehydrogenase concentration after the acute exposures, it resulted in a significant increase ($p < 0.05$) after the 0.259 g l^{-1} chronic exposure (Table 10.16).

Although the chronic exposures showed no significant changes in the pyruvate kinase concentration in the plasma, it resulted in a significant decrease ($p < 0.005$) after the 0.345 g l^{-1} acute exposure (Table 10.16).

10.4 Discussion.

Fish live in close association with their external environment and are sensitive to any changes that may occur within this environment (Casillas and Smith, 1977). Water quality should be considered as one of the major factors responsible for individual variations in haematology (Van Vuren and Hattingh, 1978). Thus, if water quality is affected by pollutants (e.g., copper or zinc) to such an extent that acute stress is attained, it will be reflected in the values of one or more of the haematological variables (Van Vuren, 1986).

The most important function of white blood cells or leucocytes is the immune reaction to protect the body against stressors, including infections, pathogens and chemical irritants (Christensen *et al.*, 1978). The leucocytes of *O. mossambicus* include lymphocytes, which form antibodies during the immunological process, the granulocytes (neutrophils and eosinophils) and monocytes, which take part in the elimination of foreign substances, and lastly the thrombocytes, which play an important role during blood coagulation (Nussey *et al.*, 1995a; 1995c). The increase in leucocytes (leucocytosis) observed during this study, especially after copper "guideline" recoverings, at a neutral pH and after exposure to 0.06 $mg.l^{-1}$, 1 $mg.l^{-1}$ & 1.5 $mg.l^{-1}$ aluminium at pH 5.2, is ascribed to the stimulation of the immune system. Leucocytic conditions ensure that the experimental fish are protected against possible infection due to metal mediated damage of the gill surface (Wepener, 1997). Similar increases were found following exposure of fish to copper (Van Vuren *et al.*, 1994; Nussey *et al.*, 1995b) and zinc (Flos *et al.*, 1987; Wepener, 1990). Differential white blood cell counts indicate that metals appear to induce increases in lymphocytes and eosinophils with concomitant decrease in monocytes and neutrophils (Nussey *et al.*, 1995c). Leucocyte production may be stimulated by the nuclein products of tissue destruction, as in humans (Guyton, 1991). The tissue types that are most likely to be histologically damage in fish, are the gills, kidneys and liver (Van Rensburg, 1989; Wepener, 1990; Gey van Pittius, 1991; Van der Merwe, 1992).

The decreases in leucocyte (leucopaenia) after copper and zinc exposures and recoverings, at the acidic pH and manganese at 0.345 gl^{-1} could be attributed to the bioconcentration of these metals in the liver and kidneys (Agrawal and Srivastava, 1980; Van Rensburg, 1989), as well as a decrease in immature lymphocytes (Srivastava *et al.*, 1979). The kidneys play an important role in the excretion of metals from the blood and bioconcentration of copper (Buckley *et al.*, 1982; Stagg and Shuttleworth, 1982; Van Vuren *et al.*, 1994) and zinc (Holcombe *et al.*, 1979; Wepener, 1990) in the kidney could cause blockage and suppression of the leucopoietic tissue. A similar decreases was observed when *Clarias gariepinus* was exposed to copper (Van Vuren *et al.*, 1994). Decreases in white blood cells were also observed *Colisa fasciatus* (Mishra and Srivastava, 1979) and *Tilapia sparrmanii* (Wepener, 1990) were exposed to zinc.

Leucopaenia may also be a result of increased secretions of corticosteroid hormones. These hormones is a nonspecific response to any environmental stressor (Iwama *et al.*, 1976; Ellis, 1981). A corticosteroid, cortisol, plays an important part to prevent the development of inflammation and also in the healing of inflammation. Cortisol decreases the migration of white blood cell into the inflamed area and also suppresses the immune system by causing a decrease in lymphocyte production, especially the T-cells and antibodies from lymphoid tissue (Guyton, 1991).

Red blood cells or erythrocytes are produced in the haemopoietic tissue, which is situated in the spleen and head kidney (Bond, 1979; Hoffbrand and Pettit, 1980; Smith, 1982; Heath, 1987; Grey and Meyer, 1988; Kita and Itazwa, 1989). The most important function of the red blood cells is that these cells contain haemoglobin which enables them to transport oxygen to all tissues in the body (Hoffbrand and Pettit, 1980; Hibiya, 1982; Grey and Meyer, 1988). Any unfavorable condition, such as hypoxia which was observed in this study, results I a decrease in the amount of oxygen to the tissue, increases the production rate of erythrocyte (erythrocytosis). There is a common element in the response to most, if not all forms of environmental stress (Pickering, 1993). This response involves the hypothalmic-pituitary-interrenal axis and the sympathetico-chromaffin systems, which results in increased secretion of cortisol and catacholamines (Nikinmaa, 1992). In this study, the exposure *of O. mossambicus* to copper or zinc resulted in acute stress which most probably stimulated the release of catacholamines into the circulation (Wepener, 1997). Several distinct, but related, physiological responses take place upon the release of catacholamines, to increase the oxygen carrying capacity of the blood of stressed fish ((Pickering, 1993). The first response was to stimulate the rapid release of red blood cells from the spleen (haemopoietic tissue) into the general circulation (Perry and Kinkead, 1989; Wells and Weber, 1990). Rapid release of red blood cells was evident from the increased red blood cells observed, after exposure to copper (maximum "guideline" exposure and recovering 6 as well as recovering 5) and zinc (recovering 14). Similar increases were observed during other studies after exposure to copper (McKim *et al.*, 1970 - *Salvelinus fontinalis*; Singh and Singh, 1982 - *Mytus vittatus*; Cyriac *et al.*, 1989 - *O. mossambicus*; Gill *et al.*, 1997 - *Barbus conchonius*; Nussey *et al.*, 1995b - *O. mossambicus*) and zinc (Torres *et al.*, 1986 - *Scyliorhinus canicula*; Hilmy *et al.*, 1987 - *Clarias lazera*; Wepener, 1990 - *Tilapia sparrmanii*). These increases attribute to increasing the oxygen carrying capacity in the blood, as adaptation to altered respiratory homeostasis caused by the metal. According to Wepener (1997) these increases were therefore a secondary reaction to the pollutant (metal) and not the result of direct stimulation of the haemopoietic tissue. Changes in red blood cell numbers observed at pH 5.2 and combinations of pH 5.2 and different aluminium concentrations were, however, statistically not significant. The significant increase in erythrocytes (erythrocytosis) after the 0.259 g/l manganese exposure, could be due to oxygen deficiency by epithelial lifting of the gill lamellae (Wepener *et al.*, 1992). Manganese detach the secondary lamellar epithelium and fuse the adjacent secondary lamellae together, decreasing the effective gill area and increasing the diffusion distance from water to blood. This epithelial damage also decreases the permeability of the gills to oxygen. The same reaction was found after the exposure of *C. fasciatus* to chromium (Srivastava *et al.*, 1'979). During the chronic exposure to manganese, the fish developed an oxygen deficiency. The hypoxia which developed during the chronic exposure to manganese probably increased anaerobic respiration and fish thus have a higher carbon dioxide concentration in their blood.

Decreases in erythrocytes (erythropaenia) were observed in *O. mossambicus* especially after the acidic pH and copper exposure (8) and recoverings (7, 8, 9 and 10), as well as after zinc exposure (11 and 15). These decreases could be a result of inhibited production caused by erythrocyte destruction (McLeay, 1973; Srivastava and Narain, 1985). Similar decreases were recorded by Narain and Srivastava (1989) in stressed *Heteropneustes fossilis*, Van Vuren *et al.* (1994) after exposing *Clarias gariepinus* to copper and also by Nussey *et al.* (1995b) after *O. mossambicus* was exposed to copper at different temperatures. Decreases after zinc exposures were recorded by Mishra and Srivastava (1979 - *Colisa fasiatus*) and Spry and Wood (1984 - *Salmo gairdneri*). Erythropaenia could also be attributed to gill damage and impaired osmoregulation during sublethal copper or zinc exposures which

results in haemodilution which leads to a decrease in the number or red blood cells through haemolysis (Nussey *et al.*, 1995b). This causes the fish to become anaemic (Wedemeyer and Yasutake, 1977; Soivio and Viratanen, 1980; Larsson *et al.*, 1985). The development of anaemia in fish can also be attributed to the cell membrane of the red blood cells being altered. This alteration occurs through the hydrolysis of acetylcholine in the body fluids by acetylcholine esterase (Casillas and Smith, 1977). The aggregation of erythrocytes in damaged gills are also known to cause a reduction in the number of circulating red blood cells of stressed fish (Srivastava and Mishra, 1979; Narain and Srivastava, 1989). Nussey *et al.* (1995b) found that compensation is found over a prolonged period of exposure, by the number of erythrocytes being restored after a long-term exposure to copper.

Haemoglobin is a sophisticated oxygen delivery system that provides the desired amount of oxygen to the tissues, under a wide variety of circumstances (Voet and Voet, 1990). The oxygen transport function of the blood is the product of a complex integration of the effects of various physico-chemical factors, such as temperature and the concentrations of allosteric co-factors, dissolved gases, protons and other ions on the oxygen binding properties of haemoglobin (Weber and Lykkeboe, 1978; Weber, 1982). According to Blaxhall and Daisley (1973) the determination of haemoglobin can be a good indication of anaemic conditions. A decrease in haemoglobin concentration, after copper and zinc exposures and recoverings, especially at the acidic pH, aswell as the significant decrease after the 0.345 g/l acute exposure to manganese confirms that anaemic conditions occurred in *O. mossambicus*. Decreases in haemoglobin metal exposures results in haemodilution (Cyriac *et al.*, 1989). Haemodilution is regarded by Smit *et al.* (1979) as a mechanism which reduces the concentration of the pollutant in the circulatory system. This phenomenon has been confirmed for aluminium, copper, manganese and zinc (Torres *et al.*, 1986; Wepener, 1990; Nussey, 1994; Coetzee, 1996; Barnhoorn, 1997). Thus the decreases in haemoglobin concentration signifies that the fish' ability to provide sufficient oxygen to the tissues, is restricted considerably and will result in a decrease of physical activity (Grobler, 1988; Wepener, 1990; Nussey, 1994).

The haemoglobin molecules' most important feature is its ability to combine loosely and reversibly with oxygen and thus represents the oxygen capacity of blood (Guyton, 1991). The significant increases in haemoglobin after the minimum and maximum "guideline" copper exposures and recoverings and after the 0.259 g/l chronic exposure to manganese, were accompanied by increases in the number of erythrocytes. *Oreochromis mossambicus* was subjected to an oxygen tention caused by gill damage and these increases may be due to an increased production by the erythropoietic tissue. This reaction is to elevate the oxygen capacity of the blood in order to supply more oxygen to the tissues. Therefore, this is a mechanism by which the body attempts to absorb more oxygen from the surrounding medium to meet the increased oxygen demand (Buckley, 1977; Cyriac *et al.*, 1989). The body produces more haemoglobin to replace the oxidized or denatured haemoglobin formed as a result of toxic metal exposure. Increases after exposure to copper were also reported by Van Vuren *et al.* (1994), Nussey *et al.* (1995b) and Wepener (1997), whilst increases after zinc exposure were reported by Grobler (1989) and Wepener (1990). Insignificant increases that occurred at the neutral pH, could be an indication that *O. mossambicus* was adapting to the change in the environment (Nussey *et al.*, 1995b). The data obtained in this study showed a slight decrease to 15.3 $g.dl^{-1}$ in the haemoglobin concentration after exposure to pH 5.2, but in contrast to significant decreases in the haemoglobin concentration in the blood of *Tilapia sparrmanii* after exposure to sublethal concentrations of hexavalent chromium (33). With the addition of 1 $mg.l^{-1}$ aluminium, the haemoglobin concentration increased

(maximum of 18.3 $g.dl^{-1}$), which may be due to an increased production by the erythropoietic tissues to elevate the oxygen capacity of the blood. This is a process whereby the body produces an increased amount of haemoglobin to replace the oxidised or denatured haemoglobin, formed as a result of metal exposure.

Haematocrit is an important instrument for determining the amount of plasma and corpuscles in the blood (measurement of packed erythrocytes) and is used to determine the oxygen carrying capacity of blood (Larsson *et al.*, 1985). It is also defined as the volume occupied by erythrocytes in a given volume of blood and is usually measured as the number of erythrocytes per 100m*l* of blood. The haematocrit reading is valuable in determining the effect of stressors on the health of fish (Wedemeyer and Yasutake, 1977; Munkittrick and Leatherland, 1983). The significant increases in haematocrit observed after "guideline" recovering 5 and 6 (copper), exposure to 1.5 $mg.l^{-1}$ aluminium at pH 5.2, and chronic exposure to 0.259 $g.\ l^{-1}$ may be attributed to an increase in the erythrocytes due to catecholamine-induced mobilization from the spleen, swelling of the erythrocytes due to osmotic shifts (Witters *et al.*, 1987), or a decreased plasma volume caused by fluid shifts (Milligan *et al.*, 1982). Thus a high haematocrit would imply polycythaemia induced by stress, haemoconcentration due to gill damage and dehydration (Wedemeyer and McLeay, 1981; Larsson *et al.*, 1985). In contrast the significant decreased haematocrits reported after recovering 7, 8, 10 (copper), and exposure 15, 20, recovering 17, 18 (zinc) at a acid pH, after zinc exposure 15 at the neutral pH. as well as after the 0.345 g/1 acute manganese exposure, the low haematocrits would indicate anaemia and haemodilution. This is probably the result of gill damage or impaired osmoregulation (Larsson *et al.*, 1985).

The mean corpuscular volume (MCV) is an indication of the size or status of red blood cells and reflects an abnormal or normal cell division during erythropoiesis (Larsson et al., 1985). Significant decreases were recorded after copper exposure and recovering 2, 3 and 4 as well as after zinc exposure 16, at the neutral pH. Decreases were also observed at the acidic pH, after recovering 8 (copper), exposure 17 and recovering 18 (zinc), and exposure to 1 $mg.\ l^{-1}$ aluminium. These decreases in MCV indicate that erythrocytes have shrunk. The shrinking may be due to hypoxia, stress or impaired water balance, microcytic anaemia (Larsson *et al.*, 1985) or a large concentration of smaller immature erythrocytes that have been released from the erythopoietic tissue to counteract the pathological action of copper (Nussey, 1994) or zinc (Wepener, 1990). Immature erythrocytes are released to replace damaged red blood cells or to elevate the oxygen carrying capacity of blood in order to relief the hypoxic conditions experienced by *O. mossambicus* (Nussey *et al.*, 1995b). Van Vuren (1986) found similar decreases when *Labeo umbratus* was exposed to various pollutants. Insignificant increases in MCV show that erythrocytes have swollen through impaired water balance (osmotic stress) macrolytic anaemia.). The significant ($P<0.05$) increase in the mean corpuscular volume after exposure to 1.5 $mg.l^{-1}$ aluminium at pH 5.2, on the other hand, may be due to swelling of the erythrocytes due to impaired water balance (osmotic stress) or hypoxia setting in(Larsson *et al.*, 1985), or hypoxic conditions setting in Wepener *et al.*, 1992a; Van Vuren *et al.*, 1994; Nussey *et al.*, 1995b).

Increases in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) clearly indicate that the concentration of haemoglobin in red blood cells were higher in the exposed and recovering fish than in the control fish. The slight increases and decreases observed in MCH and MCHC, after copper and zinc exposures and recoverings at the neutral and acidic pH, could be ascribed to a disproportional decrease in red blood cells, haemoglobin, haematocrit and mean corpuscular volume from the control values. This

appears to be the only logical explanation, because the MCH and MCHC are calculated to indicate red blood cell swelling (Milligan and Wood, 1982). Significant increases, after copper exposure (1 and 2) as well as recoverings (2 and 6) and zinc exposure (12 and 15) at the neutral pH, indicate that the haemoglobin concentration per red blood cell increases. The MCHC is a good indicator of erythrocyte swelling (Wepener *et al.*, 1992a; 1992b). According to Soivio and Nikinmaa (1981) the MCHC is the ratio of the blood haemoglobin concentration as opposed to the haematocrit, and it is not influenced by the blood volume nor by the number of cells in the blood. The MCHC can be incorrectly interpreted only when new cells, with a different haemoglobin concentration, are released into the circulating blood. Significant decreases reported in MCH and MCHC, after zinc exposure 17 and recovering 19 at the acidic pH, were indications of erythrocyte swelling as well as decreases in haemoglobin synthesis (Nussey *et al.*, 1995b). Whilst significant increases in MCHC, observed after exposure 1, 2 , 4 and recovering 3, 4 (copper) as well as exposure 15 and 16 (zinc), were probably indicative of polycythaemia due to stress, haemoconcentration due to gill damage or dehydration (Larsson *et al.*, 1985).

The copper and zinc damages gill membranes which result in hypoxia (Sellers *et al.*, 1975; Singh and Singh, 1982; Singh, 1985; Mallat, 1985; Grobler *et al.*, 1989b; VanVuren *et al.*, 1994). In fish suffering from hypoxic stress blood acidosis commonly occurs (Thomas and Hughes, 1982). An increase in cellular respiration resulted in an increase in the carbon dioxide levels in the blood (Van Vuren, 1977). This elevated level led to an increase in the blood lactic acid concentration (Wepener, 1990), which would cause a decrease in blood pH, as observed after copper and zinc exposures and recoverings at the neutral pH. According to Hughes (1981) fishes hyperventilate during hypoxic conditions, in an effort to regain normal pH. This is responsible for the increases in blood pH, recorded After copper and zinc exposures and recoverings at the acidic pH. Blood alkalosis may also be the result of ammonia accumulation, chloride-bicarbonate alterations, and electroneutral sodium-hydrogen exchanges at the gill surface (Spry and Wood, 1984).

The toxic mode of action of copper or zinc at acute concentrations have been shown to involve damage to gill tissue and subsequent servere arterial hypoxia (Sellers *et al.*, 1975). Exposure to copper and/or zinc caused some reduction in the amount of oxygen available to fish tissues. The principal route of oxygen in fish is from the water through the gills, to the blood. The lowered oxygen tention (PO_2) observed, suggests that some type of block occurs in the gills, this was also observed by Skidmore (1970). The increased PO_2 values do not necessarily indicate that interruption of oxygen transfer across the gill surface can be discounted because experimental fish were subjected to internal hypoxia. According to Wepener (1997) possible explanations for the increase in PO_2 values include a diffusive limitation at the gill (Spry and Wood, 1985) as well as utilization by the tissues (Zaba and Harris, 1978). Increased PO_2 values argues against a diffusive limitation, but studies on effects of zinc by Spry and Wood (1985) revealed that high PO_2 values occurred dispite diffusive limitations. These authors attribute this to reduced blood oxygen capacity due to progressively developing acidosis (i.e., Root effect).

Some of the most interesting adjustments that fishes of all kinds must make in their particular environments, concern the maintenance of proper water and salt balance in their tissues (Bond, 1979). Osmoregulation is the process by which the total electrolyte content and water volume in an organism are held relatively constant (Heath, 1987). Changes in osmoregulation in fish exposed to stressors, such as metals are generally elucidated by measuring the blood plasma sodium, potassium, calcium, chloride, and/or total osmolality

(Burton, 1986). It is important for the fish to be able to maintain water and ion homeostasis and thereby survive in a changing environment. A disturbed osmotic and ion regulation may affect the ability of the fish to function normally. Regulation of osmotic concentrations (plasma ion concentrations) is accomplished by the gills, kidneys, some special organs and the integument, to some extent, in its role as barrier (Bond, 1979). Critical loss of body electrolytes, reduces the osmotic concentration and leads to the loss of water, which in turn leads to haemoconcentration and circulatory collapse. Survival through acute challenges therefore relies upon the fish's ability to reduce ion efflux while restoring sufficient uptake, to maintain body ion levels.

During sublethal exposures to copper and zinc, gills as well as other organs such as the kidneys and the intestine undergo histological alterations or necrosis (Baker, 1969; Skidmore, 1970; Wepener, 1990; Van der Merwe, 1992). Because these organs play a part in the osmoregulation, it can be expected that any histological alterations will inevitably cause changes in the osmotic regulation of fish (Lewis and Lewis, 1971; Sellers *et al.*, 1975). .It is important for the fish to be able to maintain water and ion homeostasis and thereby survive in a changing environment

The results of this project confirm previous findings in showing that low ambient pH alone and in combination with aluminium, copper, zinc and manganese exposures produce disturbances to ion balance (Muniz and Leivestad, 1980:Neville, 1985).

The significant decreases in the plasma sodium concentration ([Na]). after copper and zinc exposures and recoverings at the neutral pH, chronic exposures to 0.1732g l^{-1} manganese and exposures to 1.5 mg.l^{-1} & 2 mg.l^{1} aluminium at pH 5.2 were probably the result of the stimulation of concentration dependent sodium losses (Lauren and McDonald, 1986). Decreases can be attributed to the nett loss of sodium through damaged gills and kidneys. Structural changes in the gills and kidneys, induced by copper and zinc can cause the excretion of sodium. The uptake of sodium from the environment does not take place, because of the inhibition of the Na^+-K^+-ATPase enzyme in the gills (Heath, 1987). The Na^+-K^+-ATPase enzymes play an important role in the kidney, where glomerular filtration and reabsorption are important facets of osmoregulation. Reabsorption of sodium via the renal tubules is reduced, as a result of the inhibition of Na^+-K^+-ATPase in the kidney and intestine (Kuhnert *et al.*, 1976). Similar decreases where found by Lorz and McPherson (1976) when *Oncorcynchus mykiss* was exposed to copper and zinc, Stagg and Shuttleworth (1982) when *Platichthys flesus* was exposed to copper, Wepener (1990) when *Tilapia sparrmanii* was exposed to zinc and Nussey *et al.* (1995b) when *O. mossambicus* was exposed to copper. In contrast to these decreases, the significant increases after the acidic exposures and recoverings to copper and zinc, reflects both haemoconcentration and an efflux of potassium ions from the intracellular compartment of white muscle (Wood and Donald, 1982). Wepener (1990) suggests that it is possible that the initial exposures led to displacement of sodium and calcium as substrate from Na^+-K^+-ATPase and Ca^{2+}-ATPase in the gills. These increases can, therefore be attributed to "accidental active uptake". This explanation has been suggested by Wright (1980) as the uptake mechanism of ions by freshwater amphipods. Increases in [Na] where also found by Wepener (1997) within the first six hours, when *Tilapia sparrmanii* was exposed to a Cu, Fe and Zn mixture. Increases in [Na] also causes haemoconcentration due to disturbed water balance (Larsson *et al.*, 1985).

Potassium is the principal cation and is intimately involved in nerve and muscle function (Arms and Camp, 1987). The significant increases ($P<0.05$) in the plasma potassium

concentration ([K]) (exposures 3, 4, 6 and recovering 2, 4 (copper), and exposure and recovering 18 (zinc) at neutral pH; after copper exposure 9 and 10 at the acidic pH; after exposure to 1 $mg.l^{-1}$ aluminium at pH 5.2; after 0. 1732 g/1 acute, 0.259 g/1 and 0.345 g/1 chronic exposures), were commonly observed in acid stressed fish (Booth *et al.*, 1988). In all probability increases at the neutral pH occurred to abolish the osmotic differences in the intracellular fluid caused by the decrease in sodium. Furthermore, the precipitation of manganese in the form of carbonates and oxides on the gills may stop the reabsorption of potassium (Nix & Ingols, 198 1). Also found by Grobler (1988) and Wepener (1990) after *Tilapia sparrmanii* was exposed to zinc, and Nussey *et al.* (1995b) after *O. mossambicus* was exposed to copper. Thus this increase in [K] can be ascribed as osmotic adaptation (Brenner *et al.*, 1976). Whilst increases at the acidic pH reflect haemoconcentration and an efflux of potassium ions from the intracellular compartment of the white muscle (Wood and McDonald, 1982). These increases can also be as a result of the exchanging of potassium ions with the hydrogen ions at cellular level to get rid of the excess hydrogen ions (Coetzee, 1996). Significant decreases in[K], after recovering 7 (copper) and recovering 11 (zinc) can be ascribed to ion loss via the urine, with subsequent decrease influx due to inhibition of Na^+-K^+-ATPase in the gill membrane (Lauren and McDonald, 1985).

The significant decrease in the potassium concentration, in the acute and chronic exposures, could be due to potassium loss in the urine through the damaged kidneys (Wepener, 1990). This condition, known as hypokalemia, could cause muscle weakness, irritability, paralysis as and disturbed heart activity in mammals.

Physiological functions of calcium include: ion regulation and membrane permeability (Wedemeyer and Yasutake, 1977; Soivio and Virtanen, 1981), muscle and nerve cell function (Gregory and MacFarlane, 1981), skeletal bone metabolism (Heath, 1987), and blood coagulation (Larsson *et al.*, 1985). It has been reported that the toxicity of metals is counteracted by calcium and other antagonistic metallic cations (Lewis and Lewis, 1971). Copper, zinc and aluminium caused in general, no drastic changes in the plasma calcium concentration ([Ca]). The significant decreases after zinc exposure 18 (pH 5.2), exposure to 2 $mg.l^{-1}$ aluminium at pH 5.2 and 0.239g l^{-1} as well as 0.345g $^{-1}$ acute manganese exposure could be due to impaired tubular reabsorption in the kidney, or impaired intestinal uptake of calcium (Larsson *et al.*, 1985). According to Eddy (1981) a low [Ca] may lead to an increase in membrane permeability. Significant increases in plasma calcium concentrations (hypercalcemy noted after aluminium exposure to pH 5.2, 0.06 $mg.l^{-1}$ (pH 5.2) & 1 $mg.l^{-1}$ (pH 5.2) as well as after the 0.1732 gl^{-1} acute, 0.259 g l^{-1} and 0.345 gl^{-1} chronic exposures to manganese might be the result of an increase in the secretion of parathormone by the parathyriod. This causes the withdrawal of calcium from bone, resulting in an increase in the plasma calcium concentration. Increases in calcium could also be caused when calcium is displaced from the bronchial areas by cations such as Mn^{2+} (Eddy, 1981; McDonald *et al.*, 1989 Wepener, 1990).. Significant and insignificant increases in [Ca] is probably caused when calcium is displaced from the branchial areas by divalent cations, such as copper or zinc (Eddy and Bath, 1979). This is also reflected in the decreases of [Na] and [Cl] (Nussey *et al.*, 1995b).

The significant decreases in plasma chloride concentration ([Cl]), after exposure and recovering 4 of copper at the neutral pH as well as after exposure to 1 $mg.l^{-1}$ aluminium at pH 5.2, may be attributed to the netto loss of chloride by the damaged gills or an excretion of chloride ions via the kidney. Also reported by Nussey *et al.*(1995b) after copper exposures. In contrast to this decrease the significant increases in [Cl] after copper exposure and

recovering 7 (acidic pH), as well as after zinc exposure 14 (neutral pH), all zinc exposures and recoverings (17, 18, 19 and 20) at the acidic pH as well as 0.1732g l^{-1} manganese exposures are probably caused by the stimulation of the bicarbonate chloride-exchange mechanism in the kidney (Pitout and Smit, 1988). Due to the osmotic imbalance in the sodium concentration, chloride retention has occurred to abolish the equilibrium (Wedemeyer and Yasutake, 1977).

Significant decreases in the total osmolality were reported after copper exposure 1, 2, 3, 4 and 5 as well as recovering 4. These decreases can be associated with a rise in tissue water content (Heath, 1987) and blood volume (Courtois and Meyerhoff, 1975). These changes suggest that the gills of *O. mossambicus* in copper have become more permeable to water which enters the body osmotically (Heath, 1984). Increased gill permeability was most probably due to the displacement of Ca^{2+} from the branchial areas, discussed earlier. Similar decreases were also reported by Christensen *et al.* (1972), Nussey *et al.* (1995b) and Wepener (1997), after sublethal copper exposures. The significant increases in total osmolality after exposure to copper (exposure and recovering 7) and zinc (exposure 17, 18, 19, 20 and recovering 17, 19) at the acidic pH could be attributed to haemoconcentration (Wepener, 1997) and increased sodium, chloride and/or calcium concentrations.

Plasma proteins in fish are homologous to those of mammals and play the dominant role in metal transport in mammals and in fish (Roesijadi & Robinson, 1994). According toLarsson *et al* (1985), the decrease in plasma protein concentrations could be due to haemodilution, kidney damage and certain liver damages. Agrawal & Srivastava (1980) found kidney damaged after exposing *C. fasciatus* to manganese. Therefore, the drastic decreases after these exposures could be due to kidney damage. Wepener (1990) also found a decrease in protein concentration of *Tilapia sparmanii* after chronic manganese exposure. The increases, after the acute and chronic exposures, could be due to impaired water balance (Larsson et al.,1985). The increase in protein concentration going with a loss of water to overcome the ionic imbalance (Soivio & Virtanen, 1980).

Glucose occupies a central role in metabolism, both as a fuel and as a precursor of essential structural carbohydrates and other biomolecules. The significant increases in blood glucose levels after exposure to pH 5.2 and after all three acute manganese exposures reveal a disruption in carbohydrate metabolism, which is probably a hypophysis-adrenal response (Christensen *et al.*, 1972). This is a stress response, involving an increase in blood sugar with the eventual secretion of glucocorticoids and catecholamines (Nath and Kumar, 1987). Catecholamines may deplete glycogen reserves in stressed fish by stimulating glucogenolysis and gluconeogenesis (Heath, 1987). Exposure to pH 5.2 caused a significant increase in the blood glucose levels. It appears that at this low aluminium concentration, the toxicity of the acid was reduced rather than increased. This was also found by Neville (1985), with exposure of juvenile rainbow trout, *Oncorhynchus mykiss* exposed to pH 4 and 2.8 µM inorganic aluminium, where it appeared that the low concentration of aluminium accumulated on the gill tissue at that pH, afforded some protection to the gill epithelium. Exposure to 1 $mg.l^{-1}$ & 1.5 $mg.l^{-1}$ at pH 5.2, once again caused a significant increase in the blood glucose levels, while exposure to 2 $mg.l^{-1}$ aluminium at pH 5.2 showed no significant change. This could be due to the possibility that the aluminium formed polymers too large to adhere closely to the gill epithelium. In reaction ten of glycolysis, the enzyme pyruvate kinase (PK) couples the free energy of phospho-enolpyruvate hydrolysis to the synthesis of ATP to form pyruvate, which, through gluconeogenesis, is converted back to glucose. The optimum pH for pyruvate kinase is in the range of pH 7.2 - 7.8 (Randall and Anderson,

1975). It is therefore clear that the addition of 1 $mg.l^{-1}$, 1.5 $mg.l^{-1}$ & 2 $mg.l^{-1}$ aluminium exacerbated the effect of the low pH on the pyruvate kinase activity, by causing significant decreases in the activity of this enzyme. The decrease in enzyme activity in the plasma shows a possibility that there may be an increase in the pyruvate kinase activity elsewhere. This may be an indication that there is an increase in glycolysis in the muscle tissue of the fish (Knox *et al.*, 1980) The non-carbohydrate precursors that can be converted to glucose, include the glycolysis products lactate and pyruvate, citric acid cycle intermediates and most amino acids.

Significant increase in lactate concentrations after exposure the metals conerned implies an increase in anaerobic metabolism (Heath, 1987), which can occur as a result of gills damaged by aluminium, manganese and the acid medium of the environment. This hypoxic state, was also found with exposure to hexavalent chromium, when anaerobic cell respiration takes place with muscle glycogen as fuel (Van Waarde *et al.*, 1983). During aerobic conditions, the pyruvate formed by glycolysis is further oxidised by the citric acid cycle and oxidative phosphorylation to CO_2 and water. During anaerobic conditions, however, the pyruvate is converted to a reduced end product, which is lactate (homolactic fermentation). Much of the lactate is exported from the muscle cells and carried by the blood to the liver, where it is reconverted to glucose.

Normally the lactate produced diffuses from the tissue and is transported through the bloodstream to highly aerobic tissues, such as the heart and liver. The aerobic tissue can catabolise lactate, through respiration, or can convert it back to glucose, through gluconeogenesis. However, if lactate is produced in large quantities, it cannot be readily consumed. Then the blood pH falls and the Bohr-effect functions to increase oxygen supplies to the tissues (Voet and Voet, 1990). The decrease of pH in the capillaries lowers the oxygen affinity of haemoglobin, allowing even more efficient release of the last traces of oxygen. Lactate accumulation in animals thus occurs when the need for tissues to generate energy exceeds their capacity to oxidise the pyruvate produced in glycolysis.

The activity of glucose-6-phosphate dehydrogenase, which is the key regulatory enzyme in the pentose-phosphate pathway, was determined, in order to determine the possible effect of low pH and aluminium, and manganese on this pathway. In the pentose phosphate pathway, additional energy rich compounds are produced. Glucose-6-phosphate dehydrogenase oxidises glucose-6-phosphate to fructose-6-phosphate, which is converted to pyruvate during glycolysis, which in turn is converted to glucose. The toxicological action of aluminium and manganese are mainly confined to the gills and there may be a higher need for energy in the gills for the active regulation of the osmotic balance of the fish. Therefore there may be an increase in glucose-6-phosphate dehydrogenase activity in the gills to produce this additional energy for the osmoregulatory process (Bhaskar and Govindappa, 1985). This is probably the reason for the increase in glucose-6-phosphate dehydrogenase activity in *O. mossambicus* after exposure to pH 5.2 and 0.06 $mg.l^{-1}$, 1 $mg.l^{-1}$, 1.5 $mg.l^{-1}$ and 2 $mg.l^{-1}$ aluminium at pH 5.2, and 0.259g l^{-1} manganese Exposure to low pH and combinations of low pH and different aluminium concentrations, caused little activity in the fish. Increases correspond with significant increases in red blood cell numbers after metal exposures. Usually, a decrease in red blood cell numbers result in a decrease in glucose-6-phosphate dehydrogenase activity (Fairbanks, 1967).

Acetylcholine is a transmitter for the chemical transmittance of nerve impulses that is released from the presynaptic membrane and causes a postsynaptic potential (PSP). The

magnitute of the PSP is related to the amount of acetylcholine released. The system could not function, however, unless the acetylcholine was rapidly removed again, for otherwise it would gradually accumulate and maintain a continuous PSP. Acetylcholine is broken down by the enzyme acetylcholinesterase. Acetylcholinesterase plays an important role in the regulation of nerve impulse transmission at the cholinergic synapses (Schmidt-Nielsen, 1990).

Acetylcholine esterase may indicate the presence of particular groups of pollutants as opposed to a generalised response (Heath, 1987). During this study, manganese exposures and exposures to pH 5.2 and 1.5 $mg.l^{-1}$ & 2 $mg.l^{-1}$ aluminium at pH 5.2 caused a significant decrease in the acetylcholine esterase activity. When acetylcholine esterase is inhibited, the accumulation of acetylcholine desensitises the membrane receptors, which after an initial period of repetitive firing can no longer respond, so that neuromuscular transmission fails. Nerve impulse transmission is therefore blocked at cholinergic synapses. Locomotor activity would therefore be reduced to such an extent that the fish remained motionless at the bottom of the tank, as observed during the exposures.

10.5 References

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11. Effects of Coal Mining Effluent on the Number and Species Diversity of Macroinvertebrate Fauna in the Upper Olifants River Catchment.

11.1 Introduction

The upper catchment area of the Olifants River is being subjected to increased mining and agricultural activities, industrial development and urbanization. As a result of this, the water quality of the Olifants River and some of its tributaries has been deteriorating since 1983. This causes reason for concern as one of the downstream users in the Olifants River Catchment area is the Kruger National Park. The Kruger National Park requires water of good quality to sustain its terrestrial and aquatic ecosystems. It is therefore necessary to determine to what extent activities upstream of the Olifants River, especially in the Witbank, Middelburg and Phalaborwa areas, influence the water quality of the Olifants River (Van Vuren *et al.*, 1995).

11.2 Materials and Methods

This study is part of a larger project for the Water Research Commission, Report No. K5/608 : Lethal and sublethal effects of metals on the physiology of fish : an experimental approach with monitoring support. This study was conducted in the upper reaches of the Olifants River and Klein Olifants River between Davel and Middelburg in coal mining areas from March 1993 until February 1994. The aim of this study was to investigate the effect of coal mining effluent on the numbers and species diversity of macroinvertebrate fauna.

For the purpose of the macroinvertebrate fauna sampling, a total of fourteen localities (Figure 6.1) were chosen where seasonal sampling were done. A locality X, situated on the East Rand (receiving organic and industrial effluent) was chosen as a reference site. A comparison was made between the number and species of macroinvertebrates found at locality X and those organisms found at the Olifants River and to establish values for large and small numbers of organisms. Sampling procedures and further analysis of the macroinvertebrate fauna were conducted according to standard techniques (Chapter 2).

11.3 Results

11.3.1 Identification and distribution of macroinvertebrates.

Data of the macroinvertebrates sampled are given in Tables 11.1 to 11.4. Each table portrays the quantitative presence of macroinvertebrates for a specific season.

Summer

Table 11.1 summarizes the number and diversity of benthic organisms sampled during summer.

Locality 1 - At this locality there is organic enrichment of the system (mainly from the surrounding farming area) and algae. Benthic macroinvertebrates consist of Tubificidae (*Tubifex* and *Limnodrilus*) and Chironomidae (*Chironomus*) while other aquatic organisms present were Cladocera, Copepoda, Baetidae (*Baetis*), Lestidae (*Lestes* - damselfly nymph), Corixidae (waterboat men) and Ceratopogonidae (*Culicoides*).

Locality 2 - This locality at Van Dycks Drift receives some mining and thermal effluent from the surrounding mines and the power station, and some organic enrichment due to the degradation of plant materials. Only a few water organisms such as Cladocera and Copepoda were present and the benthic organisms present were *Limnodrilus* (Tubificidae) and *Chironomus* (Chironomidae).

Locality 3 - (Steenkool Spruit) Organic enrichment of the system (cattle grazing in the vicinity and an informal settlement) and silt and algae contribute to the muddiness of the water. Organisms present at this locality included *Limnodrilus* (Tubificidae), Cladocera, Copepoda, *Sigara* (Corixidae), Pyralidae (Lepidoptera), *Hydroporus* (Dytiscidae), *Chironomus* (Chironomidae) and *Culicoides* (Ceratopogonidae).

Locality 4 - Koring Spruit receives some organic enrichment form the degradation of plant materials, cattle grazing near the river and recreational activities such as angling. The presence of the Tubificidae (*Tubifex*, *Limnodrilus* and *Branchiura sowerbyi*) and Chironomidae (*Chironomus*) confirms the fact that there is some form of organic enrichment of the system at this locality. Other water organisms present were leeches, water fleas, copepods and waterboat men (Corixidae - Hemiptera).

Locality 5- This locality represents a stream entering a mining area. A few aquatic earthworms, leeches, copepods and biting midges were present at this locality.

Locality 6- This locality is situated in the Boesman Spruit downstream from a mining area. Degradation of plant material (organic pollution) and high values for phosphates and nitrates may have contributed to the few Chironomidae and other water insects present at this locality.

Locality 7- presents a small number of benthic macroinvertebrates such as *Limnodrilus* (Tubificidae - aquatic earthworms), *Chironomus* (Chironomidae - midges) and *Culicoides* (Ceratopogonidae - biting

Table 11.1 The number and diversity of macroinvertebrate larvae sampled during summer 1994/1995

ORGANISMS / LOCALITY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Annelida (Aquatic earthworms)														
Oligichaeta														
Haplotaxida														
Tubificidae														
Tubifex	195	-	-	877	-	-	-	-	-	146	97	-	292	-
Limnodrilus	877	9740	2435	-	7938	-	584	-	1753	633	877	1169	1315	3799
Branchiura sowerbyi	-	-	-	97	-	-	-	-	-	-	-	731	195	584
Hirudinae (Leeches)														
Rhynchobdella														
Glossiphoniidae	-	-	-	-	-	146	-	-	-	-	-	-	-	-
Helobdella	-	-	-	1315	-	-	-	-	2240	-	-	-	-	-
Cladocera (Waterfleas)	1266	1071	3603	974	-	-	-	-	3068	-	-	-	-	97

Table 11.1: (continued)

Copepoda	2143	146	1266	3117	292	49	779	-	4334	-	-	146	584	244
Ostracoda (Seed shrimps)	-	-	-	-	-	-	-	-	1899	-	-	-	-	-
Ephemeroptera (Mayflies)														
Baetidae														
Baetis	195	-	-	-	-	-	-	-	487	146	97	-	146	2045
Odonata (Dragon-, damselflies)														
Anisoptera														
Libellulidae														
Orthemis	-	-	-	-	-	-	-	292	-	-	-	-	-	-
Plathemis	-	-	-	-	-	-	-	-	-	49	-	-	-	-
Gomphidae														
Gomphus	-	-	-	-	-	-	-	-	-	-	-	-	49	-
Zygoptera														
Lestidae														
Lestes	49	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemiptera (Bugs)														
Corixidae	97	-	-	-	-	97	-	-	-	-	-	-	-	-
Sigara	-	-	390	877	-	49	-	-	-	-	-	-	-	341
Trichoptera (Caddis flies)														
Hydroptilidae	-	-	-	-	-	-	-	-	-	97	-	-	-	-
Hydroptila	-	-	-	-	-	-	-	-	146	-	-	-	-	49
Lepidoptera (Aquatic caterpillars)														
Pyralidae	49	-	-	-	-	-	-	-	-	-	-	-	-	-
Coleoptera (Beetles)														
Dytiscidae	97	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroporus	-	-	195	-	-	-	-	-	-	-	-	-	-	-
Diptera (Flies,Mosquitoes,Midges														
Culicidae	-	-	-	-	-	-	49	-	-	-	-	-	-	-
Culex	-	-	-	-	-	-	-	-	-	97	-	-	-	-
Simulidae	-	-	-	-	-	-	-	-	974	-	-	-	-	-
Chironomidae (Midges)														
Chironomus larvae	1753	438	1023	3312	-	244	925	14610	2629	1315	2484	49	487	5601
Chironomus pupae	-	49	-	-	-	-	-	-	97	97	49	-	49	146
Pentaneura	-	-	-	-	-	-	-	-	-	-	-	-	-	390
Ceratipogonidae (Biting midges)														
Bezzia	97	-	292	-	49	244	49	-	-	-	-	49	49	49
Gastropoda (Snails, Limpets)														
Pulmonata														
Physidae	-	-	-	-	-	-	-	-	438	-	-	-	-	-
Pelecypoda (Clams, mussels)														
Sphaeriidae	-	-	487	-	-	-	-	-	-	-	-	244	-	-

midges). Other aquatic organisms present were Copepoda (copepods) and *Culex* (Culicidae - mosquitoes). The small number of organisms present at this locality could be due firstly to organic effluent from the Naauwpoort Sewage Works, secondly to recreational activities at Witbank Dam during summer, and thirdly to thermal pollution from the Duva Power Station.

Locality 8 - (Suur Stream) At this locality organic enrichment of the system occurs by degradation of plant material and industrial effluent from the nearby industries that enters the river. Low pH,

sometimes as low as 3, and high levels of iron and nitrate occur in the system. The only benthic organisms surviving in this system are a few Odonata and Chironomidae.
Locality 9 - The Olifants River receives some effluent from the Suur Stream, as well as effluent from sewage works in Witbank (organic enrichment). There are many algae present in the system, along with high levels of nitrates and nitrites. Benthic organisms present at this locality were Tubificidae, Hirudinae, Cladocera, Copepoda, Ostracoda (seed shrimps), Ephemeroptera, Trichoptera, Culicinae (mosquito larvae) and Chironomidae (larvae and pupae).

Locality 10 - The Spook Spruit system is affected by effluent from mines, brick-works and surrounding farming areas. Tubificidae were present in the system while water insects such as *Baetis* (Ephemeroptera), *Gomphus* (Odonata), *Hydroptilidae* (Trichoptera) and *Culex* (Diptera) occurred in small numbers.

Locality 11 - At Olifants River Lodge, organic enrichment is caused by waterfowl. Only a few Tubificidae, Chironomidae and Ephemeroptera were present.

Locality 12- (Woesalleen) An organically enriched system (cattle grazing near the river) receiving mining and thermal effluent from the nearby mines and the Arnot Power Station respectively. The only benthic organisms present at this locality were a few Tubificidae, Copepoda, Chironomidae and Ceratopogonidae.

Locality 13 - At Middelburg Dam, there are many recreational activities during summer which affect the occurrence of the macroinvertebrate fauna. Only a few Tubificidae (*Tubifex*, *Limnodrilus* and *Branchiura sowerbyi*), water insects (Copepoda, Ephemeroptera, Odonata and Ceraropogonidae) and Chironomidae (*Chironomus* larvae and pupae) were present in this dam.

Locality 14 - Aasvoëlkrans has large concentrations of algae during summer. Water organisms such as *Baetis* (Ephemeroptera), *Sigara* (Hemiptera), *Hydroptila* (Trichoptera) were present, while Tubificidae (*Limnodrilus* and *Branchiura sowerbyi*) and Chironomidae (*Pentaneura* and *Chironomus*) represented the benthic macroinvertebrate fauna in the area.

Autumn

Table 11.2 summarizes the number and diversity of benthic organisms sampled during autumn.

Locality 1 - Increases in both the benthic organisms, and the water insects occurred at the control locality near Davel.

Locality 2 - Benthic organisms such as *Tubifex* and *Limnodrilus* (Tubificidae) were present in lower numbers than during summer (Table 1), while *Chironomus* (Chironomidae) were absent from Van Dycks Drift. There was a definite increase in the number of water organisms such as Hirudinae, Cladocera, Copepoda, Ephemeroptera and Hemiptera.

Locality 3- At Steenkool Spruit, there was a slight decrease in the number and variety of water insects and benthic macroinvertebrates during autumn.

Table 11.2 The number and diversity of macroinvertebrate larvae sampled during autumn 1994.

ORGANISM / LOCALITY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Coelenterata (Hydroids, Jellyfish)														
Hydroida														
Hydridae														
Hydra	-	-	-	-	49	-	-	-	-	-	-	-	-	-
Nematoda (Roundworms)														
Dorilaimida														
Dorylaimidae														
Tobrilus	-	-	-	-	-	-	-	-	487	-	-	-	-	-
Annelida (Aquatic eartworms, Polychaeta)														
Oligochaeta (Aquatic earthworms)														
Haplotaxida														
Tubificidae														
Tubifex	584	487	-	-	-	-	-	-	292	-	-	-	341	-
Limnodrilus	1461	877	3263	2825	11591	6185	584	-	1753	877	925	1753	3263	1688
Branchiura sowerbyi	-	-	-	-	-	-	-	-	-	-	49	779	-	-
Hirudinea (Leeches)														
Rhynchobdella														
Glossiphoniidae														
Helobdella	97	49	-	-	3263	49	731	-	1656	-	-	-	633	-
Cladocera (Waterfleas)														
Daphnidae	4042	925	244	1218	2581	341	633	-	-	-	49	49	195	-
Copepoda														
Cyclopoida														
Cyclops	2776	1851	438	2581	2630	2338	390	146	3214	-	-	-	2045	-
Ostracoda (Seed shrimps)	3506	-	-	-	-	-	-	-	-	-	-	-	-	-
Decapoda														
Caridina nylotica	-	-	-	-	-	-	97	-	-	-	-	-	-	-
Ephemeroptera (Mayflies)														
Baetidae	-	-	-	-	-	49	-	-	-	-	-	-	-	-
Baetis	828	-	-	-	-	-	292	-	292	97	-	-	1656	97
Cloeon	584	341	-	195	195	-	-	-	-	-	-	-	292	-
Odonata (Dragonflies, Damselflies)														
Anisoptera (Dragonflies)														
Libellulidae	-	-	-	-	-	-	-	-	-	49	-	-	-	-
Libellula	-	-	-	-	-	-	-	195	-	-	-	-	-	-
Zygoptera														
Lestidae														
Lestes	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aeshnidae	-	-	-	-	-	-	49	-	-	49	-	-	-	-
Hemiptera (Bugs)														
Corixidae (Water boatmen)	146	49	-	-	-	292	-	-	-	-	-	-	49	-
Sigara	-	-	-	1510	-	-	-	-	-	-	-	-	-	-

Table 11.2: (continued)

Trichoptera (Caddis flies)														
Hydroptilidae														
Hydroptila	-	-	-	-	-	-	49	-	-	49	-	49	-	-
Coleoptera (Beetles)														
Dytiscidae (Predaceous diving beetles)	-	-	-	-	-	97	-	-	-	-	-	-	-	-
Hydroporus	-	-	-	-	49	-	-	49	-	-	-	-	-	-
Dytiscus	-	-	-	-	-	-	-	49	-	-	-	-	-	-
Diptera (Flies, mosquitoes, midges)														
Tipulidae (Crane flies)	-	-	-	49	-	49	-	-	-	-	-	-	-	-
Culicidae (Mosquitoes, Phantom midges)														
Culicinae														
Culex	-	-	-	-	49	49	-	-	-	-	-	-	-	-
Simulidae (Black flies)	-	-	-	-	-	-	-	-	-	-	-	49	-	-
Chironomidae (Midges)														
Chironomus	97	-	2484	3701	-	244	633	2532	3263	877	1023	-	-	1169
Chironomus pupae	-	-	-	-	-	-	-	-	-	-	-	-	-	49
Pentaneura	-	-	-	-	487	-	-	-	-	97	-	-	-	-
Ceratopogonidae (Biting midges)														
Palpomyia	-	-	49	-	49	-	-	-		146	536	-	-	-
Bezzia	-	-	-	-	-	-	-	-		-	-	-	-	-
Gastropoda (Snails, Limpets)														
Pulmonata														
Physidae	-	-	-	-	-	-	-	-	49	-	-	-	-	-
Pelecypoda (Clams, mussels)														
Sphaeriidae	-	-	-	-	-	-	-	-	-	49	-	-	-	97

Locality 4 - A slight increase in the number of *Limnodrilus* and *Chironomus* stresses the fact that there was still some organic enrichment of the system at Koringspruit during autumn. Tipulidae (biting midges), Corixidae (waterboat men) and Baetidae (mayflies) were also present.

Locality 5 - There was a definite increase in not only the number, but also the variety of water insects (Cladocera, Copepoda, Ephemeroptera, Coleoptera and Tipulidae) and benthic macroinvertebrates such as Tubificidae and Chironomidae.

Locality 6- There was a slight increase in the number of water insects and benthic macroinvertebrates at this locality within the mining area.

Locality 7 - presents some benthic macroinvertebrates (*Limnodrilus* and *Chironomus*) and an increase of aquatic organisms such as Cladocera, Copepoda, Decapoda (*Caridina nylotica*), Ephemeroptera, Odonata and Trichoptera. A decrease in recreational activities at Witbank Dam during autumn could be the reason for the increase in organisms at this locality.

Locality 8 - During autumn there was a very slight increase in benthic organisms in the Suur Stream. Odonata and Chironomidae were present in increased numbers and the presence of Copepoda and Coleoptera was noted.

Locality 9 - At this locality in the Olifants River, there appeared to be a decrease in the number of benthic organisms such as Tubificidae, Hirudinae, Copepoda and Ephemeroptera with the

Cladocera, Ostracoda and Chironomidae being totally absent during autumn.

Locality 10- Tubificidae such as *Limnodrilus* and *Branchiura sowerbyi*, Chironomidae such as *Chironomus* and *Cullicoides* (Heleidae) were present at Spook Spruit in smaller numbers than during summer (Table 6.1).

Locality 11 - During autumn there was a definite decrease in not only the number of benthic organisms (Tubificidae and Chironomidae) but also in the number of water organisms such as Baetidae, Libellulidae, Hydroptilidae and Ceratopogonidae.

Locality 12 - Small numbers of Simulidae (Black flies), Hydroptilidae (Trichoptera) and Copepoda occurred at Woesalleen, with a very slight increase in the number of Tubificidae from summer to autumn.

Locality 13 - An increase in benthic macroinvertebrates and water insects occurred at Middelburg Dam during autumn, probably due to a decrease in recreational activities.

Locality 14 - During autumn only Tubificidae and Chironomidae were present at Aasvoëlkrans in moderate numbers.

Winter

Table 11.3 indicates the number and diversity of macroinvertebrates sampled during winter.

Table 11.3 The number and diversity of macroinvertebrate larvae sampled during winter 1994.

ORGANISM / LOCALITY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nematoda (Roundworms)														
Dorilaimida														
Dorylaimidae	779	–	–	–	–	97	–	–	–	97	–	–	49	–
Enoplida														
Tobrilus	–	–	–	–	49	–	–	–	–	–	–	–	–	–
Annelida														
Oligochaeta (Aquatic earthworms)														
Haplotaxida														
Tubificidae														
Tubifex	–	–	–	–	–	–	–	196	–	–	–	–	–	–
Limnodrilus	17045	1364	12029	3117	1364	12175	1071	682	390	1364	2581	–	12175	–
Branchiura sowerbyi	–	–	–	–	–	–	–	–	–	536	–	–	–	–
Lumbriculida	–	–	–	–	–	–	49	–	–	–	–	–	–	–
Hirudinea (Leeches)														
Rhynchobdella														
Glossiphoniidae														
Helobdella	–	–	–	146	1169	–	536	–	–	–	683	–	1023	–
Cladocera (Waterfleas)														
Daphnidae	2532	2045	6623	97	–	146	–	–	49	292	292	–	196	–
Bosminidae	2289	–	1997	244	–	–	–	–	–	–	–	–	–	–
Copepoda														
Cyclopoida														
Cyclops	4091	1753	1315	292	3360	292	779	146	–	–	49	–	2386	390
Macrocyclops	–	–	–	–	–	1315	–	–	–	–	–	–	292	–

Table 11.3: (continued)

Ostracoda (Seed shrimps)	1607	-	-	-	-	-	-	244	-	-	-	-	-	-
Ephemeroptera (Mayflies)														
Baetidae														
Baetis	49	682	-	-	-	-	1364	97	-	-	4042	-	-	4334
Odonata (Dragon- and Damselflies)														
Anisoptera (Dragonflies)														
Gomphidae														
Gomphus	-	-	-	-	-	97	-	-	-	-	-	-	-	-
Hemiptera (Bugs)														
Corixidae (Water boatmen)														
Sigara	49	146	197	-	-	731	49	-	-	-	-	-	-	-
Pleidae (Pigmy backswimmers)														
Plea	-	-	-	-	146	-	-	-	-	-	-	-	-	-
Belostomatidae (Giant water bugs														
Lethocerus	-	-	-	-	-	-	-	-	-	-	-	-	49	-
Trichoptera (Caddis flies)														
Hydroptilidae	-	-	-	-	-	-	779	-	-	-	-	-	-	877
Hydroptila	-	146	-	-	-	-	-	49	-	-	341	-	-	-
Coleoptera (Beetles)														
Dytiscidae(Predaceous diving beetles)														
Hydroporus	-	-	-	-	-	-	-	49	-	-	-	-	-	-
Elmidae (Riffle beetles)	-	-	-	-	341	-	-	-	-	-	-	-	-	-
Diptera (Flies,mosquitoes,midges														
Tabanidae (Horseflies)														
Chrysops	-	-	-	-	-	-	-	49	-	-	-	-	-	-
Tabanus	-	-	-	-	-	-	-	96	-	-	-	-	-	-
Psychodidae (Moth flies)	-	-	-	-	-	341	-	-	-	96	-	-	-	-
Chironomidae (Midges)														
Chironomus	1607	2386	49	4675	292	197	1802	341	-	3019	6136	438	244	3312
Chironomus pupae	-	-	-	-	-	-	-	-	-	-	-	-	-	97
Ceratopogonidae (Biting midges)														
Palpomyia	49	49	390	-	-	-	1558	536	-	-	-	-	-	-
Bezzia	-	-	-	-	-	49	-	-	-	-	-	-	-	-
Gastropoda (Snails, Limpets)														
Pulmonata														
Physidae	-	-	-	-	-	341	-	-	-	96	-	-	-	-

Locality 1 - At the control locality, only a few Hemiptera were present, while larger numbers of Tubificidae, Nematoda, Cladocera, Odonata and Chironomidae occurred.

Locality 2- At Van Dycks Drift, the same tendency for autumn occurred for winter with water insect larvae and large numbers of Tubificidae, Cladocera, Copepoda and Chironomidae present.

Locality 3 - An increase in the number of Tubificidae, Cladocera and Copepoda occurred at Steenkool Spruit but a decrease was observed for the Chironomidae.

Locality 4 - An overall decrease in macroinvertebrate diversity occurred with only some Tubificidae, Hirudinae, Cladocera and Chironomidae present at this locality.

Locality 5 - This locality presented a few Nematoda, water insect larvae (Hemiptera and Coleoptera) and Chironomidae. Larger numbers of Tubificidae and Copepoda were present.
Locality 6 - At Boesman Spruit there was a slight increase in the number of Tubificidae, while smaller numbers of water insect larvae, Nematoda, Chironomidae and Pulmonata were present.

Locality 7 - There was a decrease in the number of the water insect larvae species with only a few Copepoda, Ephemeroptera, Hemiptera and Trichoptera present. The Tubificidae, Chironomidae and Ceratopogonidae were more abundant.

Locality 8 - At Suur Stream, Chironomidae were the only macroinvertebrates remaining during winter.

Locality 9 - At this locality in the Olifants River, only a few water insect larvae (Trichoptera - Hydroptila) were present while the water leeches and copepods were less abundant than during autumn. Larger numbers of Nematoda and Tubificidae occurred during winter than during autumn, while the Cladocera and Chironomidae occurred in large numbers during winter after being absent during autumn.

Locality 10 - Ephemeroptera and Trichoptera were present. Only a few Chironomidae were present while the Tubificidae and Coleoptera occurred in large numbers and Tabanidae occurred for the first time at Spook Spruit.

Locality 11 - At Olifants River Lodge, Tubificidae and Cladocera were present in small numbers while a few moth flies (Psychodidae) occurred for the first time.

Locality 12 - Nematoda (Dorylaimidae), Cladocera (Daphnidae), Chironomidae and Gastropoda (Physidae) were more abundant during winter than during autumn. Tubificidae occurred in smaller numbers than during autumn and no water insect larvae were present.

Locality 13 - Following the absence of Hirudinae (leeches) and Chironomidae (midges) at Middelburg Dam during autumn, these aquatic organisms reappeared in abundant numbers. Larger numbers of Cladocera (water fleas), Ephemeroptera (mayflies) and Trichoptera, and smaller numbers of Tubificidae and Copepoda were present. This is probably due to almost no recreational activities.

Locality 14 - This locality at Aasvoëlkrans presented some Cladocera, Ephemeroptera, Trichoptera and Chironomidae in larger numbers than during autumn.

Spring

Table 11.4 presents the number and diversity of macroinvertebrate larvae sampled during spring.

Locality 1 - An overall increase in the numbers of Nematoda, Tubificidae, Cladocera, Copepoda and water insect larvae (Hemiptera and Coleoptera) was observed, while Ostracoda and Chironomidae were present in smaller numbers.

Locality 2 - An overall increase in the macroinvertebrate numbers and diversity occurred at Van Dycks Drift during spring with only the Ephemeroptera and Trichoptera occurring in smaller numbers.

Table 11.4: The number and diversity of macroinvertebrate larvae sampled during spring 1994

ORGANISM / LOCALITY	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Coelenterata (Hydroids, Jellyfish)														
Hydroida														
Hydridae														
Hydra	–	97	–	–	–	–	–	–	–	–	–	–	292	49
Nematoda (Roundworms)														
Dorylaimida														
Dorylaimidae	2727	925	–	779	49	–	–	–	–	584	49	–	97	–
Annelida (Aquatic earthworms)														
Oligichaeta														
Haplotaxida														
Tubificidae														
Tubifex	–	1315	–	–	–	–	–	97	–	–	–	–	–	–
Limnodrilus	19480	4188	29220	8766	1510	24350	1169	925	487	14610	1412	49	1656	6915
Hirudinae (Leeches)														
Rhynchobdella														
Glossiphoniidae														
Helobdella	49	–	–	244	49	49	–	–	–	–	1023	–	390	–
Cladocera (Waterfleas)														
Daphnidae	4675	34090	2873	97	–	–	146	–	–	341	97	–	3506	146
Bosminidae	1218	–	1607	146	–	–	–	–	97	–	–	–	–	–
Copepoda														
Cyclopoida														
Cyclops	7305	6575	1071	97	1899	146	974	–	292	390	438	146	6575	292
Macrocyclops	–	–	–	–	292	–	–	–	–	–	–	–	1315	–
Ostracoda (Seed shrimps)	390	2289	682	828	–	828	–	–	–	–	–	–	–	–
Collembola (Springtails)														
Isotomidae														
Isotomo	97	–	–	–	–	–	–	–	–	–	–	–	–	–
Ephemeroptera (Mayflies)														
Baetidae														
Baetis	–	146	–	–	–	–	244	–	–	–	828	–	–	3799
Caenidae														
Caenis	–	–							49					
Hemiptera (Bugs)														
Corixidae	97	–	146	–	–	–	–	–	–	49	–	–	49	–
Sigara	–	244	–	97	–	–	–	–	–	–	–	–	–	–
Notonectidae														
Notonecta	–	–	–	–	–	–	–	–	49	–	–	–	–	–
Trichoptera (Caddis flies)														
Hydroptilidae														
Hydroptila	–	146	–	–	–	–	–	–	–	–	97	–	–	–
Hydropsychidae														
Leptonema	–	–	–	–	–	–	–	–	–	–	–	–	–	49

Table 11.4: (continued)

Coleoptera (Beetles)														
Dytiscidae														
Hydrovatus	97	-	-	-	-	-	-	-	-	-	-	-	-	-
Deronectes	-	-	-	-	-	-	-	-	97	-	-	-	-	-
Cybister	-	-	-	-	-	-	-	-	49	-	-	-	-	-
Diptera (Flies,Mosquitoes,Midges														
Tipulidae (Crane flies)														
Tipula	-	-	-	-	97	-	-	-	-	-	-	-	-	-
Psychodidae (Moth flies)														
Chironomidae (Midges)														
Chironomus	438	3068	1412	2094	925	2240	1218	195	3019	3799	2386	1461	2386	4773
Chironomus pupae	49	-	-	49	-	-	-	-	49	-	-	244	49	-
Ceratipogonidae (Biting midges)														
Bezzia	49	-	49	-	-	-	97	97	97	-	195	-	49	146
Gastropoda (Snails, Limpets)														
Pulmonata														
Physidae	146	-	-	-	-	-	-	-	-	-	-	-	-	-
Pelecypoda (Clams, mussels)														
Sphaeriidae	341	-	-	-	-	-	-	49	-	-	-	-	-	-

Locality 3 - Present at this locality were Tubificidae (Limnodrilus), Ostracoda, Hemiptera, Chironomidae and Heleidae (Biting midges) in large numbers, and Cladocera and Copepoda in smaller numbers.

Locality 4 - At Koringspruit a slight increase in the number of Tubificidae signifies organic enrichment of this system. Nematodes, leeches, seed shrimps and bugs occurred in large numbers, with the waterfleas, copepods and midges were present in smaller numbers.

Locality 5 - At this locality a slight increase in the numbers of Tubificidae, Chironomidae and Tipulidae occurred while only a few Copepoda and Hirudinae were present.

Locality 6 - There was a decrease in the number of Copepoda, while an increase in the number of Tubificidae, Hirudinae, Cladocera and Chironomidae occurred

Locality 7 - After the winter there was a decrease in the number and diversity of macroinvertebrates occurring at this locality. Some Tubificidae, Cladocera, Copepoda, Ephemeroptera and Chironomidae were present.

Locality 8 - At Spook Spruit it was evident that the effluent from the mines, brick-works and surrounding farming areas affected the occurrence of macroinvertebrates in this system. Only some Tubificidae, Chironomidae and Pelecypoda (clams, mussels) were present.

Locality 9 - Although a slight increase in the numbers of macroinvertebrates occurred, the numbers of Ephemeroptera present decreased.

Locality 10 - Nematoda, Tubificidae, Cladocera, Copepoda, Hemiptera and Chironomidae were more abundant during spring than during winter at Woesalleen.

Locality 11 - There was a slight decrease in the number of benthic organisms (Tubificidae) and crustaceans (Cladocera), while an increase in the numbers of Nematoda, Hirudinae, Copepoda and Trichoptera occurred.

Locality 12 - During spring there was again a slight increase in macroinvertebrates in the Suur Stream. A few Tubificidae, Cladocera and Chironomidae were present during this season.

Locality 13 - At this locality in the Olifants River there appeared to be an increase in the number of Hydridae, Cladocera, Copepoda, Chironomidae and Ceratopogonidae. Smaller number of Tubificidae and Hirudinae also occurred.

Locality 14 - During spring at Aasvoëlkrans smaller numbers of Copepoda, Ephemeroptera, Trichoptera and Chironomidae were observed. The numbers of Hydridae, Tubificidae and Cladocera occurring in spring were larger than in winter.

11.3.2 Metal accumulation by macroinvertebrates

Data on the metal concentrations of the macroinvertebrates are given in Tables 6.5 to 6.8. Each table portrays the metal concentrations accumulated by the macroinvertebrates sampled during a specific season. Due to the presence of moderate numbers of macroinvertebrates, these organisms were analyzed according to families.

Summer

The data obtained for the benthic organisms during summer are given in Table 11.5.

Iron and aluminum occurred in the highest concentrations while manganese, lead and copper occurred in low concentrations.

Outstanding high metal concentrations were observed for the macroinvertebrates occurring at localities 3, 7, 8 and 9. The Ostracoda at locality 11 also presented high metal concentrations. The high metal concentrations at locality 3 may be due to the fact that this locality is just below a mining area and thus receiving effluent from the mine. At locality 7 the high metal concentrations observed for the organisms analyzed, were due to effluent from the surrounding farming area resulting in organic and metal enrichment of this specific locality.

Table 11.5: Metal concentrations (wet mass) accumulated by the macroinvertebrate larvae during summer 1994/1995

LOCALITY	ORGANISM	Al µg/g	Cr µg/g	Cu µg/g	Fe µg/g	Mn µg/g	Ni µg/g	Pb µg/g	Zn µg/g
1	Tubificidae	**22968.8**	**15906.3**	**2781.3**	**80312.5**	4156.3	**11468.8**	**6343.8**	**6906.3**
	Chironomidae	6194.3	795.6	175.7	10895.3	662.2	670.6	250	587.8
	Ceratopogonidae	**53000**	**59000**	**10875**	**1007500**	**27750**	**45625**	**19500**	**28000**
	Cladocera	**19950**	11725	**2200**	**88500**	**2775**	**8675**	**4125**	**17225**
	Copepoda	**23571.4**	**16875.1**	**2857.1**	128571.4	**3964.3**	**12464.3**	**5892.9**	**13035.7**
	Baetidae	10510.9	4750	869.6	33369.6	1130.4	3369.6	1554.4	2319.3
	Corixidae	32125	**29187.5**	**5187.5**	219375	**5875**	**22500**	**9812.5**	**28875**
	Lestidae	7559.5	5642.9	928.6	28809.5	1059.5	3869.1	1773.8	**6059.5**
	Dytiscidae	**29850**	**23900**	**4700**	**129500**	**4700**	**17400**	**7900**	22150
2	Tubificidae	4241.3	69	13.1	4191.2	151.7	45.2	16.4	59.8
	Glossiphoniidae	2160.5	603.2	89.6	6815.9	303.5	493.8	235.1	221.4
	Copepoda	2767.9	3113.1	458.3	16547.6	577.4	2208.3	988.1	1583.3
	Ceratopogonidae	13941.2	7338.2	1147.1	**46470.6**	1000	**5294.1**	**2191.2**	3529.4
3	Tubificidae	0	0	0	0	0	0	0	0
	Chironomidae	5552.3	1830.7	286.3	12903.2	790.3	1286.3	806.5	725.8
	Ceratopogonidae	57.3	97.7	11.6	442.7	20.5	60.5	46.2	34.7
	Cladocera	328.1	111.5	16.8	1174	68.7	73.8	38.9	55.2
	Copepoda	85.3	106.4	14.4	411.3	22.2	76.2	32.3	39.4
	Corixidae	373.9	76.2	15.7	1130.9	34.3	57.1	28.9	43.1
	Sphaeriidae	330.1	28.1	7.7	625.2	86.4	30.1	19.5	32.8
	Dytiscidae	201.8	106.5	15.2	1049.2	63.4	69.1	43.7	71.9
	Pyralidae	157.8	121.8	16.4	710.8	34.5	80.9	34.5	159.1
4	Tubificidae	4807.9	478.9	57	5859.7	385.1	323.7	137.7	265.8
	Glossiphoniidae	595.7	187.5	26.1	2944.6	215.5	123.7	45.7	141.4
	Chironomidae	2184.9	97.7	19.9	2584.9	119.1	66.5	24	94.9
	Copepoda	5032.9	3671.1	401.3	18289.5	703.9	2375	703.9	1710.5
	Cladocera	7000	4758.9	803.6	**50535.7**	1133.9	3098.2	839.3	2348.2
	Corixidae	1839.4	665.2	87.1	4852.9	460.4	412.9	121.1	382.4
5	Tubificidae	1861.3	63.1	12.3	2580.7	234.9	47.7	12.1	70.9
	Glossiphoniidae	6833.3	6464.3	880.9	**53333.3**	1619.1	4166.7	1511.9	3559.5
	Copepoda	5864.6	5531.3	843.8	31875	718.8	3812.5	1312.5	2656.3
	Ceratopogonidae	6480.8	3163.5	1125	41442.3	1221.2	**6000**	1278.9	4009.6
6	Tubificidae	15812.4	159.2	48.3	14723.8	460.6	87.9	37.9	593.8
	Chironomidae	10790.3	4379	572.6	30080.7	991.9	2879	798.4	3379
	Ceratopogonidae	**19196.4**	**10250**	**1607.1**	**48035.7**	**2517.9**	**7089.3**	1982.1	**5839.3**
	Copepoda	**20452**	**12425**	**1750**	**79250**	1925	**8225**	**2000**	**5850**
	Corixidae	5161.1	2351.7	296.6	15974.6	822	1491.5	572	**5025.4**
7	Tubificidae	1313.3	1623.3	210	8200	216.7	1140	366.7	753.3
	Chironomidae	2773.4	3382.8	390.6	19843.8	460.9	2117.2	820.3	3468.8
	Ceratopogonidae	408.1	800.3	100.6	4051.7	96.3	533.1	229.9	209.8
	Copepoda	849.4	950.3	134.6	6105.8	133	1166.7	246.8	429.5
8	Chironomidae	9572.5	247.8	50.9	14810	660.2	180.1	46.2	160.6
	Anisoptera	1269.8	110.7	24.6	3191.2	120.4	81.8	26.5	55.8

Table 11.5: (continued)

9	Tubificidae	7027.8	2588.9	544.4	30333	1705.6	5938.9	911.1	2988.9
	Chironomidae	1509.5	569.1	117.9	6773.8	244.1	407.1	94.1	276.2
	Glossiphoniidae	482.1	317.9	63.6	1808.6	75.3	218.5	72.2	190.7
	Ostracoda	16375	**10589.3**	**2482.1**	**708928.6**	**10571.4**	**7750**	**2214.3**	**10392.9**
	Cladocera	5233.9	3943.6	733.9	**73225.8**	895.2	2588.7	975.8	3225.8
	Copepoda	4076.9	2072.1	437.5	13750	506.6	1706.7	745.2	1793.3
	Baetidae	5555.6	2494.4	550	1150-	405.6	1855.6	1011.1	1727.8
	Simulidae	1479.938	479.9	174.4	6404.3	195.9	649.7	199.1	351.9
	Hydroptilidae	1348.8	2284.9	604.7	14186.1	494.2	**5970.9**	732.6	1813.9
	Physidae	262.1	102.5	17.8	708.4	75.9	51.3	16.4	92.5
10	Tubificidae	3733.7	2016.3	472.8	16739.1	1364.1	1603.3	407.6	1320.7
	Chironomidae	3231.9	935.6	286.1	**8092.8**	**858.2**	778.4	121.1	1353.2
	Baetidae	1443.9	765.3	237.2	**106122.4**	1505.1	676	114.8	1227
	Culicidae	3136.4	2359.1	618.2	19318.2	600	1977.3	0	2263.6
	Libellulidae	5113.9	658.2	183.7	6683.7	1301	561.2	81.6	807.8
	Hydroptilidae	1780.9	1126.5	1450.6	21265.4	515.4	941.4	197.5	1228.4
11	Tubificidae	2109.2	445.4	177.8	7816.9	397.9	818.7	184.9	410.2
	Chironomidae	2485.8	819.1	207.3	6869.9	463.4	662.6	160.6	780.5
	Baetidae	1238.9	1158.3	258.3	43361.1	650	908.3	230.6	538.9
12	Tubificidae	5679.6	131.2	34.8	8011.1	383.2	106.1	26.8	92.3
	Chironomidae	4250	2986.8	1315.8	**43552.6**	1171.1	**5684.2**	1263.2	2539.5
	Ceratopogonidae	5868.4	2513.2	**1671.1**	**106315.8**	**2236.8**	**6078.9**	1381.6	**5657.9**
	Copepoda	8454.5	8159.1	1568.2	**45227.3**	1227.3	**5681.8**	1954.6	4295.5
	Sphaeriidae	726.9	450.7	83.1	3560.9	138.9	270.6	13.2	139.8
13	Tubificidae	10921.1	2796.1	664.5	89078.9	1559.2	2388.2	1000	2703.9
	Copepoda	13181.8	9500	**2022.7**	55000	1613.6	**6909.1**	**2704.6**	**8045.5**
	Chironomidae	8250	8214.3	1482.1	**45357.1**	1464.3	**6107.1**	1392.9	2964.3
	Ceratopogonidae	5053.6	4866.1	991.1	31339.3	678.6	3758.9	848.2	3178.6
	Gomphidae	968.7	47.1	16.4	3247.4	39.7	52.7	13.4	57.5
	Baetidae	163.1	93	18.4	542.4	28.6	63.6	20.8	112.9
14	Tubificidae	1516.9	34.8	14.8	1333.1	273.6	41.8	14.2	177.5
	Chironomidae	1434.7	100	56.6	4877.2	494.3	177.6	67.9	157.9
	Hydroptilidae	1479.4	610.8	296.4	9432.9	1180.4	1177.8	342.8	1221.7
	Cladocera	1327.8	1147.2	266.7	7305.6	202.8	919.4	330.6	708.3
	Copepoda	3330.7	1786.3	383.1	**91532.3**	1443.6	1318.6	556.5	1064.5
	Baetidae	579.2	275	55.4	2244.1	327.9	205.9	58.3	194.1
	Corixidae	351.3	319.1	65.6	1375.7	91.5	236	125	201.8
	Ceratopogonidae	437.1	383.7	85.7	2115.4	119.8	279.7	119.8	230.8

Bold print : High concentrations

Autumn

The data obtained for the macroinvertebrates during autumn are given in Table 11.6.

High metal concentrations were observed for the Chironomidae at localities 1, 3 and 7, Tubificidae at locality 1, the Cladocera and Copepoda at localities 1, 3 and 7. The high metal concentrations at locality 1 are due firstly to organic effluent from the Naauwpoort Sewage Works and secondly to thermal pollution from the Duva Power Station.

Table 11.6: Metal concentrations (wet mass) accumulated by the macroinvertebrate larvae sampled during autumn 1994

LOCALITY	ORGANISM	Al µg/g	Cr µg/g	Cu µg/g	Fe µg/g	Mn µg/g	Ni µg/g	Pb µg/g	Zn µg/g
1	Tubificidae	13877.6	5969.4	301	48775.5	841.8	4877.6	1158.2	3653.1
	Ostracoda	4258.1	13008.1	403.2	57983.9	1395.2	10967.7	2129	4322.6
	Cladocera	1510.6	4207.5	159.6	2707.5	515.9	3824.5	819.2	4920.2
	Copepoda	7059.2	3071.4	151.3	27927.6	447.4	3562.5	782.9	3328.9
	Chironomidae	**42440.2**	**48442.4**	190.2	44891.1	586.9	4331.5	885.9	**6239.1**
	Glossiphoniidae	2096.8	5451.6	145.2	27500	411.3	4717.7	1177.4	3330.7
	Baetidae	2007.8	1574.2	83.9	12421.9	277.3	1328.1	369.1	1798.8
2	Tubificidae	1135.4	630.2	23.1	4330.4	66.2	492.6	125.7	185.3
	Cladocera	692.4	684.8	21.3	3643.7	57.2	560.9	107	348
	Copepoda	470.7	440.8	20.5	3180.3	287.1	370	97.4	299.8
	Baetidae	1033.9	654.2	20.3	4118.6	59.3	572.9	130.5	268.6
	Corixidae	732.1	716.9	25.1	3888.9	56.5	589.6	150.5	283.2
	Glossiphoniidae	202.3	885.6	10.1	4470.3	87.9	728.8	191.7	260.6
	Chironomidae	468.9	258.1	12.5	2431.2	54.2	237.1	84.1	83.7
3	Tubificidae	2163.4	195.5	8.9	5200.4	110.7	186.8	27.5	97.9
	Chironomidae	605	519.1	32.8	2289	102.6	436.2	125.3	326.4
	Cladocera	271.9	1140.4	23.4	6630.4	105.3	1045.3	159.4	325.4
	Copepoda	**30625**	**31166.7**	2375	**233333.3**	4166.7	**29541.7**	4208.3	14625
	Ceratopogonidae	6467.7	2806.5	116.9	19838.7	245.9	2427.4	681.5	923.4
4	Tubificidae	5414.7	2308.8	602.9	18500	385.3	1923.4	505.8	682.4
	Chironomidae	1259.6	88.2	4.3	1687.7	46.2	71	8.1	33.6
	Cladocera	4833.3	4020.8	130.2	26927.1	411.5	3447.9	708.3	1760.4
	Copepoda	3349.5	1925.9	85.7	15138.9	259.3	1537	497.7	2773.2
	Corixidae	59.2	346.6	18.8	2206.6	32.1	260.9	79.8	195.6
	Baetidae	2179.9	1399.7	39.8	10302.6	176.8	1148.1	286.6	383.8
	Notonectidae	1753.3	1330.9	39.6	8021.6	196	1122.3	289.6	422.7
5	Tubificidae	19555.3	1229.7	135.4	35375.5	737.2	2022.7	126.5	677.9
	Glossiphoniidae	15750	20966.7	816.7	**153833.3**	11166.7	16550	3466.7	9100
	Hydridae	8411.8	11617.7	264.7	61911.8	1279.4	10117.7	2514.7	3352.9
	Copepoda	8425	9675	325	66250	1537.5	8525	1700	**8000**
	Cladocera	**44687.5**	**39062.5**	**1500**	**221875**	**5437.5**	**34000**	**10375**	44937.5
	Chironomidae	23178.6	25071.4	607.1	**123214.3**	2357.1	**19035.7**	**7535.7**	**6285.7**
	Ceratopogonidae	**35527.8**	22138.9	805.6	**239166.7**	2444.4	**19861.1**	3638.9	**6055.6**
	Culicidae	19333.3	20694.4	500	**108888.9**	1944.4	**17638.9**	4722.2	**5277.8**
	Baetidae	10171.1	8539.5	276.3	73552.6	1328.9	7052.6	2236.8	1855.3
	Dytiscidae	1662.5	1864.6	45.8	9979.2	202.1	1456.3	391.7	352.1
6	Tubificidae	4743	491.5	20.7	8717.6	464.5	393.6	31.9	94.1
	Tipulidae	7463.5	3286.5	244.8	19427.1	0	3119.8	1114.6	781.3
	Chironomidae	14288.5	6423.1	461.5	62019.2	0	6644.2	1692.3	2230.8
	Baetidae	9243.9	4792.7	298.8	35182.9	0	4243.9	1426.8	676.8
	Cladocera	7967.7	1629.3	129.3	24762.9	698.3	1601.3	441.8	428.9
	Dytiscidae	2152.9	1000	66.8	6529.1	95.9	889.6	256.1	348.3
	Corixidae	3579.6	1437.5	107.9	10946.9	524.6	1407.2	386.4	484.9
	Culicidae	5929.1	2410.5	186.6	15559.7	0	2727.6	429.1	1022.4
	Glossiphoniidae	9920.7	4262.2	335.4	28902.4	0	4548.8	914.6	2865.9
	Copepoda	**49000**	17916.7	1527.8	**126666.7**	0	**19666.7**	**3833.3**	**22305.6**

Table 11.6: (continued)

7	Corixidae	1000	2690.5	83.3	17589.3	330.4	2229.2	613.1	2720.2
	Tubificidae	27861.1	19722.2	1361.1	**124166.7**	**8833.3**	**18944.4**	**4888.9**	**6805.6**
	Cladocera	**51916.7**	**26958.3**	**1958.3**	**187500**	0	**27791.7**	**6750**	**7791.7**
	Copepoda	28833.3	**39979.2**	**1604.2**	**173125**	1104.2	**28229.2**	**5250**	**7562.5**
	Glossiphoniidae	3005.2	2005.2	139.2	11855.7	1025.8	1762.9	554.1	564.4
	Decapoda	511.9	495	26.9	2581.2	71.9	417.6	49.8	76.9
	Baetidae	8350	4943.8	343.8	31562.5	56.3	4562.5	1293.8	968.8
	Aeshnidae	19390.6	10703.1	703.1	64062.5	0	10765.6	2921.9	1453.1
	Hydroptilidae	17812.5	17050	750	86750	287.5	**15487.5**	2162.5	3437.5
	Dytiscidae	11341.7	5233.3	400	33416.7	0	5491.7	1308.3	1575
8	Tubificidae	911.9	2571.4	69.1	25428.6	647.6	2057.1	507.1	390.5
	Chironomidae	1626.4	2804.6	66.1	16235.6	583.3	2304.6	652.3	479.9
	Ceratopogonidae	1753.4	3435.8	87.8	21520.3	570.9	2888.5	709.5	807.4
	Baetidae	581.8	4309.1	109.1	21727.3	563.6	3831.8	886.4	781.8
	Hydroptilidae	642.9	4994.9	107.1	24234.7	607.1	4244.9	1066.3	1102
	Libellulidae	22068.2	7668.2	163.6	55136.4	940.9	6340.9	1004.6	1304.6
	Ostracoda	7387.5	5087.5	167.5	31575	682.5	4162.5	447.5	1152.5
	Sphaeriidae	280.6	250.6	49.8	1544.8	841.1	183.2	65.9	83.5
9	Tubificidae	1950.2	1192.9	30.3	**110388.4**	515.8	929.6	280.3	309.5
	Chironomidae	2377	728	33.2	5852.8	275.6	560.1	111.8	241
	Ceratopogonidae	397.1	1803.3	40.4	8878.7	431.9	1501.8	386	522.1
	Cladocera	947.8	3731.3	82.1	20261.2	447.8	2932.8	940.3	705.2
10	Tubificidae	3675.1	236.3	16.9	5581.2	319.5	207.2	21	123.6
	Cladocera	5666.7	16857.1	845.2	**133333.3**	2809.5	**28166.7**	2214.3	**5107.1**
	Simulidae	5310.6	1348.5	196.9	59090.9	530.3	5583.3	1916.7	2734.9
	Hydroptilidae	22812.5	24187.5	**1895.8**	**237916.7**	4208.3	49416.7	**4416.7**	**9562.5**
11	Chironomidae	14177.9	10572.1	600.9	93413.5	2134.6	14548.1	1317.3	2894.2
	Tubificidae	6670.3	7496.4	391.3	66702.9	3170.3	6253.6	768.1	3125.2
	Ceratopogonidae	1585.1	3292.6	106.4	23510.6	6053.2	3489.4	1276.6	2202.1
	Copepoda	3871.2	7931.8	363.6	46893.9	7537.8	7734.9	1583.3	3477.3
	Cladocera	1415.2	6464.3	125	33973.2	2102.7	5196.4	1066.9	1674.1
	Corixidae	877.6	2750	68.9	14642.9	405.6	2467.9	596.9	1081.6
	Baetidae	2115.5	968.9	37.4	7929.9	371	1013.5	195.9	432.3
12	Copepoda	1993.2	4263.5	128.4	30472.9	655.4	4662.2	1635.1	1959.5
	Chironomidae	6414.7	1328.6	59.8	21438.2	357.5	1124.3	176.7	436.8
	Libellulidae	623.2	135.6	7.3	2206.2	26.5	112.2	27.5	68.3
	Dytiscidae	792.9	530.9	30.1	4103.9	171.7	643.1	177.7	250.8
13	Tubificidae	878.4	1931.2	68.8	12568.8	419.7	1598.6	383	1222.5
	Glossiphoniidae	249.7	420.9	17	2501.4	127.7	327.6	60.5	254.9
	Chironomidae	2937.5	1635.4	112.5	11875	564.6	1372.9	439.6	747.9
	Copepoda	704.6	2284.1	62.5	13314.2	407.2	1625	433.2	797.3
	Baetidae	1994.4	2977.4	62	16597.7	310.2	2347.7	477.4	546.9
	Physidae	503.5	152.5	23	1527.6	917.2	87.2	19.6	164.9
14	Tubificidae	339	1039.6	23.4	6142.1	142.1	732.9	250.9	187.9
	Chironomidae	339.4	1013.7	22.8	4644.1	121.4	706.2	250	302.9
	Baetidae	1136.2	3529.5	106.7	15955.1	417.1	2602.5	362.4	855.3
	Sphaeriidae	40.8	11.2	3.6	119.3	114.5	16.9	13.2	14.7

Bold print : High concentrations

Winter

The data obtained for the metal concentrations in the macroinvertebrates during winter are given in Table 11.7.

Manganese, lead and copper occurred in the lowest concentrations, while iron and aluminum were the metals present in the highest concentrations.

During this period outstanding high metal concentrations were observed for the Copepoda at localities 1, 3 and 9, the Nematoda at localities 2, 8 and 11 and the Ceratopogonidae at localities 3 and 7.

A variety of water insect larvae, such as Dytiscidae (Predaceous diving beetles), Elmidae (Riffle beetles), Tabanidae (Horseflies), Belastomatidae (Giant water beetles) and Chironomidae (midges) had low metal concentrations as did the leeches (Glossiphonidae), aquatic earthworms (Lumbricidae) and mollusks (Physidae). Other macroinvertebrates such as Anisoptera, Ceratopogonidae, Baetidae and Tubificidae had metal concentrations varying from high to low.

Table 11.7: Metal concentrations (wet mass) accumulated by the macroinvertebrate larvae sampled during winter 1994

LOCALITY	ORGANISM	Al µg/g	Cr µg/g	Cu µg/g	Fe µg/g	Mn µg/g	Ni µg/g	Pb µg/g	Zn µg/g
1	Tubificidae	1293.5	110.8	40.2	4087.7	214.9	97.2	15.4	91.4
	Chironomidae	1227.1	539.3	21.7	4024.9	164.9	417.7	87.1	79.1
	Ceratopogonidae	4187.5	18250	359.4	96093.8	**3468.8**	**14593.8**	**2718.8**	**4531.3**
	Ostracoda	1348.6	2632.2	64.9	14615.4	403.9	2038.5	483.2	540.9
	Cladocera	1714.7	3682.4	100	20500	579.4	2811.8	564.7	532.4
	Copepoda	723.6	789.6	53.8	9330.2	209.4	1435.9	159.4	303.8
	Corixidae	494.7	1642.3	37.2	7832.5	170.2	1271.3	255.3	299.2
	Baetidae	741.1	2113.5	44.3	9893.6	274.8	1540.8	352.8	875.9
	Nematoda	1729.2	3229.2	465.3	14652.8	416.7	2027.8	534.7	1437.5
2	Chironomidae	2829.8	324.4	30.9	5097.7	1198.7	473.1	56.2	130.3
	Tubificidae	3785.3	5500	203.8	26032.6	1883.2	4176.6	516.3	812.5
	Copepoda	3101.4	4871.6	418.9	52027	1864.9	9513.5	1412.2	**2141.9**
	Cladocera	2540	6090	140	29650	1540	4960	830	1335
	Hydroptilidae	3828.6	7078.6	721.4	83428.6	1700	**16228.7**	1271.4	**2771.4**
	Ceratopogonidae	2458.3	6779.8	404.8	51726.2	1333.3	9422.6	1125	1083.3
	Baetidae	3385.7	4482.1	103.6	33250	1203.6	3635.7	782.1	925
	Corixidae	4273.4	**87718.8**	265.6	45390.6	**2742.2**	7343.8	**1585.9**	2343.8
3	Tubificidae	5465.9	550.5	41.9	13300.1	329.3	461.9	70.8	131.9
	Chironomidae	2670.5	8551.1	193.2	38238.6	863.6	6812.5	857.9	1517.1
	Ceratopogonidae	2769.7	8710.5	197.4	37894.7	802.6	6230.3	1355.3	**2513.2**
	Cladocera	5186.7	4216.7	123.3	26566.7	823.3	3510	536.7	790
	Copepoda	897.3	3804.8	85.6	33219.2	541.1	3342.5	458.9	1215.8
	Corixidae	905.7	5012.3	94.3	28401.6	553.3	3877.1	918	1057.4
4	Tubificidae	6968.1	2350.5	188.7	30465.7	2446.1	4392.2	348	887.3
	Glossiphoniidae	1268.3	7884.2	164.6	34878.1	1628.1	6128.1	847.6	1780.5
	Chironomidae	1486.4	43.2	6.7	1856.6	806.5	40.2	4.8	32.2
	Copepoda	1395.8	6010.4	161.5	28489.6	1677.1	4661.5	947.9	1536.5
	Cladocera	2256.9	5958.3	416.7	57083.3	1875	10298.6	1229.2	2284.7

Table 11.7 : (continued)

5	Tubificidae	7103.7	7024.4	432.9	61341.5	1518.3	**10219.5**	1201.2	**2329.3**
	Glossiphoniidae	1443.5	2769.4	86.3	13779.8	297.6	2187.5	288.7	683
	Chironomidae	3465.4	4753.9	103.9	23461.5	484.6	3823.1	846.2	1130.8
	Copepoda	1468.8	8125	393.8	52937.5	1168.8	**10075**	1156.3	2031.3
	Pleidae	2193.4	4136.8	301.9	42075.5	886.8	7839.6	834.9	1042.5
	Elmidae	1395	2965	420	49850	1000	9345	1020	**2205**
	Nematoda	**127234**	7952.1	**867**	**257446.8**	**4234**	**38515.9**	962.8	**3542.6**
6	Tubificidae	3555.9	65.9	13.9	7055	412.4	57	9.6	58.1
	Chironomidae	4639.5	8040.7	145.3	36627.9	1209.3	5680.2	1418.6	1767.4
	Ceratopogonidae	6927.6	8671.1	230.3	43157.9	1092.1	6368.4	**1539.5**	1736.8
	Copepoda	6308.3	**9916.7**	216.7	46750	1275	8083.3	**1691.7**	**2491.7**
	Cladocera	7295.5	4761.4	**488.6**	64318.2	1397.7	**12068.2**	1465.9	**2102.3**
	Corixidae	1641.2	1391.2	35.9	8815.8	809.7	1042.9	226.3	215.8
	Anisoptera	**12202.5**	257.8	17.5	6976.2	276.6	228.2	25.1	108.5
	Nematoda	2776	2343.8	484.4	14895.8	552.1	**25526**	286.5	1598.9
	Physidae	2988	38.6	9.4	3246.7	447.1	30.9	8.6	47.3
7	Glossiphoniidae	1587.5	4287.5	158.3	22270.8	808.3	3722.9	420.1	687.5
	Lumbricidae	3864.8	2528.7	69.7	18872.9	436.5	2071.7	448.8	571.7
	Tubificidae	4537.5	7425	175	46625	1925	6168.8	1287.5	1668.8
	Copepoda	4216.7	7450	383.3	**170555.6**	**9616.7**	8205.6	1111.1	3027.8
	Ceratopogonidae	489.9	1403.6	35.9	6681.6	502.2	1126.7	262.3	520.2
	Chironomidae	4341.4	307.9	86.5	23774	1567.3	2685.7	466.4	1036.1
	Hydroptilidae	2290.8	1627.6	191.3	25000	1038.3	3936.2	538.3	1581.6
	Baetidae	4169.1	7911.8	279.4	39191.2	1621.3	6694.9	485.3	1492.7
	Corixidae	4272.4	4873.1	235.1	33731.3	828.4	5712.7	716.4	891.8
8	Tubificidae	1488.3	1562.5	82	9355.5	898.4	1166	318.4	464.8
	Copepoda	1746.6	2962.8	158.8	15979.7	439.2	2317.6	516.9	797.3
	Chironomidae	1076.1	2004.4	158.7	14434.8	445.7	1447.8	341.3	467.4
	Ceratopogonidae	2500	5914.3	357.1	36857.1	1285.7	4514.3	771.4	1064.3
	Ostracoda	846.3	2126.2	114.7	9977.1	247.7	1433.5	451.8	373.9
	Baetidae	1814.1	1756.2	254.1	9607.4	419.4	1336.8	291.3	555.8
	Hydroptilidae	569.4	1344.1	81.8	6250	359.6	899.7	311.7	425.9
	Tabanidae	162.2	236.6	35.6	1228.6	211.4	162.7	47.4	101.2
	Dytiscidae	344.2	512.3	52.5	2569,6	252.1	357.1	111.9	138.7
9	Tubificidae	468.8	1066.1	53.8	4775.8	149.1	715.2	244.4	235.4
	Cladocera	672.5	1180.5	68.2	6029.4	121.7	795.5	272.7	316.9
	Psychodidae	987.9	1325.8	187.9	7969.7	177.3	969.7	266.7	686.4
10	Tubificidae	2275.2	1585.4	46.6	10124.2	**16582**	1216.6	237.3	416.4
	Chironomidae	409.3	53.3	7.6	2000.2	258.3	42.9	7.9	34.2
	Cladocera	5426.4	1053.5	21.5	6563.6	170.6	771.7	112	173.1
	Nematoda	**39375**	**60312.5**	**9937.5**	**281250**	**6250**	**36500**	**7500**	**14187.5**
	Physidae	731.2	807.8	112.7	3901.7	303.5	515.9	183.5	342.5
11	Tubificidae	207.5	160.2	8.4	1897.5	54.3	260.3	61.5	78.3
	Glossiphonidae	313.1	508.3	25.9	4845.1	110.1	361.2	97.4	164.8
	Chironomidae	952.7	511.5	34.1	7945.1	263.7	352.8	97.8	182.9
	Cladocera	3266.7	4983.3	261.1	26722.2	588.9	3683.3	816.7	1061.1
	Copepoda	8031.3	**12625**	**718.5**	65625	1328.1	8828.1	**2609.4**	**4296.9**
	Hydroptilidae	2024.3	3062.5	173.6	15451.4	378.5	2253.5	541.7	819.4
	Baetidae	870.6	656.7	47.5	5660.2	253.5	502.6	138.2	279.1

Table 11.7: (continued)

12	Chironomidae	980.3	2799.3	138.2	15460.5	309.2	2082.2	549.3	582.2
13	Tubificidae	255.2	350.3	30.1	2477.7	272.3	253.7	88.8	163.5
	Glossiphoniidae	243.4	566.5	42.6	2945.5	197.5	418.2	113.7	215.4
	Chironomidae	9125	3548.6	187.5	15937.5	451.4	2288.2	739.6	711.8
	Cladocera	3110.7	3204.9	180.3	55614.8	881.1	2622.9	479.5	1053.3
	Copepoda	1095.7	1467.6	81.8	8641.9	333.3	1083.3	274.7	291.7
	Belastomatidae	182.1	105.6	12.5	667.1	237.3	78.4	23.2	74.3
	Nematoda	**23666.7**	**39583.3**	**5166.7**	**190000**	**4000**	**24000**	**3833.3**	**17750**
14	Chironomidae	306.8	336.7	27.9	1838.4	150.4	230.9	71.4	177.2
	Copepoda	728.4	1123.9	63.6	6670.5	127.3	782.9	220.5	281.8
	Hydroptilidae	617.8	1038.6	52.9	5671.8	145.4	738.9	172.9	272
	Baetidae	264.3	235.2	18.2	1342.4	162.7	169	48.4	120.9

Bold print : High concentrations

Spring

Data on the metal concentrations accumulated by the macroinvertebrates during spring are presented in Table 11.8.

A variety of water insect larvae, such as Chironomidae, Tipulidae (Crane flies), Baetidae (mayflies), Hydroptilidae (Caddisflies), Notonectidae (back swimmers) and Dyriscidae, as well as some mollusks (Gastropoda - Physidae and Pelecypoda - Sphaeriidae) had low metal concentrations. High metal concentrations were observed for macroinvertebrates such as the Crustacean Ostracoda and Cladocera, hydroids (Hydra) and water insect larvae Corixidae and Isotomidae.
Variable metal concentrations were observed for macroinvertebrates such as Copepoda (crustacea), Helobdella (leeches), Tubificidae (aquatic earthworms) and Ceratopogonidae (Biting midges).
The metal values during spring varied from high concentrations for iron and aluminum to low manganese, lead and copper concentrations.

11.4 Discussion

11.4.1 Identification and Distribution of Macroinvertebrates

Kotze (1997) described the surface water of the Olifants River as generally more alkaline than acidic. pH varied form 3.32 (locality 12) to 9.40 (locality 7 : Kotze, 1997). Water temperature ranged from 7.2°C (locality 10 during winter) to 28.7°C (locality 2 during summer 1995 and locality 1 during summer 1994 : Kotze, 1997). Factors such as algal blooms (locality 14 causing low oxygen levels), agricultural and mining activities (locality 11 causing low turbidity levels due to increased siltation) had a direct effect on the surface water of the Olifants River and eventually influencing the aquatic macroinvertebrates occurring at the various localities.

In Table 11.9 the total number of benthic organisms during the four seasons for the Olifants River are compared to locality X, a reference site receiving organic and industrial effluent. A comparison is made between the total number of organisms at the Olifants River and the total number of organisms at locality X to establish values for small and large numbers of benthic organisms.

Table 11.8: Metal concentrations (wet mass) accumulated by the macroinvertebrate larvae sampled during spring 1994

LOCALITY	ORGANISM	Al µg/g	Cr µg/g	Cu µg/g	Fe µg/g	Mn µg/g	Ni µg/g	Pb µg/g	Zn µg/g
1	Tubificidae	5912.9	228.4	86.7	15530.3	672.3	202.7	66.3	279.9
	Glossiphoniidae	144875	75250	**12000**	**723750**	**14500**	49125	19625	**30000**
	Copepoda	16725	7175	1025	75500	3125	4812.5	1537.5	4025
	Cladocera	**34428.6**	17857.1	2964.3	**136428.6**	4464.3	12392.9	3071.4	11500
	Ostracoda	**71500**	**64125**	**9000**	**371250**	**9875**	**41375**	**12125**	**34125**
	Chironomidae	9042.7	3487.8	554.9	31585.4	1085.4	2085.4	829.3	1512.2
	Ceratopogonidae	53687.5	35062.5	4375	**336250**	5187.5	21562.5	9125	17250
	Corixidae	**162083.3**	**48083.3**	**6833.3**	**453333.3**	7416.7	29166.7	9833.3	**30250**
	Dytiscidae	2149	1033.1	201.9	7367.6	344.4	574.5	278.2	567.9
	Isotomidae	71083.3	**46083.3**	6750	**925833.3**	**13166.7**	**28416.7**	**7666.7**	**20083.3**
	Nematoda	3153.8	2435.8	403.8	13782.1	519.2	1730.8	0	1596.2
	Sphaeriidae	179.1	133.1	24.7	849.5	96.3	2137.7	17.4	46.7
	Physidae	6762.1	236.4	35.6	3839.9	156.8	127.8	49.9	69.8
2	Tubificidae	6205.4	469	12.7	9561.9	1371.6	249.2	141.2	429
	Chironomidae	3067.3	528.5	87.6	6582.9	1867.5	236.4	130.4	389.9
	Copepoda	**31285.7**	**27571.4**	**3607.1**	**114285.7**	6500	**11464.3**	6285.7	12392.9
	Cladocera	8589	784.2	107.9	13981.2	1695.2	410.1	148.1	399.8
	Ostracoda	22000	**27142.9**	2750	**96071.4**	**7875.1**	**10321.4**	6464.3	12857.1
	Hydroptilidae	**26650**	**39850**	**4450**	**105000**	5450	**16250**	9100	13350
	Hydridae	**36300**	**40700**	**4950**	**137500**	4300	**16150**	9150	**20500**
	Corixidae	**176750**	**193000**	**24500**	**717500**	**34500**	**79750**	**36500**	**77750**
	Baetidae	1459.9	1625.9	152.7	5782.4	166	683.2	316.8	841.6
	Nematoda	353125	**28895.8**	**3020.8**	**758333.3**	4000	9166.7	1104.2	9125
3	Tubificidae	4464.9	88.1	16.2	6158.9	286.2	45	14	93.5
	Chironomidae	6130.8	2595.9	305.2	17180.2	1029.1	1145.4	313.9	1267.4
	Ceratopogonidae	41250	**54625**	**5312.5**	**176875**	**10500**	**23187.5**	7687.5	16875
	Cladocera	**25297.6**	6363.1	625	59464.3	1571.4	2744.1	1142.9	2291.7
	Ostracoda	**50859.4**	**18921.9**	2250	**185312.5**	2687.5	8750	2500	6546.9
	Copepoda	**97000**	**112750**	**19625**	**435000**	**9500**	**44625**	**19125**	**57000**
	Corixidae	**65750**	**45650**	**4800**	**222000**	4500	**18050**	8150	**17200**
4	Tubificidae	5528.6	934.7	218.2	15987.3	1880.6	581.2	226.1	735.7
	Glossiphoniidae	6637.5	1991.7	450	19541.7	1833.3	1420.8	666.7	1808.3
	Chironomidae	1197.3	112.2	32.5	2628.3	1171.2	77.7	25.8	117
	Ostracoda	14925	15250	2550	68500	5200	7625	4700	8825
	Cladocera	**25625**	76750	**11750**	**278750**	**8375**	**38500**	**17500**	**62625**
	Copepoda	**53625**	**90000**	**16250**	**382500**	**8750**	**45125**	**16625**	**39375**
	Corixidae	17875	**27958.3**	**4791.7**	**145000**	3375	**14583.3**	4791.7	**13291.7**
	Nematoda	2046.8	3828.1	632.8	18515.6	453.1	2171.8	992.2	2031.3
5	Tubificidae	1543.4	889.2	115.3	7500	851.8	580.8	187.1	908.7
	Glossiphoniidae	4023.8	2148.8	288.7	18333.3	672.6	1267.8	479.2	872
	Copepoda	3012.3	1128.5	193.7	12500	1352.1	741.2	198.9	968.3
	Chironomidae	2313.4	2211.3	588	10035.2	911.9	1278.2	510.6	2042
	Psychodidae	**1744.5**	**1648.4**	219.8	9807.7	620.9	942.3	390.1	1046.7
	Tipulidae	386.3	282.2	46.7	2833.2	86.5	163.9	82.4	142.7
	Nematoda	50150	**32550**	**4650**	**226000**	4350	**32200**	**2200**	**14700**
6	Tubificidae	1905.9	50.9	14.7	3968.4	466.3	33.9	17	83.7
	Chironomidae	713.2	52.9	9.9	3424.9	289.3	34.5	11.1	43.8
	Glossiphoniidae	2720.6	2416.7	348	15196.1	642.2	1651.9	426.5	2127.5
	Ostracoda	3995.3	2643.6	396.2	15432.7	605.8	1692.3	490.4	2663.5
	Copepoda	3886.4	3761.4	426.1	17329.6	431.8	2130.7	988.6	1232.9

Table 11.8: (continued)

7	Tubificidae	28830	5560	1210	73300	2010	3710	980	4040
	Chironomidae	2427.3	1051.4	148.9	9875.9	195	682.6	171.9	604.6
	Ceratopogonidae	**48279.4**	8720.6	1397.1	**80000**	2720.6	5808.8	1455.9	5514.7
	Cladocera	15647.7	7045.5	1079.6	54204.6	829.6	4181.8	2011	4704.6
	Copepoda	9116.7	**17666.7**	**2283.3**	**94500**	1983.3	**11783.3**	2100	6450
	Baetidae	5821.4	4919.6	660.7	37053.6	758.9	3250	1151.8	2839.3
8	Tubificidae	618.2	229.5	34.3	1716.6	104.9	130.9	85.2	92
	Chironomidae	353.2	190.1	25.8	1009.1	33	110.8	64.9	51.2
	Ceratopogonidae	197.7	180.9	28.7	755.8	45.4	100.7	71.2	84.3
	Spaeriidae	393.4	71	14.5	1806.2	227.5	65.2	33.1	198.8
9	Tubificidae	**24062.5**	6125	1037.5	31625	1200	3912.5	2750	**47012.5**
	Chironomidae	4487.9	1245.9	229.8	13750	639.1	731.9	296.4	889.1
	Ceratopogonidae	4494.2	3139.5	401.2	25639.5	622.1	1750	906.9	2034.9
	Copepoda	1891	3173.1	461.5	13461.5	429.5	2384.6	871.8	2660.3
	Cladocera	5821.4	6261.9	809.5	31547.6	916.7	4345.2	1964.3	3273.8
	Caenidae	1192.5	1292	194.7	8827.4	269.9	840.7	289.8	389.4
	Dytiscidae	532.4	667.6	82.4	3216.2	158.1	429.7	237.8	294.6
	Notonectidae	510.7	796	115	4509.2	147.2	593.6	236.2	394.2
10	Tubificidae	721.9	14.7	4.3	1268.9	124.6	12.7	3	34.4
	Chironomidae	1315	28.6	7.5	1868.9	164.5	19.4	5.5	32.2
	Copepoda	4235.9	2816	377.4	28207.6	750	1575.5	679.3	316
	Cladocera	5065.8	3269.7	394.7	11776.3	447.4	1782.9	940.8	1625
	Corixidae	4437.5	3181.3	418.8	19875	425	1825	518.8	2600
	Nematoda	**60083.3**	9091.7	1550	85916	1191.7	4733.3	1183.3	1975
11	Chironomidae	5564.1	834.9	97.8	7884.6	479.2	445.5	174.7	278.9
	Glossiphoniidae	396.4	220.2	28.2	1401.3	119.8	117.3	39.8	137.2
	Tubificidae	1962.8	1128.1	115.7	5661.2	371.9	588.8	188	855.4
	Ceratopogonidae	1552.4	1143.2	125	4737.9	221.8	550.4	227.8	407.3
	Copepoda	3266.2	1821.4	220.8	7922.1	240.3	879.9	389.6	944.8
	Baetidae	410.1	287.6	43.4	1913.3	81.6	214.9	142.2	107.8
	Hydroptilidae	427.1	368.1	53.8	1718.8	75.5	296.9	174.5	281.3
	Nematoda	***821851.9***	***14703.7***	***4824.1***	***475925.9***	***25240.7***	***11046.3***	7527.8	***23250***
12	Chironomidae	814.2	364	65.9	3361.5	174.8	288	195.9	205.2
	Copepoda	515.1	493.9	88.7	3598.8	78.6	365.9	215.7	322.9
	Tubificidae	1179.2	1068.8	204.2	10291.7	277.1	858.3	387.5	577.1
13	Tubificidae	2415.3	1612.9	286.3	8790.3	451.6	1205.7	778.2	987.9
	Glossiphoniidae	322.2	252	33.3	1702	49.5	169.2	103.5	144.4
	Hydridae	445.1	340.3	50.6	1900.3	59.3	223.9	158.2	162.6
	Corixidae	1376.5	787	106.5	4058.6	103.4	487.7	311.7	677.5
	Chironomidae	189.1	181.2	27.2	942	42.8	102.9	80.4	88.4
	Copepoda	208.4	123.1	19.5	709.5	85.1	66.7	51.5	58.2
	Cladocera	290.2	183.4	28.5	957.9	79.1	109.7	76.2	132.8
	Ceratopogonidae	237.1	191.5	26.9	1341.2	34.9	110.6	76.4	72.2
	Nematoda	1992.1	3592.9	614.3	16785.7	428.8	2128.6	771.4	1850
14	Tubificidae	1563.7	643.9	104.9	4716.9	329	438.7	166.3	395.1
	Chironomidae	528.9	296.7	41.9	1759.7	263.4	211.4	95.5	222.1
	Cladocera	1769.6	2906.9	348	12500	338.2	1887.3	813.7	1210.8
	Copepoda	3180.6	2629.6	324.1	22083.3	467.6	1791.7	476.9	1726.9
	Hydridae	2513.6	2609.1	381.8	20136.4	681.8	1768.2	659.1	1050
	Hydropsychidae	1522.7	1556.8	184.7	5823.9	289.8	988.6	480.1	497.2
	Baetidae	410	53	13.4	597.4	304.5	46.3	16.2	104

Bold print : High concentrations

Table 11.9: Comparison of The Olifants River and Locality X

CLASSIFICATION	SEASONS							
	Winter		Spring		Summer		Autumn	
	OR	Loc X	OR	Loc X	OR	Loc X	OR	Loc X
Coelenterata	-	1060	438	916	-	4990	49	3507
Annelida	69695	134524	117953	1346573	40324	70419	46055	122120
Ostracoda	1851	138155	5017	379561	1899	49049	3506	50351
Collembola	-	20	97	-	-	-	-	390
Ephemeroptera	10568	167	5066	49	3116	-	4918	420
Hemiptera	1367	137	731	127	1851	460	2046	939
Trichoptera	2192	1209	292	188	292	263	147	1162
Coleoptera	390	118	243	176	292	167	244	99
Diptera	27808	187601	30730	146235	37745	78689	17681	56082
Gastropoda	437	5177	146	12569	438	11026	49	20421
TOTAL SPECIES	**22**	**19**	**22**	**22**	**18**	**36**	**25**	**24**

From the comparisons, locality X clearly shows large numbers of the Coelenterata, Nematoda, Annelida, Ostracoda and Diptera, when compared to the total number sampled. In contrast water insect larvae found at the Olifants River localities were more abundant.

When comparing species diversity (Table 11.9), a large number of species is evidently present at the reference locality. The sampling sites in the Olifants River showed less species diversity, probably due to the effect of mine and other effluent on various benthic species.

Seasonal differences in the number of organisms and diversity of species in the Olifants River are evident. There was an increase in the number of certain organisms such as Annelida (Tubificidae), Cladocera and Copepoda towards winter and also spring. This might be due to a drop in water level at most of the localities, resulting in the concentration of macroinvertebrates per unit volume water. The decrease in the number of water insect larvae (Ephemeroptera, Odonata, Hemiptera, Coleoptera and Diptera) and mollusks towards winter is the result of lower temperatures and nutrient availability. During summer, with the start of the rainy season (water volume increases) higher temperatures and nutrient availability resulted in an increase in water insect larvae and especially Chironomidae. Lower numbers of. The volume increase of streams could lead to the lower numbers per sample of Nematoda, Cladocera and Copepoda observed during summer. However, information on the life cycle of a species is another important factor when considering increases/decreases in the number of organisms as well as species diversity at the Olifants River for certain periods of the year (Gaufin & Tarzwell, 1952; Pennak, 1978).

The large numbers of Tubificidae, Cladocera, Copepoda and Chironomidae were present at most of the localities throughout the sampling period, can be attributed to their physiological tolerance to agricultural, industrial and mining effluents (Vangenechten *et al.*, 1986), as well as the nature and stability of the stream beds (Chutter, 1971). Availability of food (Aagaard & Sivertsen, 1979; Vangenechten *et al.*, 1986) and the presence or absence of predators are further determining factors for species abundance (Kajak, 1979; Haines, 1981; Vangenechten *et al.*, 1986). Tubificidae and Chironomidae are species considered to be tolerant to various forms of pollution (Gaufin & Tarzwell, 1956; Brinkhurst, 1966; Aagaard & Sivertsen, 1979; Moon & Lucostic, 1979). Determining factors for both these species survival in surface waters include temperature (Gaufin &

Tarzwell, 1956), alkalinity, water hardness, dissolved oxygen and pH (Brinkhurst, 1966; Brkovic-Popovic & Popovic, 1977; Godfrey, 1978; Moon & Lucostic, 1979). Considering the presence and abundance of Tubificidae, Cladocera, Copepoda and Chironomidae, it is evident that some form of tolerance had been developed by these species ensuring their survival in a polluted system (Gaufin & Tarzwell, 1956).

Decreased numbers of Ostracoda, some water insect larvae such as Ephemeroptera, Odonata, Hemiptera, Trichoptera, Lepidoptera and Coleoptera as well as Gastropoda and Pelecypoda occurred at some of the localities throughout the sampling period. Factors contributing to low species diversity and restricted is probably caused by the activity of predators (Kajak, 1979; Haines, 1981) and food availability (Aagaard & Sivertsen, 1979; Vangenechten *et al.*, 1986). Physico-chemical conditions of the surface water affecting especially water insect larvae abundance, is pH (Bell, 1971; Haines, 1981), low dissolved oxygen and alkalinity (Godfrey, 1978). Moon and Lucostic (1979) as well as Bell (1971) confirmed Ephemeroptera, Odonata and Plecoptera as being fairly sensitive to changes in chemical conditions of the surface water. Bell (1971)Further stated that low pH conditions may cause aquatic insect emergence to be one of the most critical stages of their life cycle. However, safe pH levels for aquatic insects can vary from one family to another (Bell, 1971; Kelly, 1988).

When conditions are favourable for organisms which can adopt to pollution, they thrive and build high populations. For this reason, the number and diversity of organisms found in polluted water are significant in offering clues to the intensity of pollution and the degree of recovery.

11.4.2 Metal Accumulation by Macroinvertebrates

Kotze (1997) indicated a few areas in the Olifants River catchment regarding metal pollution of immediate concern. These areas included localities 6 (high Cu, Zn, Fe, Ni and Pb), 3 (Fe, Ni, Mn and Pb), 10 (Zn, Al, Ni, Mn and Cu), 12 (Mn and Ni) and 13 (Cu, Fe, Pb and Cr) where the water at these localities were all subjected to coal mining activities in the upper reaches (Kotze, 1997). Low pH at localities 3,6 and 10 had a negative impact not only on the aquatic life, but also influenced bioavailability of metals (Kotze, 1997). Kotze (1997) further indicated an increase in mean copper, chromium, nickel and lead concentrations in the water of the Olifants River moving downstream from localities 8 to 14. These levels might have been due to sewage treatment effluent containing industrial effluent discharges, urban runoff from Middelburg town and coal mining at locality 8 (Kotze, 1997). Witbank and Loskop Dams contained high levels of metals and thus also acting as a sink for metal pollution occurring in their sub catchment. High concentrations of copper, zinc, iron, nickel, lead and chromium at locality 7 (Witbank Dam) could be ascribed to effffluent (coal mining) from the Steenkool Spruit and the Boesman Spruit. Locality 13 (Middelburg Dam) presented high levels of zinc, iron, nickel, lead and chromium due to mining influences in the upper reaches of Woesalleen Spruit (Kotze, 1997).

Iron, copper and chromium concentrations in the sediment at all the localities to exceed the EPA-guideline value, while manganese and zinc concentrations showed the opposite trend (Steenkamp *et al.*, 1994; Kotze,1997)

Metals released into an ecosystem tend to accumulate in sediments and thus become part of the ecosystem (Steenkamp *et al.*, 1994). Metals can be reintroduced into the water in a bioavailable form, transformed into a more or less toxic form or migrate from the sediment into the

macroinvertebrates from which they can be taken up into the food chain (Burton, 1992). Whether the contaminants will remain in place or contaminate the ecosystem, is difficult to determine (Steenkamp *et al.*, 1994).

During the sampling period iron and aluminum concentrations were high in the macroinvertebrates analyzed. This tendency is probably due to mining and industrial effluents, which are the general sources of elevated metal concentrations in surface water. High iron and aluminum concentrations were observed for the sediment (Van Vuren *et al.*, 1995). Aluminum averages 82 % of the mass of the earth's crust (Freedman, 1989) and high iron concentrations could be related to the presence of Fe-hydroxides, Fe-oxides and organic carbon on these particles (Venter, 1995). The possibility is thus that both iron and aluminum either migrated from the sediment to the macroinvertebrates or were reintroduced from the sediment to the water in which these organisms survive.

The following sequence for metal concentrations in the macroinvertebrates sampled and analyzed can be derived from the results obtained : Cu < Pb < Mn < Cr < Zn < Ni < Al < Fe. Macroinvertebrates such as Nematoda, Tubificidae, Crustacea (Cladocera, Copepoda and Ostracoda) and some water insect larvae (Hydroptilidae, Corixidae, Chironomidae and Ceratopogonidae) presented outstanding high metal concentrations throughout the sampling period. Brown (1977) confirmed that the concentrations of metals in sediment and water and their consequent bioavailability to aquatic life may vary with chemical and physical factors such as pH, temperature (Dixit & Witcomb, 1983), sediment load and water hardness. Metal contaminants introduced into the aquatic system, from both mining and industrial activities, usually exists in relatively unstable chemical forms and are, therefore, predominantly accessible for biological uptake (Förstner, 1982). Accumulation of metal concentrations by aquatic invertebrates are determined by several factors. Firstly, the organism's stage of development (Burrows & Whitton, 1983) - some immature and also molting stages are more sensitive to metal concentrations than the mature stages (Martin, 1970; Spehar *et al.*, 1978; Burton *et al.*, 1985). Secondly, the metal content of the habitat. Metal levels in the organism differ because of the specific association of the organism with the substrate (Dixit & Witcomb, 1983). Thirdly,varying physiological abilities to exclude metals from organism bodies (Dixit & Witcomb, 1983). Each group of species have different element concentrations as each concentrate a particular element through different mechanisms (Martin, 1970). According to brown (1977) the mechanism of adaptation for insect involve decreased permeability of the insect to metals and increased efficiency of any regulatory mechanism (Bryan & Hummerstone, 1973). Fourthly, feeding habits of the organisms. Organisms with different feeding habits may concentrate metals to different levels (Anderson, 1977; Brown, 1977; Burrows & Whitton, 1983; Uimonen-Simola & Tolonen, 1987; Amiard, 1992).
The above mentioned aquatic invertebrates have thus been exposed to a variety of environmental contaminants. Although accumulating these contaminants by means of different path ways to extraordinary high levels, these organisms have survived, thus becoming more tolerant to pollution (Dixit & Witcomb, 1983; Freedman, 1989).

Toxic metal pollution from either mining or industries effluent, reduced species numbers and abundance. The presence of a species will depend on its environmental tolerance, but its abundance will be determined by the resources available to it.

11.5 Occurrence evaluation index

The Occurrence Evaluation Index (Table 11.10) was compiled for the aquatic invertebrates sampled at the different localities of the upper Olifants River catchment. The water quality data, as well as metal concentrations for the water column and sediment compartment were taken into consideration when determining the sensitivity of the different aquatic organisms to mining effluent.

The water quality data of the index was compared with water quality guidelines suggested by Kempster *et al.*, 1982; Kühn (1991) and prescribed by Environment Canada (1987). From the variables determined for the water it was evident that only phosphate and ammonia levels were higher than the prescribed guidelines. The water metal concentrations were well above the guidelines indicating a possible detrimental effect on the survival of macroinvertebrates in this river system.

Very high iron and aluminum concentrations were observed for the sediment analysed. The iron sediment concentrations could be related to the presence of Fe-hydroxides, Fe-oxides and organic carbon on these particles (Venter, 1995).

A great diversity and number of aquatic macroinvertebrates occurred at the different localities. In Table 6.10 the organisms were presented from the least abundant to the most abundant species. Metal concentrations for the various organisms were included to give an indication of the metal levels these organisms were exposed to. Aluminum and iron concentrations averaged high levels for the organisms analysed. High aluminum and iron concentrations were also observed for the water and sediment analysed (Table 11.10). Thus aluminum and iron from both the water column and sediment compartment contributed to elevated levels in the macroinvertebrates (Brown, 1977). Aquatic macroinvertebrates occurring in the upper Olifants River catchment have to some extend adapted to high metal concentrations, whether from the water column or sediment compartment (Scullion & Edwards, 1980). These organisms such as the Chironomidae, Tubificidae and Crustacea thrived and built large populations (Table 11.10). The exposure of these organisms and their consequent survival in large numbers might also be due to factors such as developmental stage when exposed to metal concentrations (Getsova & Valkova, 1962; Spehar *et al.*, 1978; Wright, 1980), feeding habits (Kelly, 1988), the organism's ability to exclude or regulate metals by their physiological abilities (Dixit & Witcomb, 1983), the availability of food and the presence/absence of predators (Vengenechten *et al.*, 1986).

The lower numbers of organisms (water insect larvae, Coelenterata, Gastropoda and Pelecypoda) may be indicative of their sensitivity towards very high metal concentrations in the surrounding water column and sediment compartment (Roback & Richardson, 1969).

Thus, the population of organisms found in the Olifants River system is significant in offering clues to the intensity of pollution and the consequent degree of recovery.

Table 11. 10: Occurrence Evaluation Index

Water quality data			Macroinvertebrates			Metal Concentrations in the Sediment (µg/g dry weight)		
Constituent	Average ± SD n =	Min - Max	Organisms	Average ± SD (µg/g wet weight)	Min - Max	Variable	Average ± SD n =	Min - Max
PH (log[+H])	7.6 ± 0.4	7.0 - 7.9	Odonata - Libellulidae	Al 3 050.5 ± 3 908.8	623.2 - 7 559.2	Iron (µg/g Fe)	36 126.4 ± 9 111.5	27 452.4 - 53 372.4
Conductivity (mS/m)	540.9 ± 513.0	1.0 - 1 235.8	- Gomphidae	Cr 1 941.9 ± 3 205.5	47.1 - 5 642.9	Copper (µg/g Cu)	106.5 ± 60.1	54.1 - 220.7
Total Dissolved Solids (mg/l TDS)	734.9 ± 157.7	505.7 - 893.8	- Lestidae	Cu 317.4 ± 529.3	7.3 - 728.6	Nickel (µg/g Ni)	451.4 ± 747.2	76.5 - 1 958.1
Total Hardness (mg/l $CaCO_3$)	91.5 ± 6.4	83.3 - 99.6		Fe 11 421.0 ± 15 067.9	2 206.2 - 28 809.5	Manganese (µg/g Mn)	764.2 ± 308.8	513.4 - 1 191.7
Calcium (mg/l Ca^{2+})	91.5 ± 6.4	83.3 - 99.6		Mn 375.2 ± 592.6	26.5 - 1 059.5	Zinc (µg/g Zn)	213.2 ± 153.6	95.7 - 505.7
Magnesium (mg/l Mg^{2+})	55.6 ± 14.5	32.9 - 68.9		Ni 1 344.7 ± 2 186.4	52.7 - 3 869.1	Chromium (µg/g Cr)	257.9 ± 193.9	110.4 - 588.4
Sodium (mg/l Na^{+})	38.9 ± 7.8	33.2 - 50.3		Pb 604.9 ± 1 012.3	157.8 - 1 773.8	Lead (µg/g Pb)	86.5 ± 105.1	35.9 - 300.2
Chloride (mg/l Cl^{-})	25.0 ± 9.1	13.4 - 35.9		Zn 2 061.8 ± 3 462.1	57.5 - 6 059.5	Aluminium (µg/g Al)	43 002.0 ± 12 922.4	33 384.3 - 68 393.3
Nitrate (mg/l NO_3)	0.9 ± 0.5	0.4 - 1.8	Lepidoptera - Pyrallidae	Al 16 174.9 ± 28 367.3	157.8 - 71 083.3			
Sulphate (mg/l SO_4)	397.7 ± 108.6	227.1 - 493.1	Diptera - Tipulidae	Cr 12 235.0 ± 19 267.4	212.8 - 46 083.3			
Phosphate (mg/l PO_4)	0.3 ± 0.1	0.1 - 0.4	- Simulidae	Cu 11 420.2 ± 27 474.2	16.4 - 67 500.0			
Potasium (mg/l K)	10.0 ± 3.1	6.4 - 14.6	- Psychodidae	Fe 177 560.0 ± 369 673.0	710.8 - 925 833.0			
Chromium (mg/l Cr)	0.2 ± 0.01	0.22 - 0.25	- Tabanidae	Mn 2 690.5 ± 5 207.7	34.5 - 13 166.7			
Iron (mg/l Fe)	2.2 ± 1.3	1.1 - 4.2	- Tanypodinae	Ni 8 219.2 ± 12 376.8	80.9 - 28 416.7			
Zinc (mg/l Zn)	0.4 ± 0.3	0.2 - 0.8		Pb 2 625.0 ± 3 855.8	34.5 - 7 666.7			
Manganese (mg/l Mn)	1.3 ± 1.4	0.2 - 3.0		Zn 4 611.3 ± 7 948.5	101.2 - 20 083.3			
Copper (mg/l Cu)	0.02 ± 0.01	0.01 - 0.05	Coelenterata - Hydroida	Al 13 086.2 ± 20 130.3	445.1 - 36 300.0			
Nickel (mg/l Ni)	0.3 ± 0.1	0.2 - 0.3		Cr 14 549.8 ± 22 675.1	340.3 - 40 700.0			
Lead (mg/l Pb)	0.1 ± 0.1	0.1 - 0.2		Cu 1 794.1 ± 2 738.1	50.6 - 4 950.0			
Silica (mg/l SiO_2)	3.3 ± 0.4	2.9 - 3.9		Fe 53 178.9 ± 73 591.3	1 900.3 - 137 500.0			
Ammonia (mg/l N)	0.1 ± 0.1	0.1 - 0.2		Mn 1 680.4 ± 2 289.9	59.3 - 4 300.0			
Fluoride (mg/l F^{-})	1.1 ± 0.2	0.8 - 1.3		Ni 6 050.7 ± 8 779.8	233.9 - 16 150.0			
Aluminium (mg/l Al)	1.9 ± 1.1	0.8 - 3.6		Pb 3 322.4 ± 5 053.0	158.2 - 9 150.0			
				Zn 7 237.5 ± 11 494.2	162.6 - 20 500.0			
			Coleoptera - Elmidae	Al 5 447.5 ± 9 783.1	201.8 - 29 850.0			
			- Dytiscidae	Cr 3 872.0 ± 7 666.6	106.5 - 23 900.0			
				Cu 621.6 ± 1 534.2	15.2 - 4 700.0			
				Fe 21 970.2 ± 41 494.2	1 049.2 - 129 500.0			
				Mn 665.3 ± 1 516.5	0.0 - 4 700.0			
				Ni 3 034.6 ± 5 634.9	69.1 - 17 400.0			

Table 11.10: (continued)

				Pb 1 189.5 ± 2 544.3	43.7 - 7 900.0			
				Zn 2 861.0 ± 7 247.2	71.9 - 22 150.0			
			Gastropoda - Physidae	Al 2 249.4 ± 2 748.3	262.1 - 6 762.1			
				Cr 337.2 ± 303.2	38.6 - 807.8			
				Cu 52.8 ± 43.5	9.4 - 112.7			
				Fe 3 215.4 ± 978.5	1 527.6 - 3 901.7			
				Mn 392.7 ± 318.6	138.9 - 917.2			
				Ni 206.5 ± 194.4	30.9 - 515.9			
				Pb 54.9 ± 73.6	8.6 - 183.5			
				Zn 152.8 ± 116.5	47.3 - 342.5			
			Pelecypoda - Sphaeriidae	Al 325.1 ± 232.5	40.8 - 726.9			
				Cr 99.4 ± 86.8	11.2 - 250.6			
				Cu 19.7 ± 16.5	3.6 - 49.8			
				Fe 942.2 ± 624.6	119.3 - 1 806.2			
				Mn 240.3 ± 299.5	75.9 - 841.1			
				Ni 414.1 ± 846.5	16.9 - 2 137.7			
				Pb 27.6 ± 19.9	13.2 - 65.9			
				Zn 78.3 ± 66.0	14.7 - 198.8			
			Diptera - Culicidae	Al 12 631.2 ± 9 478.2	5 929.1 - 19 333.3			
				Cr 11 552.5 ± 12 928.7	2 410.5 - 20 964.4			
				Cu 343.3 ± 221.6	186.6 - 500.0			
				Fe 62 224.3 ± 65 993.7	15 559.7 - 108 889.0			
				Mn 972.2 ± 1 374.9	0.0 - 1 944.4			
				Ni 10 183.3 ± 10 543.3	2 727.6 - 17 638.9			
				Pb 2 575.7 ± 3 035.7	429.1 - 4 722.2			
				Zn 3 150.1 ± 3 009.0	1 022.4 - 5 277.8			
			- Ceratopogonidae	Al 12 146.4 ± 18 168.2	57.3 - 53 687.5			
				Cr 10 526.1 ± 16 102.1	97.7 - 59 000.0			
				Cu 1 230.8 ± 2 404.1	11.5 - 10 875.0			
				Fe 97 146.3 ± 206 320.0	442.7 - 100 700.0			
				Mn 2 646.1 ± 5 691.5	20.5 - 27 750.0			
				Ni 7 657.3 ± 10 422.3	60.5 - 45 625.0			
				Pb 2 325.0 ± 4 216.2	46.2 - 19 500.0			
				Zn 4 366.3 ± 6 732.2	34.7 - 28 000.0			

Table 11.10 (Continued)

			Trichoptera - Hydropsychidae - Hydroptilidae	Al 6 733.8 ± 9 695.8	427.1 - 26 650.0			
				Cr 8 667.8 ± 12 280.0	368.1 - 39 850.0			
				Cu 877.8 ± 1 271.9	52.9 - 4 450.0			
				Fe 26 531.1 ± 28 444.6	1 718.8 - 86 750.0			
				Mn 1 271.7 ± 1 738.2	75.5 - 5 450.0			
				Ni 9 721.3 ± 13 986.1	296.9 - 49 416.7			
				Pb 1 723.8 ± 2 618.2	172.9 - 9 100.0			
				Zn 3 053.8 ± 4 120.6	272.0 - 13 350.0			
			Hemiptera - Corixidae	Al 23 139.2 ± 51 014.3	59.2 - 176 750.0			
				Cr 18 135.2 ± 42 740.9	76.2 - 193 000.0			
				Cu 2 289.3 ± 5 523.8	15.7 - 24 500.0			
				Fe 94 169.6 ± 181 384.0	1 130.9 - 717 500.0			
				Mn 3 050.3 ± 7 506.2	32.1 - 34 500.0			
				Ni 9 282.9 ± 18 125.3	57.1 - 79 750.0			
				Pb 3 633.0 ± 8 194.7	28.9 - 36 500.0			
				Zn 8 850.9 ± 18 262.5	43.1 - 77 750.0			
			Annelida - Hirudinea	Al 10 422.0 ± 33 757.4	243.4 - 144 875.0			
				Cr 7 274.3 ± 17 653.4	187.5 - 75 250.0			
				Cu 830.0 ± 2 794.9	17.0 - 12 000.0			
				Fe 60 133.2 ± 169 210.0	1 401.3 - 723 740.0			
				Mn 2 218.6 ± 4 144.2	49.5 - 14 500.0			
				Ni 5 209.4 ± 11 629.9	117.3 - 49 125.0			
				Pb 670.2 ± 868.8	39.8 - 3 466.7			
				Zn 3 021.4 ± 7 01.3	137.2 - 30 000.0			
			Nematoda - Dorylaimida - Enoplida	Al 123 932.0 ± 241 262.0	1 729.2 - 821 852.0			
				Cr 17 376.6 ± 18 798.1	2 343.8 - 60 312.5			
				Cu 2 718.1 ± 2 944.3	403.8 - 9 937.5			
				Fe 196 125.0 ± 229 858.0	13 782.1 - 758 333.0			
				Mn 4 302.9 ± 6 910.7	416.7 - 25 240.0			
				Ni 15 812.3 ± 14 537.2	1 730.8 - 38 515.9			
				Pb 2 241.3 ± 2 657.1	0.0 - 7 527.8			
				Zn 7 753.7 ± 7 781.1	1 437.5 - 23 250.0			
			Ephemeroptera - Baetidae	Al 2 794.2 ± 3 051.8	163.1 - 10 510.9			
				Cr 2 659.3 ± 2 532.4	53.0 - 9 434.8			

Table 11.10: (Continued)

				Cu 202.1 ± 224.5	13.4 - 869.6			
				Fe 20 911.7 ± 23 342.9	542.4 - 106 122.0			
				Mn 490.9 ± 458.5	0.0 - 1 621.3			
				Ni 1 960.1 ± 1 782.5	46.3 - 6 694.9			
				Pb 508.6 ± 480.9	16.2 - 1 554.4			
				Zn 887.2 ± 737.2	104.0 - 2 839.3			
			Diptera - Chironomidae	Al 5 087.9 ± 7 523.5	189.1 - 42 440.2			
				Cr 2 686.2 ± 4 617.9	28.6 - 25 071.4			
				Cu 218.3 ± 333.2	4.3 - 1 482.1			
				Fe 19 676.5 ± 28 482.7	942.0 - 139 444.0			
				Mn 610.6 ± 561.3	0.0 - 2 357.1			
				Ni 2 347.9 ± 4 184.3	19.4 - 19 035.7			
				Pb 607.4 ± 1 224.4	4.9 - 7 535.7			
				Zn 1 112.6 ± 1 587.3	32.2 - 6 555.6			
			Crustacea - Copepoda	Al 10 882.6 ± 18 040.7	85.3 - 97 000.0			
				Cr 11 495.8 ± 21 471.7	106.4 - 112 750.0			
				Cu 1 463.2 ± 3 708.6	14.4 - 19 625.0			
				Fe 65 569.1 ± 91 003.2	411.3 - 435 000.0			
				Mn 3 328.4 ± 11 244.4	0.0 - 75 378.8			
				Ni 7 309.7 ± 10 498.0	40.2 - 45 125.0			
				Pb 2 248.5 ± 4 768.5	4.9 - 19 125.0			
				Zn 5 579.2 ± 10 496.9	32.2 - 57 000.0			
			- Ostracoda	Al 19 349.5 ± 23 619.7	846.3 - 71 500.0			
				Cr 16 151.8 ± 18 726.6	2 126.2 - 64 125.0			
				Cu 2 017.7 ± 2 706.8	64.9 - 9 000.0			
				Fe 155 965.0 ± 223 689.0	9 977.1 - 708 929.0			
				Mn 3 952.6 ± 4 110.1	247.7 - 10 571.4			
				Ni 9 701.7 ± 11 657.9	1 433.5 - 41 375.0			
				Pb 3 200.0 ± 3 727.1	447.5 - 12 125.0			
				Zn 8 180.3 ± 10 093.9	373.9 - 34 125.0			
			Annelida - Tubificidae	Al 5 682.5 ± 7 111.7	0.0 - 28 830.0			
				Cr 2 273.5 ± 3 785.8	0.0 - 19 722.2			
				Cu 248.0 ± 467.6	0.0 - 2781.3			
				Fe 18 892.2 ± 24 802.8	0.0 - 124 167.09			

Table 11.10: (Continued)

				Mn 1 250.5 ± 2 579.7	0.0 - 16 582.0			
				Ni 2 040.2 ± 3 446.0	0.0 - 18 722.2			
				Pb 555.2 ± 1 135.1	0.0 - 6 343.8			
				Zn 1 875.7 ± 6 558.2	0.0 - 47 012.5			
			Crustacea - Cladocera	Al 9 133.0 ± 12 878.3	271.9 - 51 916.7			
				Cr 8 413.9 ± 14 494.7	111.5 - 76 750.0			
				Cu 864.4 ± 2 043.5	16.8 - 11 750.0			
				Fe 53 400.8 ± 65 720.8	957.9 - 278 750.0			
				Mn 1 321.6 ± 1 750.2	0.0 - 8 375.0			
				Ni 7 528.8 ± 10 751.8	109.7 - 38 500.0			
				Pb 2 045.6 ± 3 732.6	76.2 - 17 500.0			
				Zn 5 877.6 ± 13 981.7	132.8 - 62 625.0			

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12. General discussion and conclusions

12.1 Water and Sediment

Aquatic ecosystem contamination can be confirmed by examining the water, sediment and organisms occurring in such an environment. This is important to assess because the quality of the aquatic environment will determine the health and existence of aquatic organisms, as well as of the users reliant on the resource. This section of the study therefore investigated the extent of occurrence of various physical and chemical water quality variables, as well as metal concentrations in the water and sediment of selected localities in the upper and lower catchments of the Olifants River.

Evaluation of the data for the macro- and trace elements (metals) in the water at selected sites indicated that many of the concentrations exceeded the water quality guidelines (Canadian, South African-DWAF.) for aquatic ecosystems. This is alarming because many of these constituents have negative impacts on aquatic life (see Tables 9.1 & 9.2), thereby posing a potential threat to ecosystem health. Evaluation of the physical and chemical water quality variables of selected sites (Table 12.1 and Figure 12.1) showed that localities 2, 3, 6, 8, 10, 12 and 17 were severely impacted. Elevated levels of certain variables (e.g. total dissolved salts & sulphates) suggest that runoff originating in the catchments of these localities is being impacted by mining. This is further confirmed by low pH-values at localities 3, 5, 8, 9, 10 and 12, which indicates acid mine drainage from the many coal mines in the upper catchment of the Olifants River.

Nutrient enrichment (elevated phosphates, nitrates and nitrites) occurred at many sites in the catchment, but in particular at localities 3, 4, 6, 10, 11, 14, 15 and 17. Point source pollution from sewage treatment works and non-point sources from agricultural runoff and informal settlements are the main contributors to these elevated levels of nutrients. This is clearly evident at Localities 11 and 14 where the high phosphate also caused excessive growth of algae and aquatic weeds in the river at the two localities. The 1 mg/l phosphate standard for effluent water is therefore not acceptable and should be revised to also take into account drought and low flow periods when there is practically no dilution. The elevated phosphate effluents in the Selati River are the main contributor to the high nutrient levels detected in the lower Olifants River catchment at Locality 17.

It is evident from the evaluation of metal concentrations in the water and sediment (Table 12.2 and Figure 12.2) that most of the sites along the Olifants River are being affected by metal pollution. Acid mine drainage at localities 3, 5, 8, 10 and 12 is most likely responsible for the release of metals from the sediment, resulting in the high metal loads detected in the water at these sites. The negative impact of the Selati River on the Olifants River is also stressed by its contribution to the elevated metal levels detected in the water at Locality 17.

However, efficient evaluation of the metal pollution in the catchment is difficult because the data is based on single seasonal samples. Furthermore, most guideline values set by the Department of Water Affairs and Forestry are primarily based on dissolved metals, while

this study focussed on total metal concentrations. The contribution of natural processes such as geological weathering to metal levels in the Olifants River catchment is also unknown, thereby complicating the overall evaluation.

Table 12.1 Evaluation of the physico-chemical water quality variables at selected sites in the Olifants River, to indicate problematic areas that need to be addressed.

Variable	Locality																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temp.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
0_2-sat.	✓	✓	x	✓	✓	⊗	✓	x	x	✓	✓	✓	✓	✓	✓	✓	✓	✓
Turbidity	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	☺	✓	✓	✓	x	x
pH	✓	✓	✓	✓	✓	✓	⊗	x	⊗	✓	x	✓	✓	✓	✓	✓	✓	✓
TAL	☺	✓	✓	✓	x	✓	⊗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
TDS&EC	✓	x	✓	✓	✓	⊗	✓	⊗	✓	⊗	✓	✓	✓	✓	✓	✓	⊗	⊗
$S0_4$	☺	x	✓	✓	✓	⊗	✓	⊗	✓	⊗	✓	⊗	x	✓	✓	☺	⊗	⊗
Cl^-	✓	x	x	✓	✓	x	x	✓	x	✓	x	x	✓	x	x	✓	⊗	x
Na^+	✓	x	x	✓	✓	x	✓	x	x	✓	x	x	✓	x	x	☺	⊗	⊗
K^+	✓	x	x	✓	✓	x	✓	x	x	x	x	x	✓	x	x	☺	⊗	⊗
Mg^{2+}	✓	x	✓	✓	✓	⊗	✓	⊗	✓	x	✓	⊗	✓	✓	✓	☺	x	x
Ca^{2+}	☺	x	✓	✓	x	⊗	✓	⊗	✓	⊗	✓	⊗	✓	✓	✓	☺	x	x
F^-	☺	✓	⊗	✓	✓	x	x	⊗	x	x	x	x	x	x	x	x	⊗	⊗
$P0_4$-P	✓	✓	⊗	✓	✓	✓	✓	✓	⊗	x	⊗	✓	✓	⊗	x	✓	⊗	✓
NO_3-NO_2	⊗	✓	⊗	X	✓	x	☺	x	⊗	x	x	✓	☺	⊗	x	☺	x	✓
NH_4-N	✓	☺	x	X	x	x	✓	⊗	x	x	x	✓	x	x	✓	✓	✓	x

☺ - Levels of variable generally well within guideline limits. Locality seems to be unimpacted by pollutants containing/influencing this variable. Levels occurring at this site seem to be of no threat to the health of the aquatic ecosystem. There is a possibility that this site can be used as a reference site for this specific variable in future studies in this area.

✓ - Levels were generally within guideline limits. Levels of variable detected seem to bear no direct threat to aquatic life occurring at this site.

x - Values exceeded the guideline limits and/or seemed to be impacted by some source of pollutant containing/influencing this variable. Concentrations detected could have a negative effect on the aquatic ecosystem and will have to be investigated and addressed.

⊗ - Levels of variable detected exceeded guideline limits and/or other sites investigated by large margin. Seems to be heavily polluted by a source containing/influencing variable. Major possibility of negatively impacting ecosystem at present (especially sensitive organisms). Urgent need for improvement !

Despite these difficulties, the present study clearly indicates that the Olifants River is subjected to metal pollution. Specific impacts of observed metal concentrations on aquatic communities of this river system should be investigated, if possible by the use of on-site toxicity testing.

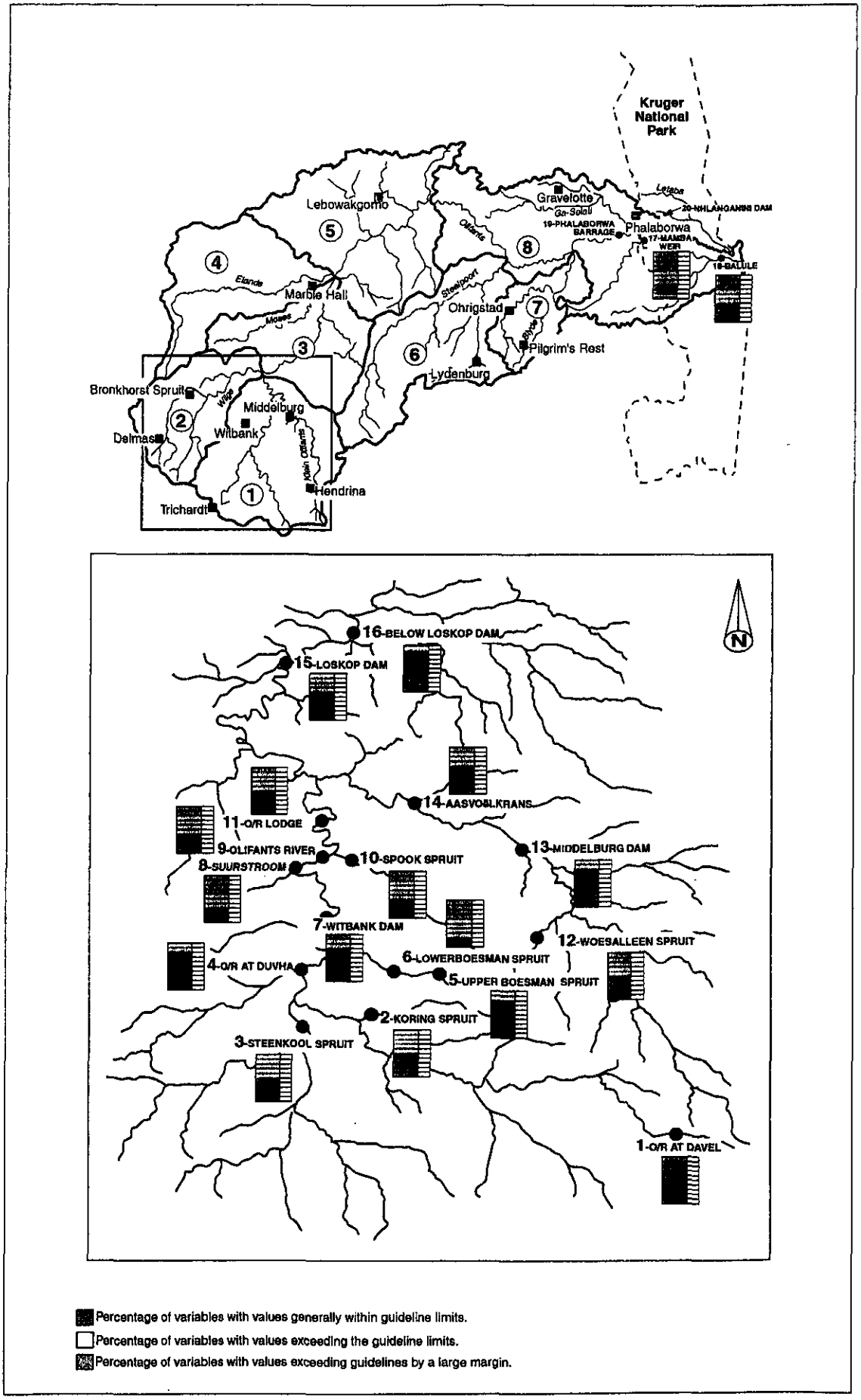

Figure 12.1: Evaluation of the physico chemical water quality variables at selected sites in the Olifants River. Increments of 10% are given. See legend for explanation of colours.

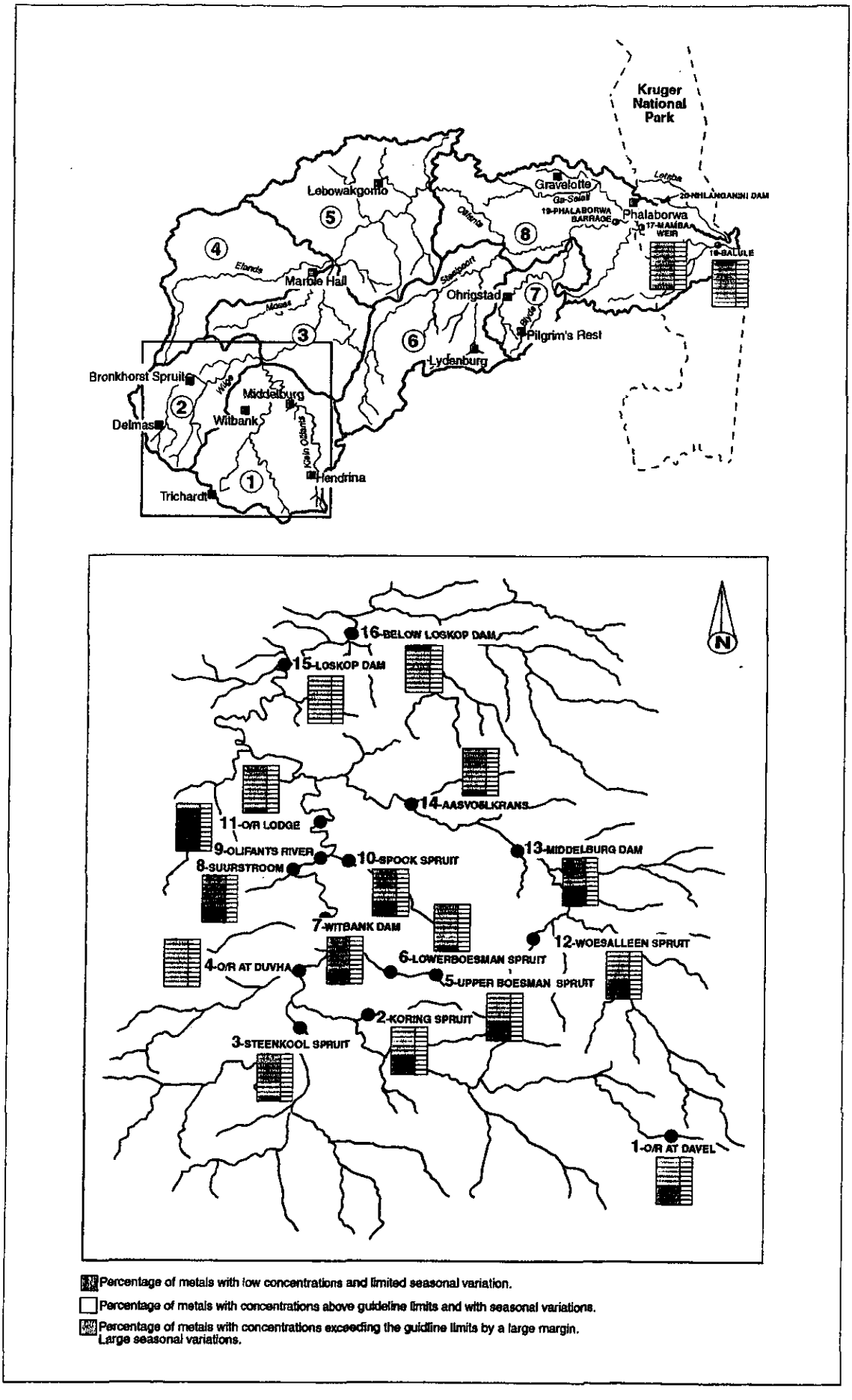

Figure 12.2: Evaluation of metal concentrations detected in the water at selected sites in the Olifants River. Increments of 10% given. See legend for explanation of colour

12.2 Bioaccumulation of selected metals in the organs and tissues of fish.

Fish are mentioned in the literature as good bioaccumulative indicators of metal pollution because they are known to readily accumulate metals from their environment. This can be detrimental to the health of both the organism itself, as well as to consumers, be they animals or humans. The investigation of metal bioaccumulation in fish is important because it supports the monitoring of the chemical and physical quality of water and sediment in aquatic ecosystems. It is also important for the assessment of the spatial and temporal extent of accumulation as well as organism health. Fish are an important food source to humans and it is therefore necessary to investigate the potential consumption of contaminated fish.

Metals were bioaccumulated mainly by the gills, but copper and iron concentrations were the highest in the liver tissue. It is therefore suggest that these organs be used in a general biomonitoring programme for the assessment of the extent of bioaccumulation of metals in fish tissues/organs. The lowest concentrations of the selected metals were found in the muscle and skin tissue. However, this should also be included in the biomonitoring programme, as it is the edible part of the fish. Since the skin only forms a small percentage of the edible part of the fish, its tissue may for the purpose of analysis be replaced by vertebrae which, in general, appear to accumulate higher levels of metals. Nevertheless, it is suggested that skin, muscle, gills, liver and vertebrae are included in a metal bioaccumulation monitoring programme.

Although different sampling techniques were used, it was not possible to capture fish of a specific size throughout the sampling period. For the present data set both positive and negative correlations between size and metal levels were detected, but in many cases these relationships were not significant. Evaluation of the present data and the literature suggests that, for each data set, size of the fish must be considered, especially if it is not possible to select a specific size range of fish. The data from young, fast-growing fish should be considered as a separate data set. The present study indicates that there were generally no significant difference or specific trends in metal bioaccumulation between different sexes of fish. This indicates that the organs and tissues sampled during this study do not give a true reflection of the preferences of bioaccumulation between sexes. Gonads will be the most obvious tissues to sample for differences in accumulation between sexes, but according to the literature levels of metals usually vary between different species as well as seasons. Gonads will therefore be of limited value in bioaccumulation studies aimed at investigating the extent of metal pollution in aquatic ecosystems.

Metal concentrations varied mostly between *O. mossambicus* and *L. umbratus* as well as *C. gariepinus,* and *L. umbratus* which can possibly be attributed to the differences in general feeding behaviour of the species. It is, however, not only the levels in food

source that can be an important pathway for uptake but also the intake of sediment particles associated with the food. Metals adsorbed onto sediment particles play an important role in their availability to aquatic organisms and usually become more available as pH levels decrease. The low pH in the stomachs of fish could therefore cause increased levels of bioavailable metals which could in turn be taken up and accumulated by fish. This route of metal uptake should be investigated, especially at contaminated sites.

Temporal variation in metal accumulation by fish occurred due to variation in metal concentrations in the water and sediment at a locality. This is the result of seasonal variation in climatic conditions (e.g. rainfall, temperature) as well as fluctuations in pollutant inputs into the river system over a period of time. Seasonal patterns of accumulation varied between different localities because of variations within their subcatchments. In some cases, decreased accumulation occurred during high flow periods; this is ascribed to the diluting effect of more water on pollutant concentrations. In other cases, increased flow caused fish to be exposed to higher levels of metals, due to their increased contact with metal-polluted sediment in the more turbid waters of the high flows or flushing of dams, such as the case with the

Table 12.2 Evaluation of the metal concentrations detected in the water and sediment at selected localities in the Olifants River, in an attempt to identify problematic areas.

Variables	Locality																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Water																		
Cu	x	x	⊗	x	x	x	x	⊗	✓	x	x	x	x	⊗	x	x	x	x
Zn	x	x	⊗	x	x	x	⊗	⊗	✓	⊗	x	x	⊗	x	x	x	x	x
Al	x	x	x	x	x	x	✓	⊗	✓	✓	x	✓	✓	x	x	x	x	⊗
Fe	x	x	x	x	x	x	x	x	✓	✓	x	✓	x	x	x	x	x	x
Ni	✓	✓	⊗	x	✓	x	⊗	⊗	✓	⊗	x	x	⊗	⊗	x	x	x	x
Mn	✓	✓	✓	⊗	✓	⊗	✓	⊗	✓	⊗	✓	x	✓	✓	x	⊗	✓	x
Pb	x	x	⊗	x	✓	⊗	⊗	x	✓	x	⊗	x	⊗	⊗	x	x	x	x
Cr	✓	✓	⊗	x	x	✓	⊗	x	x	x	⊗	✓	x	x	x	x	x	x
Sediment																		
Cu	x	✓	✓	x	x	x	x	x	x	⊗	x	x	x	x	⊗	x	x	✓
Zn	✓	✓	✓	✓	✓	x	⊗	⊗	x	⊗	⊗	⊗	⊗	✓	x	x	x	✓
Al	✓	✓	✓	x	⊗	⊗	x	⊗	x	⊗	✓	⊗	⊗	✓	⊗	x	⊗	✓
Fe	x	✓	✓	x	x	⊗	✓	⊗	x	⊗	x	⊗	✓	✓	⊗	x	⊗	x
Ni	x	✓	✓	x	x	⊗	✓	⊗	x	⊗	x	x	✓	✓	x	x	⊗	x
Mn	⊗	x	✓	✓	✓	⊗	✓	✓	x	⊗	⊗	⊗	x	x	x	x	x	x
Pb	✓	✓	✓	x	x	⊗	✓	⊗	x	⊗	✓	⊗	x	✓	x	x	x	✓
Cr	x	x	✓	x	x	x	x	⊗	x	⊗	x	x	✓	✓	x	x	⊗	⊗

✓- Concentrations of metal are low and/or slight seasonal variation occurs. This locality therefore seems to be unimpacted or only slightly impacted by sources containing this metal.

x- The concentrations detected were generally above guideline limits and the levels detected at other sites and/or seasonal fluctuations in the metal concentrations were evident. Levers occurring at this site could affect the aquatic ecosystem negatively and an attempt should be

made to reduce concentration of metal at this site. Further investigation and monitoring definitely recommended.

⊗- The concentrations of the metal detected at this site were generally far higher than the guideline limits and/or the levels detected at the other localities and/or major seasonal fluctuations occurred in the level of metal. It is obvious that these levels could be detrimental to the health of aquatic organisms (especially sensitive species) occurring at this site. Urgent need for reduction in the concentration of the metal and further monitoring is proposed.

Phalaborwa Barrage. Various factors such as water quality and variation in behaviour of fish during different seasons (e.g. decreased metabolism during winter) could also have contributed to the seasonal trends observed for bioaccumulation.

Geological differences could result in different levels of metals in different reaches of a river. However, as discussed, anthropogenic activities are responsible for point and diffuse sources of pollutants causing measurable differences in metal concentrations between localities. This caused a variation in the extent of bioaccumultion that occurred at different sites. In general, fish at Locality 11 (Olifants River at Olifants River Lodge) accumulated more Aluminium, iron, nickel, manganese and chromium than from Locality 14 (Klein Olifants River at Aasvoëlkrans). Zinc and manganese were accumulated in higher levels at Locality 15 while fish at Locality 17 accumulated higher levels of copper, aluminium, iron and nickel. The impact of the highly polluted Selati River in the lower catchment was evident in the difference between metal concentrations detected in the fish at locality 17, and locality 19 (Phalaborwa Barrage) upstream of the Olifants-Selati confluence. From this data, literature data on the metal levels in fish as well as water and sediment quality information, it can be concluded that the aquatic biota at the selected sites in the Olifants River system are to some extent subjected to metal pollution.

12.3 Experimental work

Firstly, it was clear that the haematology and osmoregulation of *O. mossambicus* were altered after the exposure to copper or zinc at a neutral and acidic pH, respectively. In most cases the acidic pH caused the opposite result to the neutral pH (e.g., [Na]: [Na] was decreased at the neutral pH, and increased at the acidic pH).

Secondly, it was clear that low pH and sublethal aluminium concentrations had a physiological effect on *O. mossambicus*. In some cases however, for example an aluminium concentration of 0.06 $mg.l^{-1}$ aluminium and pH 5.2, the toxicity of the acid was reduced rather than increased by the aluminium as was clearly seen in the blood glucose concentration. At pH 5.2 it seems the most toxic concentration of aluminium was in the range of 1.00 $mg.l^{-1}$ and of 1.50 $mg.l^{-1}$ aluminium. Higher concentrations, in this case 2.00 $mg.l^{-1}$ aluminium at pH 5.2, had statistically little effect on the haematology of *O. mossambicus*. The metabolism of *O. mossambicus* was severely affected after exposure to low pH as well as the combination of aluminium and pH 5.2.

Thirdly, the sublethal manganese exposures resulted in clinical diagnostic haematological changes.

All the changes after exposures (Cu, Zn, Al and Mn) indicate that haematological variables can be used as indicators in detecting the effects of sublethal metal exposure on fish (Tables 12.3 to 12.5). These toxicity tests, performed under controlled laboratory conditions, provide essential information concerning the sublethal effects of copper, zinc, aluminium and manganese on the haematology of fish (*O. mossambicus*). Some of the values obtained during this study fluctuated widely which indicated that the influence of the "stress" condition was handled differently by each individual. Although individual variations are a very important phenomena when conclusions are drawn, this does not mean that diagnostic tools of this nature cannot be used in the process of water quality guidelines. The more information available the better the prediction of effects of metals on fish survival. Water quality guidelines could be a source in the identification of pollution, before it causes changes in the natural population or environment. Figure 12.3 shows the levels of acceptability
of copper, zinc and aluminium at the sampling localities investigated during this project. These metals are already present in the system at levels where it could affect the survival of fish negatively.

12.4 Invertebrate work

A great diversity and number of aquatic macroinvertebrates occurred at the different localities. Metal concentrations for the various organisms give an indication of the metal levels these organisms were exposed to. Aluminum and iron concentrations averaged high levels in the organisms analysed. High aluminum and iron concentrations were also observed for the water and sediment analysed (Table 11.10). Thus aluminum and iron from both the water column and sediment compartment contributed to elevated levels in the macroinvertebrate. Aquatic macroinvertebrates occurring in the upper Olifants River catchment have to some degree adapted to high metal concentrations, whether from the water column or sediment compartment. These organisms such as the Chironomidae, Tubificidae and Crustacea thrived and built high populations (Table 11.10). These organism's exposure and their consequent survival in large numbers might also be caused by the developmental stage when exposed to metal concentrations, feeding habits, the organism's ability to exclude or regulate metals by their physiological abilities, the availability of food and the presence/absence of predators.

The lower numbers of organisms (water insect larvae, Coelenterata, Gastropoda and Pelecypoda) may be indicative of their sensitivity towards very high metal concentrations from their surrounding water column and sediment compartment.

Thus, the populations of organisms found in this system of pollution is significant in offering clues to the intensity of pollution and the consequent degree of recovery.

Table 12.3 Pathological conditions of haematology, caused in *O. mossambicus* after 96 hour exposures to copper, zinc, aluminium and manganese.

Metal	Condition											
	Leucocytes		Erythrocytes		Haemoglobin (hypoxia)		Haematocrit		Mean corpuscular volume		pH	
($mg.l^{-1}$)	Leucocytosis	Leucopaenia	Erythrocytosis	Erythropaenia	Increase	Decrease	Haemoconcentration	Haemodilution	Increase	Decrease	Alkalosis	Acidosis
[Cu]: 0.0191		⊗		⊗	⊗			⊗		⊗		●*
0.0124	⊗		⇔		⊗			⊗		⊗		⊗
0.0439	⊗		⊗		⊗		⊗			⊗		●*
0.0264	⊗		⊗		⊗			⊗		●*		●*
0.0050	⊗		⊗		●*		⊗		⊗			●*
0.2000	⊗		●*		●*		⊗		⊗			●*
pH5.2 + 0.0191		●*		⊗		⊗		⊗	⊗		⊗	
pH5.2 + 0.0124		●*		●*		●*		⊗		⊗		⊗
pH5.2 + 0.0439		⊗	⊗			⊗	⊗			⊗		⊗
pH5.2 + 0.0264		⊗		⊗		⊗		⊗		⊗		●*
[Zn] : 0.2099		⊗		⊗		⊗		⊗		⊗		●*
0.2535	⊗		⊗		⊗		⊗			⊗		●*
0.3674		⊗	⊗		⊗			⊗		⊗		●*
0.8391	⊗		⊗		⊗			⊗		⊗		●*
0.0300		⊗		●*	⊗			●*		⊗		●*
0.1000	⊗			⊗	⊗			⊗		●*		⊗
pH5.2 + 0.2099		●*		⊗		●*	⊗		●*		⊗	
pH5.2 + 0.2535		●*	⊗			⊗	⊗			⊗	●*	
pH5.2 + 0.3674		●*		⊗		●*		⊗	⊗		●*	
pH5.2 + 0.8391		●*		⊗		⊗		●*		⊗	●*	
[Al]:												
pH5.2 + 0.06	●*		⊗		⊗		⊗			⊗	N/A	
pH5.2 + 1.00	●*		⊗		⊗		⊗			●*	N/A	
pH5.2 + 1.50	●*		⊗			⊗	●*		●*		N/A	
pH5.2 + 2.00	⊗			⊗		⊗		⊗		⊗	N/A	
[Mn]:												
172.30	⊗			⊗		⊗	⊗		⊗		N/A	
259.00	⊗		⊗		⊗		⊗			⊗	N/A	
345.00		●*		●*		●*		●*		⊗	N/A	

N/A = Not available
●* = Significant increases or decrease
⊗ = Insignificant increases or decrease
⇔ = No detected change (same value)

Table 12.4 Pathological conditions of osmotic and ion regulation, caused in *O. mossambicus* after 96 hour exposures to copper, zinc, aluminium and manganese.

[Metal] ($mg.l^{-1}$)	Condition									
	Total osmolality		Plasma sodium concentration		Plasma potassium concentration		Plasma calcium concentration		Plasma chloride concentration	
	Increase	Decrease	Hypernatraemia	Hyponatraemia	Hyperkaliaemia	Hypokaliaemia	Hypercalcaemia	Hypocalcaemia	Hyperchloraemia	Hypochloraemia
[Cu]: 0.0191	↑*			↑*	⊗		⊗		⊗	
0.0124	↑*			↑*	⊗			⊗	⊗	
0.0439		↓*		↑*	↑*			⊗		⊗
0.0264		↓*		↑*	↑*			⊗		↑*
0.0050	↑*			↑*		⊗		⊗	⊗	
0.2000	⊗			↑*	↑*			⊗		⊗
pH5.2 + 0.0191	↑*		↑*			⊗	⊗		↑*	
pH5.2 + 0.0124	⇔		⊗		⊗			⊗	⊗	
pH5.2 + 0.0439	⊗		⊗		↑*		⊗		⊗	
pH5.2 + 0.0264	⇔			⊗	↑*			⊗	⊗	
[Zn] : 0.2099	⊗			↑*		⊗	⊗		⊗	
0.2535		⊗		↑*	⊗			⊗		⊗
0.3674		⊗		↑*		⊗		⊗	⊗	
0.8391	⇔			↑*		⊗		⊗	↑*	
0.0300	⇔			↑*	⊗			⊗	⊗	
0.1000	⇔			↑*		⊗		⊗	⊗	
pH5.2 + 0.2099	↑*		↑*		⊗		⇔		↑*	
pH5.2 + 0.2535	↑*		⊗		↑*			↑*	↑*	
pH5.2 + 0.3674	↑*		↑*		⊗			⊗	↑*	
pH5.2 + 0.8391	↑*		↑*		⊗			⊗	↑*	
[Al]:										
pH5.2 + 0.06		⊗		⊗		⊗	↑*			⊗
pH5.2 + 1.00	↑*			⊗	↑*		↑*			↑*
pH5.2 + 1.50	↑*			↑*		⊗		⊗		⊗
pH5.2 + 2.00	↑*			↑*	⊗			↑*		⊗
[Mn]:										
172.30	⊗			↑*	↑*		↑*		↑*	
259.00	⊗			⊗		↑*		↑*	⊗	
345.00	⊗			⊗		↑*		↑*	⊗	

N/A = Not available
↑* = Significant increases or decrease
⊗ = Insignificant increases or decrease
⇔ = No detected change (same value)

Table 12.5 Pathological conditions of metabolism, caused in *O. mossambicus* after 96 hour exposures to copper, zinc, aluminium and manganese.

[Metal] ($mg.l^{-1}$)	Condition: Choline Esterase activity		Pyruvate Kinase activity		Glucose-6-Phosphate dehydrogenase activity		Lactate concentration (Hypoxia)		Blood glucose concentration	
	Increase	Decrease	Increase	Decrease	Increase	Decrease	Increase	Decrease	Hyperglycaemia	Hyporglycaemia
[Al]:										
pH5.2 + 0.06		⊗		⊗	🍐*		🍐*		⊗	
pH5.2 + 1.00		⊗		🍐*	🍐*		⊗		🍐*	
pH5.2 + 1.50		🍐*		🍐*	🍐*		🍐*		🍐*	
pH5.2 + 2.00		🍐*		🍐*	🍐*		🍐*		⊗	
[Mn]:										
172.30		🍐*	⊗		N/A			🍐*	🍐*	
259.00	⊗		⊗			⊗		🍐*	🍐*	
345.00	⊗			🍐*		⊗	⊗		🍐*	

N/A = Not available
🍐* = Significant increases or decrease
⊗ = Insignificant increases or decrease

Table 12.6: Description of sampling localities in the study area in the Upper and Lower Olifants River as given in Figure 12.3.

1: Davel	A locality in the most upper reaches close to the origin of the Olifants River near a town called Davel.
2. Koring Spruit	A locality in the Koring Spruit South of Van Dyksdrift
3. Steenkool Spruit	A locality in the Steenkool Spruit before its confluence with the Riet Spruit
4. Duvha	A locality in the Olifants River upstream of the Witbank Dam
5. Upper Boesman Spruit	A locality in the upper reaches of the Boesmankrans Spruit before it flows through a coal mining area.
6.Lower Boesman Spruit	A locality in the lower reaches of the Boesmankrans Spruit before its confluence with the Olifants River (after passing through a mining area).
7. Witbank Dam	Witbank Dam - this impoundment on the Olifants River is the biggest municipal dam in the country, with a storage capacity of 10 402 million m^3. It provides water for urban and industrial use in the Witbank area. Compensation releases for Loskop Dam are made monthly which influences the flow of the river between these two dams.
8. Suurstroom	Suurstroom - A locality in a small stream arising from mine drainage flowing into the Olifants River between Witbank and Middelburg.
9. Olifants River	A locality in the Olifants River at the bridge on the old road to Middelburg after it passes the urban and industrial areas of Witbank.
10. Spook Spruit	A locality in the Spook Spruit before the confluence with the Olifants River.
11. Olifants River Lodge	A locality in the Olifants River between Witbank and Middelburg at Olifants River Lodge.
12. Woesalleen Spruit	A locality in the Woesalleen Spruit before its confluence with the Klein Olifants River.
13. Middelburg Dam	Middelburg Dam - an impoundment on the Klein Olifants River close to Middelburg. It has a storage capacity of 47,9 million m^3 and mainly supplies the town of Middelburg with domestic water.
14.Aasvoëlkrans	A locality in the Klein Olifants River in the vicinity of Aasvoëlkrans, after it passes through Middelburg.
15. Loskopdam	Loskop Dam - this is the largest dam in the Olifants River basin, with a storage capacity of 348,1 million m^3. The major land use sectors are irrigation, domestic and industrial.
16. Below Loskop Dam	A locality in the Olifants River just below the Loskop Dam wall.
17. Mamba Wier,	Mamba weir Kruger National park (KNP) - A locality in the Olifants River, after it crosses the western boundary of the KNP. It is ± 15 km downstream of the Phalaborwa Barrage and ± 10 km downstream of the Selati-Olifants River confluence.
18.Balule	Balule weir (KNP). This is a locality in the Olifants River ± 40 km downstream of locality 17.
19. Phalaborwa Barrage	Phalaborwa Barrage. This dam has a storage capacity of 4,5 million m^3 and provides water to the towns, mines and industries in the area.
20. Nhlanganini Dam	Nhlanganini Dam. A dam built for water provision to game in a tributary of the Letaba River, a major tributary of the Olifants River. This site was sampled as a control because there are no known anthropogenic activities affecting its water quality.

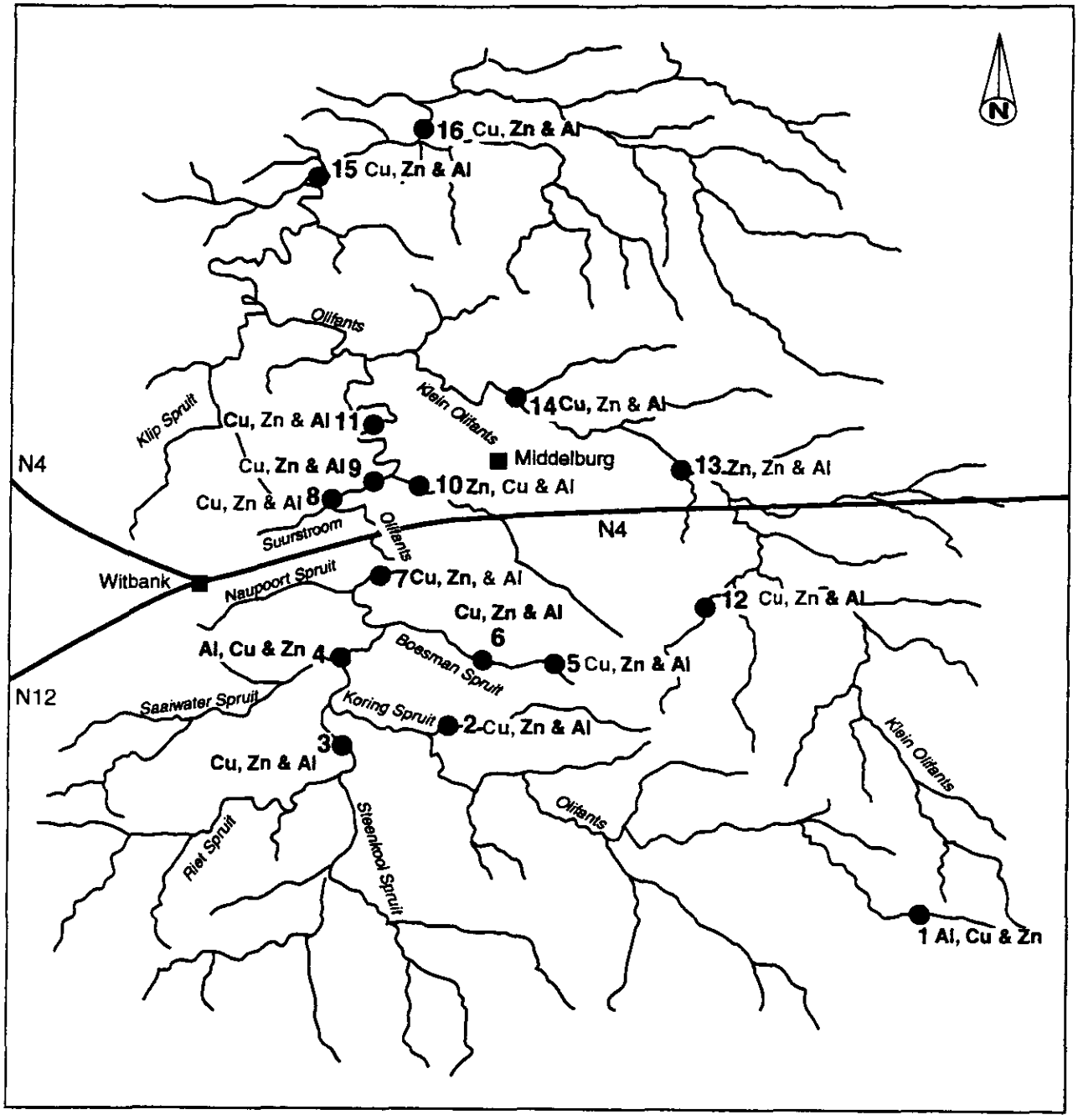

Figure 12.3: Acceptability of copper, zinc and aluminium levels to fish in the Upper Olifants River determined through sublethal experimental

- Acceptable
- Low Risk (Low Flow only)
- High Risk (High and Low Flow)
- Hazardous to aquatic life

13. Recommendations

13.1 Monitoring

The general objective of this section of the study was to investigate the water and sediment quality and bioaccumulation of metals in fish at selected sites along the Olifants River. This was achieved, showing that these catchments are impacted by present and past anthropogenic activities. The data gained from this study can not only be used in future management strategies aimed at reducing impacts on the Olifants River, but also serve as a foundation for further investigations and monitoring. It is recommended that future research should at least address the following aspects:

* To identify specific sources/bodies/organisations directly responsible for point or diffuse sources of pollution affecting water quality in severely impacted areas. Figures 12.1 and 12.2 could be used to identify specific areas of concern that need to be investigated and addressed. Special attention should be given to the areas with low pH water, while the sulphate and phosphate load of the system should be investigated and modelled. The party responsible for pollution must be informed about its impact on the river. It should then become their responsibility to develop and implement a management plan to reduce their impacts. The Department of Water Affairs and Forestry and other interested or affected parties, such as the Olifants River Forum, to ensure and enhance rehabilitation and improvement should apply pressure.

* Due to the metal loads detected in the water and sediment of the Olifants River, it is recommended that DWAF should also implement a more frequent sampling programme to investigate metal loads, maybe similar to the procedures followed for macro-constituents. As mentioned previously, special attention should be given to dissolve metal concentrations, as many of the proposed guidelines are set on this criterion. The speciation of metals in the water and sediment of the Olifants River should also be investigated to improve our understanding of the possible impacts of such metals on aquatic organisms.

* Bioaccumulation studies should in future be included in monitoring programmes because they not only identify possible metal polluted sites, but also result in the assessment of fish quality for consumption by humans. Bioaccumulation studies on fish should include at least 20 specimens of each species under investigation, to ensure more reliable results by reducing variation in the data. More than one sample of a specific tissue from a specimen (e.g. muscle tissue being abundantly available) should be sampled and investigated to ensure more reliable data. These investigations should include species with different behavioural patterns and food requirements, for example one predatory and one omnivorous species. A few surveys should also be done to investigate the extent of accumulation of a metal in different age groups of the species under investigation. The combination of organs and tissues used in this study should be sufficient to give a reliable evaluation of the metal pollution status.

* A detailed monitoring programme must be implemented on the Olifants River. This programme should in future, not only focus on the physical and chemical qualities of the river, but also include a well-designed biomonitoring component. As an initial phase the protocols of the national biomonitoring programme, currently being tested in the Crocodile River Catchment can be adopted. This should then be expanded to incorporate bioaccumulation assessment for metals and biocides. Furthermore, on-site and laboratory toxicity testing should form the major bioassays aimed at the investigation of the impacts of pollutants on resident biota of the Olifants River. It is thus evident that a multi-disciplinary approach should be followed to ensure the future existence of a sustainable freshwater system.

13.2 Experimental work

From this study it was evident that a physiological effect was exerted on *O. mossambicus* causing changes in the haematology, osmotic and ionic regulation, and metabolism of these fish. Thus copper, zinc, aluminium and manganese concentrations recorded in the water of the Upper Olifants River Catchment, even at levels which are considered non-lethal, can have a detrimental effect on aquatic organisms. It is important to note that in freshwater ecosystems where chronic stress, resulting from metal pollution, is operative for a long time, the organism's ability to adjust behaviourally and/or physiologically may be reduced. If an organism does not adapt to these changes, the population's survival will be in danger in that specific ecosystem.

It is important to remember that in general metals rarely occur singly. Thus, for the purpose of environmental protection, it is necessary to know the lethal and sublethal toxicity of mixtures under various environmental conditions to various aquatic species (e.g., algae, macro invertebrates and fish). Therefore, future field monitoring studies should include experimental studies on the lethal and sublethal effects of metals, both singly and in mixtures, on biochemical and physiological processes under various environmental conditions, because this is of utmost importance to gain more knowledge of their interactions and toxic effects.

Laboratory experiments when fish (or other aquatic organisms) are exposed to different metals, according to toxicity, under controlled conditions, will provide important information on lethal and sublethal levels of the metals and should be continued. This information is essential for the improvement of water quality guidelines for metals and the "health" status of fish can also be assessed. These experiments are also important in developing improved water quality guidelines for metals, which could aid water quality managers, engineers as well as consultants in assessing the impact of metals on the aquatic environment. Furthermore, the results also contribute to the development and expansion of an existing water quality index (WATER 2), which needs further refinement. The final product (WATER 2) for the Olifants River could be tested for suitability of use in other river systems in South Africa.