Monitoring cadmium and zinc contamination in freshwater systems with the use of the freshwater river crab, *Potamonautes warreni*

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Abstract

Zn (an essential element) and Cd (a non-essential element) levels were measured in water and sediment samples and in *Potamonautes warreni* individuals from Germiston Lake, an impacted site, and Potchefstroom Dam, a minimally impacted site. All samples for metal analysis were acid digested in triplicate at 200 to 250°C with 55% nitric and 70% perchloric acids in a ratio of 2:1 (v/v). The results revealed that the Cd levels in the water, sediment and biota were similar at the two sites, but that higher levels of sediment-bound Zn were detected at the impacted site. Cd levels in *P. warreni* were low and did not differ significantly between the two sites. Zn concentrations, were significantly higher in the organisms from the impacted site, a result which could be exacerbated by the softer water from that site. This might suggest that the levels of Zn were not well regulated by *P. warreni*. Gender-related differences were not observed for either metal at either site. While the size and mass of *P. warreni* did not affect Cd accumulation at either site, Zn levels were influenced by these parameters, but only from the more impacted site. This observation might suggest that size- and mass-related trends become evident only where high environmental Zn levels prevail. The results presented here imply that *P. warreni* could indeed prove to be a useful bioaccumulative indicator for Zn contamination. In this regard further investigations are essential before their use as bioindicators for Cd accumulation can be proven.

Introduction

Zn is fairly abundant in nature and its ores are widely distributed (Kelly, 1988). Cd, on the other hand, is a comparatively rare element (Aylett, 1979) that is usually closely associated with Zn ores (Friberg et al., 1974). This tends to lead to the release of Cd into the environment whenever Zn is released (Hem, 1972). Although small amounts of these metals are released by leaching of rocks and other natural processes, the levels of these metals in inland waters are often greatly increased by anthropogenic activities, ranging from mining to industry (Birch et al., 1996). Zn and Cd compounds can enter the bodies of aquatic animals via the gills, the general body surface and the alimentary canal, following ingestion of contaminated food particles (Jennings and Rainbow, 1979). The bioavailability of these metals. is usually correlated with the Zn^{2+} (Smies, 1983) and Cd^{2+} ions (WHO, 1992). Their bioavailability is therefor influenced more by the chemical forms of the elements (Coombs, 1979) and their interactions with other substances in solution (Eisler, 1981) rather than by the total levels of the metals present in the water (Kersten and Förstner, 1987). For example, the bioavailability of Cd to benthic aquatic organisms is limited by its strong adsorption to environmental components such as sediment and organic matter (WHO, 1992).

While Zn is an essential metal that is an important constituent of cells and upon which several enzymes depend as a cofactor (Friberg et al., 1974), Cd is a non-essential metal that is toxic even when present in very low concentrations (Wong and Rainbow, 1986). The toxic effect of Cd is exacerbated by the fact that it has an extremely long biological half-life (Webb, 1975) and is therefore retained for long periods of time in organisms after bioaccumulation (WHO, 1992). Although Zn does not appear to be toxic to most freshwater invertebrates (Timmermans, 1993), it can become toxic at elevated levels such as found in Germiston Lake (Phillips, 1980).

Anthropogenic activity can result in greatly increased levels of Cd and Zn in the environment. These elevated metal levels can have detrimental effects on both the biota inhabiting the aquatic environment as well as people who utilise this environment for food, recreation and potable water. It is therefor essential that the contamination of freshwater systems by these metals be carefully monitored. The aim of this study was to determine the levels of these metals in the freshwater river crab, *Potamonautes warreni*, collected from two differentially impacted sites and to assess the potential use of these organisms as bioaccumulative indicators of the degree of Cd and Zn contamination in these aquatic systems.

Materials and methods

Water, sediment and *P. warreni* individuals were collected from Germiston Lake and Potchefstroom Dam (Fig. 1) every second month between February 1995 and February 1996. A detailed description of these two sites is given in Sanders et al. (1997). Water samples of 200 mℓ used for the determination of dissolved metal content were filtered through a 0.45 µm pore size filter paper on site, and particulate metal content was calculated after analysis of the unaltered water samples. Surface sediment samples were collected with a stainless steel corer (5 cm diameter) fitted with a perspex lining. Only the top 5 cm of sediment was retained for analysis. All sediment samples were oven dried and sieved to allow separation into the following size categories: granules (2 000 to 4 000 µm); coarse sand (250 to 1 680 µm); fine sand (62.5 to 210 µm); and silt and clay (< 62.5 µm) (Folk and Ward, 1957). *Potamonautes warreni* was sampled with ten traps (1 m x 30 cm), baited with freshly killed fish and placed onto the

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Received 28 October1997; accepted in revised form 20 November 1998.

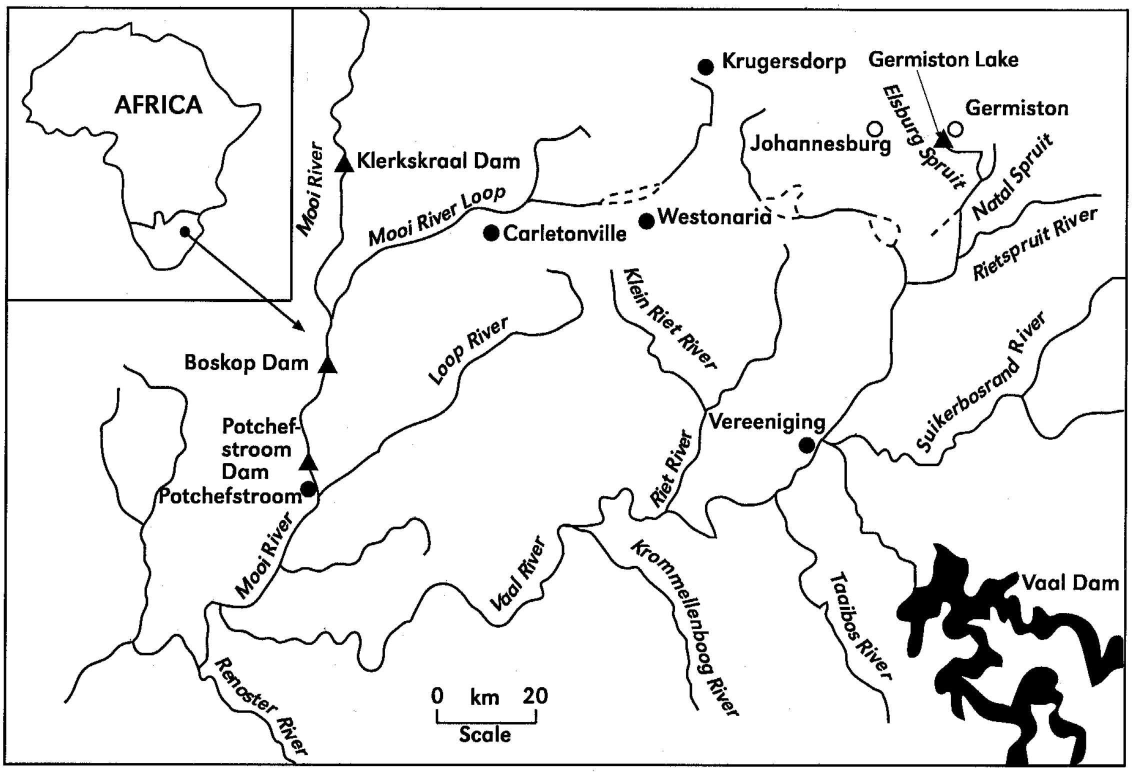

Figure 1
Geographical position of Germiston Lake and Potchefstroom Dam within South Africa

bottom substrate of the water bodies near the banks at a depth of approximately 1 to 3 m. Only hard-bodied individuals were selected and were sexed, weighed and carapace width measured prior to metal analysis. Whole organisms were dried, weighed again and thoroughly homogenised in a Wiley mill. All environmental and biological samples were digested according to the methods of Van Loon (1980) using 55% nitric and 70% perchloric acids in a ratio of 2:1 (v/v). A detailed description of all methods and techniques used is provided by Sanders et al. (1998). Cd and Zn concentrations in all samples were determined by means of flame atomic absorption spectrophotometry (Varian Spectra AA 10). Analytical standards for each metal were prepared from Holpro stock solutions, and a reference sediment standard (IAEAIRI/64) and standard tissue sample (IAEA/R1/64) were used to establish the accuracy of the instrument and preparation techniques. This procedure ensured accurate and precise determination of the trace metals in the sediment samples and biological tissue. Metal levels in the water were expressed in µg/ℓ, while those in the sediment and in *P. warreni* were expressed in µg/g dry mass.

Metal concentrations in the water, sediment (both in the total sediment sample and in each separate size fraction) and *P. warreni* from the two sites were compared using t-tests. Results for male and female *P. warreni* from each site were similarly compared. Correlations (Pearson product moment) were used to test for significant relationships between metal concentrations and the size and mass of the organisms. All statistical analyses were performed using the BMDP and STATISTICA packages, and differences between samples were accepted as being statistically significant when $p \leq 0.05$.

Results

Cd and Zn levels detected in the water did not differ significantly between the two sites (Table 1) but Zn was generally present in higher concentrations in Germiston Lake. Both dissolved and particulate Cd were below detection limits during the first four months at both sites, after which they increased slightly. Zn was more prevalent in the dissolved fraction of the water at both sites.

While sediment-bound Cd did not differ significantly between the two sites, Zn concentrations were significantly higher in the sediment from Germiston Lake, both in the total sediment ($t = -8.78$; $p < 0.01$; df = 54) and in each of the four separate size classes (Table 2):

granules:	$t = -3.09$;	$p < 0.01$;	df = 12;
coarse sand:	$t = -5.15$;	$p < 0.01$;	df = 12;
fine sand:	$t = -4.11$;	$p < 0.01$;	df = 12;
silt and clay:	$t = -5.41$;	$p < 0.01$;	df = 12.

Cd levels in *P. warreni* from Germiston Lake and Potchefstroom Dam did not differ significantly from each other, but Zn concentrations were significantly higher in the organisms from the former site ($t = 14.7$. $p < 0.01$; df = 247; Table 3), Gender-related differences were not observed for either metal at either site (Figs. 2 and 3). While Zn levels in *P. warreni* from Germiston Lake were significantly correlated to dry mass ($n = 199$; $r = -0.28$; $p < 0.01$; Fig. 4a) and carapace width ($n = 199$; $r = -0.19$; $p < 0.01$; Fig. 4b), Zn levels in *P. warreni* from Potchefstroom Dam were not significantly correlated with these variables. Correlation curves showing the relationship between Cd concentration and dry mass, and Cd concentration and carapace width in crabs collected from both localities are presented in Fig. 5. There were no size- or

TABLE 1
METAL CONCENTRATIONS (µg/ℓ) DETECTED IN THE WATER FROM GERMISTON LAKE AND POTCHEFSTROOM DAM DURING EACH SAMPLING EVENT, VALUES NOT ENCLOSED IN PARENTHESES GIVE DISSOLVED METAL CONCENTRATIONS WHILE THOSE IN PARENTHESES GIVE PARTICULATE METAL LEVELS

Metal	Feb' 95	Apr' 95	Jun '95	Aug '95	Oct '95	Dec '95	Feb '96
Germiston Lake							
Cd	N	*	*	*	4	4	2
	(N)	(*)	(*)	(*)	(*)	(*)	(*)
Zn	122	358	154	10	58	30	18
	(38)	(*)	(50)	(7)	(13)	(11)	(50)
Potchefstroom Dam							
Cd	*	*	*	*	2	4	5
	(*)	(*)	(*)	(*)	(*)	(*)	(*)
Zn	255	134	144	*	5	*	20
	(2)	(149)	(62)	(13)	(*)	(*)	(*)

***** Indicates values below detection limits

TABLE 2
METAL LEVELS (µg/g) DETECTED IN THE SEDIMENT (BOTH IN EACH INDIVIDUAL SIZE FRACTION AND IN THE TOTAL SEDIMENT SAMPLE, IN WHICH ALL SIZE FRACTIONS ARE COMBINED) FROM GERMISTON LAKE AND POTCHEFSTROOM DAM DURING EACH SAMPLING EVENT

Metal	Size	Feb'95	Apr'95	Jun'95	Aug'95	Oct'95	Dec'95	Feb'96
Germiston Lake								
Cd	Granules	N	10.5	14.7	10.8	36.1	12.8	22.7
	Coarse sand	N	14.3	18.2	10.1	27.4	30.3	25.2
	Fine sand	N	12.4	20.5	13.1	21.2	11.6	37.7
	Silt and clay	N	14.5	23.5	18.4	21.6	4.1	32.2
	Total sediment	N	51.7	76.9	52.4	106.3	58.8	117.8
Zn	Granules	1054	1228	3152	373	853	269	2357
	Coarse sand	1275	2613	3500	935	2749	565	2816
	Fine sand	550	884	3908	1234	1989	943	2569
	Silt and clay	747	1760	4680	2114	3479	1125	2707
	Total sediment	3626	6485	15240	4656	9070	2902	10449
Potchefstroom Dam								
Cd	Granules	10.4	14.6	14.3	14.4	26.8	8.5	29.0
	Coarse sand	10.6	22.0	16.7	21.7	39.8	43.7	28.3
	Fine sand	11.4	17.5	13.3	20.0	43.0	24.5	25.7
	Silt and clay	15.4	14.1	14.6	17.7	32.6	30.8	12.8
	Total sediment	47.8	68.2	58.9	73.8	142.2	107.5	95.8
Zn	Granules	198	133	268	169	150	148	134
	Coarse sand	178	145	185	189	155	145	123
	Fine sand	308	186	181	226	163	167	131
	Silt and clay	361	358	230	232	259	384	190
	Total sediment	1045	822	864	816	727	844	578

n = 1

TABLE 3
ZINC AND CADMIUM CONCENTRATIONS (µg/g) IN *P. WARRENI* FROM GERMISTON LAKE AND POTCHEFSTROOM DAM. 'ALL' GIVES THE MEAN CONCENTRATION FOUND WHEN THE TWO SEXES WERE ANALYSED TOGETHER

	Germiston Lake			Potchefstroom Dam		
	Mean	SD	Range	Mean	SD	Range
Cadmium						
Male	3.1	0.50	2.4-4.8	3.1	0.41	2.1-4.1
Female	3.1	0.39	2.2-4.1	3.2	0.53	1.8-4.5
All	3.1	0.46	2.2-4.8	3.1	0.49	1.8-4.1
Zinc						
Male	126.1	41.31	86.7-413.4	81.8	9.55	65.8-104.3
Female	120.5	32.39	70.2-245.4	82.7	12.63	52.0-111.0
All	123.5	37.48	70.2-413.4	82.3	11.39	52.0-111.0

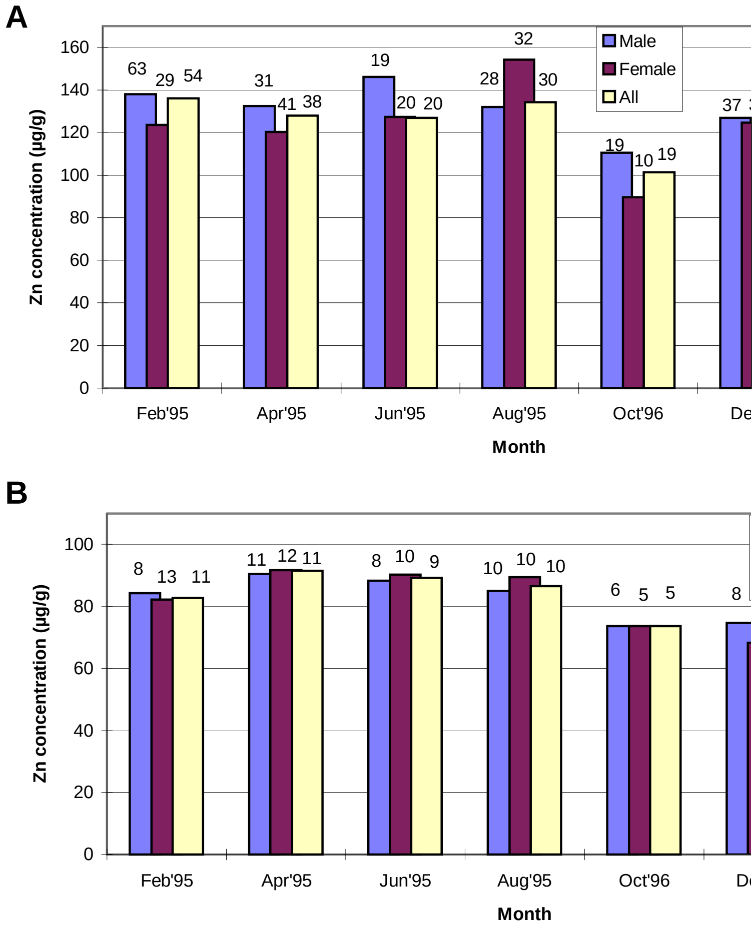

Figure 2
Mean Zn levels (µg/g dry mass) in P. warreni *collected from Germiston Lake (A) and Potchefstroom Dam (B) during each month. 'All' refers to the mean when both sexes were analysed together. Standard deviations are given above each bar.*

mass-related trends in Cd levels at either site. The number of males and females sampled during each survey is given in Table 4.

Discussion

Although determination of a mean natural concentration of Cd in surface water is difficult as the content of this metal in freshwater is greatly influenced by the underlying rock types in the drainage basin (Brewers et al., 1987), Cd in freshwater is generally present in concentrations of between 0. 1 and 10 µg/ℓ (Friberg et al., 1974). The low solubility of this metal (Khalid et al., 1981) suggests that Cd concentrations in surface waters not subjected to high Cd input should be relatively low, which was indeed the case at both Germiston Lake and Potchefstroom Dam. The Cd levels in the water from the two sites were essentially the same and exhibited similar trends (Table 1). Even after the increases in Cd levels in the water from both sites after October, which may be attributed to surface runoff following the spring rainfall, the levels of this metal in the water were still well within the 'normal'

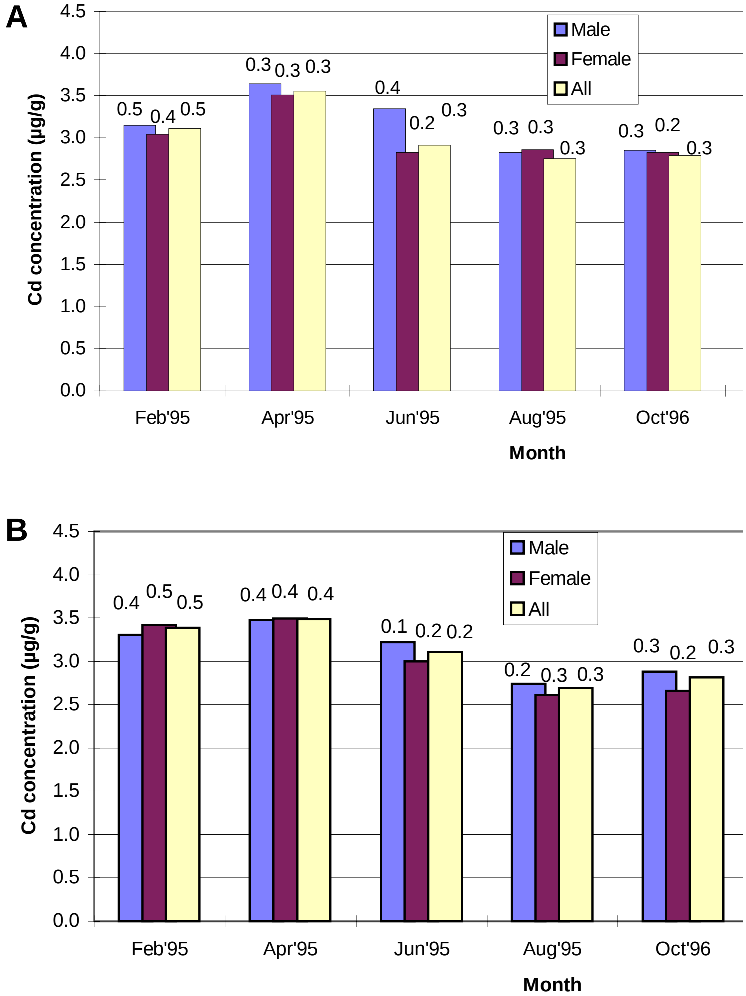

Figure 3
Mean Cd levels in P. warreni *from Germiston Lake (A) and Potchefstroom Dam (B) during each month of the study. 'All' gives the mean concentration when the sexes were analysed together. Standard deviations are given above each bar.*

range given by Friberg et al. (1974) above, but were above the target water quality guideline ranges (<60 µg/ℓ in Germiston Lake and <0.35 µg/ℓ in Potchefstroom Dam) given by the Department of Water Affairs and Forestry (1996) as general guidelines for the protection of the natural environment.

Although the differences were not significant ($p > 0.05$), Zn concentrations in the water from Germiston Lake were in most cases higher than those in the water from Potchefstroom Dam (Table 1). This may be due to the fact that carbonate, which forms insoluble complexes with Zn that subsequently precipitate out of the water (Weatherley et al., 1967), is present in lower concentrations in Germiston Lake (Sanders et al., 1998). The levels of dissolved Zn of less than 2 µg/ℓ in the water from both sites were above the target water quality ranges for the environment (Department of Water Affairs and Forestry, 1996) at both sites. Levels of particulate Zn were generally lower than levels of dissolved Zn at both the study sites, a result which is in agreement with previous studies that have also shown that most of the Zn in freshwater is present in dissolved forms (Brown, 1977; Cover and Wilhm, 1982).

Elevated metal levels in finer grained fractions are usually associated with pollution while higher concentrations in coarser particles are predominantly from the geology of the locality (Kindler and Savim, 1990). This result is more likely to result from the history of Germiston Lake as most of the sediment in this lake is unnatural and was introduced into the lake from the mines in the area. The Witwatersrand gold reefs contain Cd that could be released into the environment through leaching from rock dumps and slimes dams after mining activity (Hallbauer, 1986). The increase in the levels of sediment-bound Cd in Potchefstroom Dam during October are likely to have resulted from increased runoff associated with heavy rain and the subsequent settling of Cd-bound particles onto the substratum.

Zn concentrations in the top few centimetres of sediments in lakes and rivers are, on average, about 120 µg/g, but normal levels may range between 10 and 700 µg/g (Taylor and Demayo, 1980). Although the levels of this metal in the sediment from Potchefstroom Dam were only once within this range during the study period, those in the sediment from Germiston Lake were considerably higher than this (Table 2). It is probably due to the use of the lake as a reservoir for mine and industrial wastes for many decades, and to present-day storm-water input (Vermaak, 1985).

Zn is well regulated in crustaceans with excess metals stored in the hepatopancreas or excreted (Colvocoresses and Lynch, 1975). The total metabolic requirement for Zn in marine decapod crustaceans is in the order of 71 µg/g dry mass (White and Rainbow, 1985), and the estimated mean soft tissue concentrations in these organisms, excluding the haemolymph in the tissues, are about 50 to 208 µg/g dry mass (Depledge, 1989). Although Zn levels in *P. warreni* included the metal in the carapace and haemolymph, Zn levels found in the crabs collected from both Potchefstroom Dam in the present study (52 to 111 µg/g dry mass) and by Van Eeden and Schoonbee (1991) in a relatively polluted site (73 to138 µg/g dry mass), were in agreement with each other. The levels of this metal in *P. warreni* from

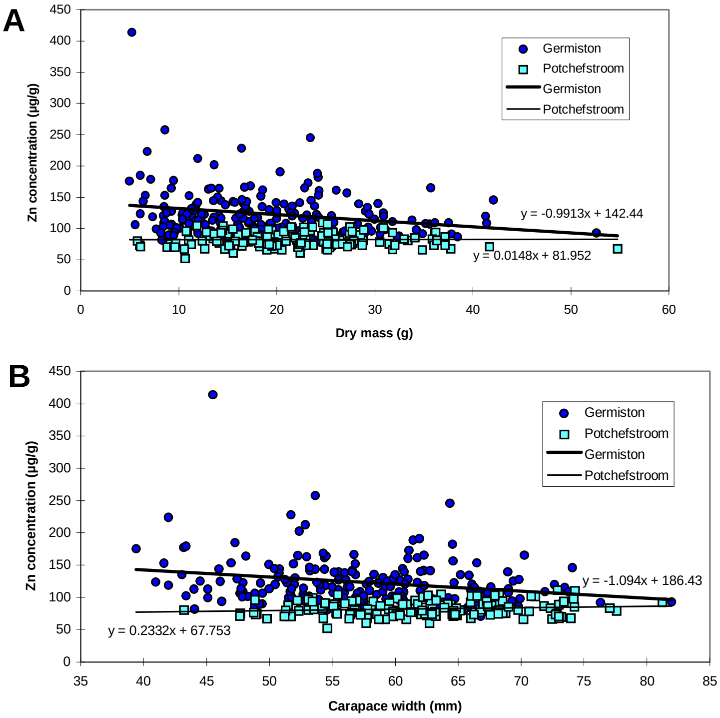

Figure 4

Correlation curves showing the relationship between Zn concentration and dry mass (A) and Zn concentration and carapace width (B) in P. warreni *collected from Germiston Lake and Potchefstroom Dam during the study*

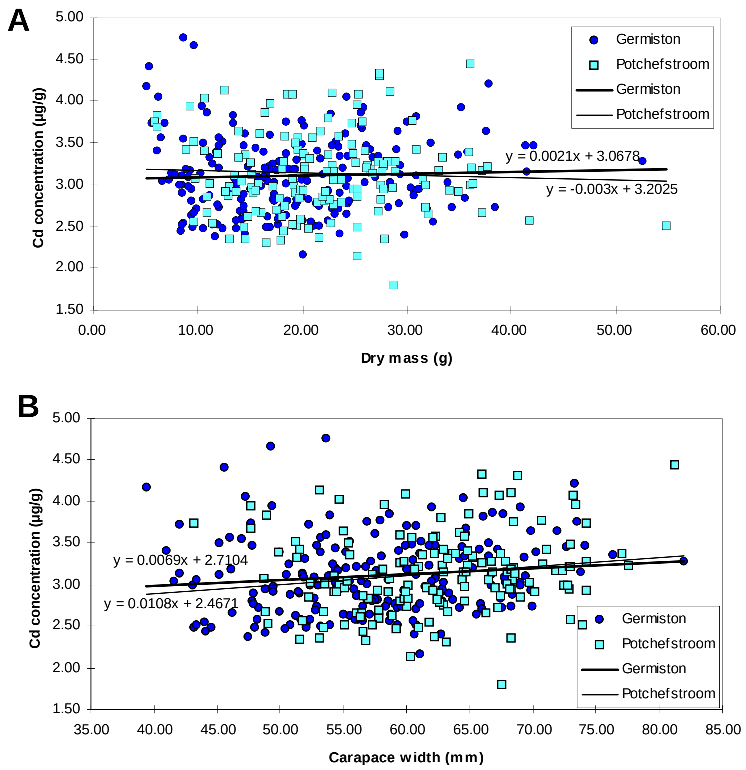

Figure 5

Correlation curves showing the relationship between Cd concentration and dry mass (A) and Cd concentration and carapace width (B) in P. warreni *collected from Germiston Lake and Potchefstroom Dam during the study*

Germiston Lake, however, were much higher (70 to 413 µg/g dry mass), thus suggesting that they contain more Zn than is required for normal physiological functions. The ability of decapods to regulate essential metal levels depends on the rate between metal uptake and excretion. In unpolluted aquatic systems, the rate of excretion is altered in such a way as to balance the rate of uptake, but when the systems become contaminated with metals, organisms may not be able to raise excretion rates sufficiently to remove all metals in the body. When this breakdown in metal regulation occurs, net accumulation begins and metal levels in the organisms begin to increase. The point of Zn regulation breakdown in a decapod is thus determined by both the Zn uptake rate inherent to that decapod and the maximum rate of Zn excretion achievable under any particular set of physico-chemical conditions (Nugegoda and Rainbow, 1989). This threshold level varies both between species and between individuals of the same species (Nugegoda and Rainbow, 1988). The high concentrations of Zn found in *P. warreni from* Germiston Lake suggest that their threshold level may have been exceeded and that their regulation mechanism may thus have broken down, resulting in the bioaccumulation of Zn by these organisms. Further investigation is, however, required in order to determine the threshold levels of Zn in these organisms from polluted sites such as Germiston Lake and elsewhere.

The higher levels of Zn in *P. warreni* from Germiston Lake as opposed to Potchefstroom Dam may have resulted not only from the increased levels of environmental Zn present in the former site, but also from the significantly softer water (60 mg/ℓ $CaCO_3$ and 220 mg/ℓ respectively) in the former site (Sanders et al., 1998). The bioavailability and toxicity of Zn is known to decrease as water hardness increases (Everall et al., 1989). This hardness effect is thought to result from the competition of Zn with calcium and magnesium ions in the water for binding sites (Zitko and Carson, 1976).

Cd is a non-essential element that is not well regulated in decapod crustaceans (Wong and Rainbow, 1986), but rather appears to be readily accumulated by aquatic invertebrates when it is present at elevated concentrations (Timmermans, 1993). This has lead to the suggestion that these organisms could be suitable biomonitors for this metal, a result which has indeed been found in previous studies with crabs (e.g. Engel and Brouwer, 1984). Cd uptake by brachyuran crabs is affected by environmental factors such as temperature (O'Hara, 1973) and calcium concentration, which influences both water hardness and salinity (Wright 1977). This creates variability in metal concentrations from different regions (Bjerregaard, 1990). Results from this study, however, did not reveal any spatial differences in the levels of Cd accumulated by *P. warreni*. This observation may have been due to the essentially similar levels of Cd present in the water and sediments from the two sites. The levels of Cd in *P. warreni* were relatively low (2 to 5 µg/g dry mass at both sites), and may just have been "natural background" levels in the organisms since the levels of Cd in the water from both sites were within the mean background range already mentioned (Friberg et al., 1974). Further investigation is required to substantiate this observation.

TABLE 4
NUMBER OF MALES AND FEMALES SAMPLED DURING EACH SURVEY

Date	Gender	Germiston Lake	Potchefstroom Dam
Feb'95	Male	31	11
	Female	16	29
Apr'95	Male	7	4
	Female	21	15
Jun'95	Male	13	10
	Female	15	10
Aug'95	Male	6	13
	Female	15	7
Oct'95	Male	16	7
	Female	6	3
Dec'95	Male	11	9
	Female	10	10
Feb'96	Male	16	9
	Female	16	10

Previous studies on the effect of gender-related tolerance to Zn in the freshwater field crab, *Oziotelphusa senex senex,* found that females were more tolerant to Zn than males. This might be attributed to females accumulating toxic ions into their organs at a faster rate than males do due to their greater metabolic activity (Radhakrishnaia, l987). Results from the present study, however, suggest that the levels of Zn attained in *P. warreni* were not influenced by the gender of the organisms at either of the sites studied. Similar results were also found on analysis of individual tissues of *P. warreni* (Du Preez et al., 1993). As has been found in studies with other crab species (Greig et al., 1982; Sadiq et al., 1982), gender-related differences in Cd levels were not observed at either of the sites.

Although the size ranges of *P. warreni* from both Germiston Lake and Potchefstroom Dam were similar (40 to 80 mm: Sanders et al., 1998), those organisms collected from the former site exhibited size-related trends, with higher Zn levels found in smaller crabs, while those collected from the latter site did not show such a trend. Sadiq et al. (1982) found low Zn concentrations in crabs from a site in the Arabian gulf and also found that Zn levels were independent of body size. Metal levels in the water, sediment and crabs from both Potchefstroom Dam and the site sampled by Sadiq et al. (1982) were much lower than those found in Germiston Lake. This phenomenon suggests that size-related differences in Zn levels may only become apparent when the organisms are exposed to elevated environmental metal levels, and when more metal is consequently accumulated by the crabs. As has been found in previous studies with other crab species (Greig et al., 1982. Sadiq et al., 1982), Cd levels were not influenced by size at either of the study sites.

Moulting has often been considered as one of the main excretory mechanisms of crustaceans since large amounts of metals may be lost with the moulted carapace (Giesy et al., 1980). Certain precautions were therefore taken to ensure that all organisms used for this study were in the moulting period. These measures included the collection of only hard-bodied organisms as well as the measurement of energy content, percentage ash and calcium concentrations (Sanders and Du Preez, 1998), which undergo dramatic changes during moult, to ensure that no significant variations in these variables were observed. The possible effects of these moult cycle parameters on metal levels in *P. warreni* have been discussed in Sanders and Du Preez (1997). The results presented suggest that while Zn levels in *P. warreni* were not significantly influenced by any of these variables, Cd levels in these organisms were influenced. These results could be expected since previous work has shown that while the carapace of *P. warreni* contains the lowest Zn concentrations (Du Preez et al., 1993), this tissue is also important for the storage of Cd in crustaceans (Davies et al., 1981). High levels of Cd is also adsorbed onto the external surface of the carapace rather than being incorporated into it (Phillips, 1980).

Conclusions

The results from this study indicate that *P. warreni* is able to regulate Zn up to a point. The levels of this metal found in the organisms varied according to the degree of metal pollution in their environment, with higher levels found in those organisms from the more impacted site, i.e. Germiston Lake. Environmental Cd levels detected at the two study sites were similarly low at both sites and may not have been bioavailable to *P. warreni.* As a result of this, Cd levels in *P. warreni* did not differ between the two sites. Thus, although it would appear that these crustaceans could indeed prove to be useful bioaccumulative indicators for Zn, the potential of *P. warreni* as a bioaccumulative indicator for Cd cannot be determined based on the results of this study. A follow-up study comparing Cd levels in crabs collected from areas that have markedly different environmental Cd levels would be required in order to assess the bioaccumulative capacity of these animals for this metal. It is therefore suggested that these organisms be incorporated into biomonitoring protocols for Zn and Cd using organisms from a selected size range are collected. The precautions listed have to be employed to counter the effects of the various moult cycle parameters on Zn levels in these organisms.

Acknowledgements

The authors wish to thank Mr Dirk Erlank and Miss Elsabe Smit for assistance with field collections and statistical analysis, respectively. The project was funded by the Rand Afrikaans University, the FRD and the Greater Germiston City Council.

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