Incidence of *Salmonella* spp. in sewage and semi-urban waste water treated by pond oxidation method at the University of the North

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Abstract

The study was carried out to determine the incidence of *Salmonella* spp. in sewage and semi-urban waste water treated by the pond oxidation system, using a *Salmonella*-specific DNA probe. The *Salmonella*-specific DNA probe was labelled following the digoxigenin (DIG) standard random primed DNA labelling technique. For the detection system, both the DIG calorimetric and chemiluminescent detection systems were used. When a total of 803 samples, collected from the four oxidation ponds A, B, C, and D, the irrigation water, overflow polluted water, the stream and the borehole, were screened, using a *Salmonella* DNA probe, 96.4% of the samples from oxidation Pond A, 51% from Pond B, 25.0% from Pond C, and 71.0% from Pond D, were *Salmonella* positive. The results also showed that 44.1% of the water samples from irrigation water, 69.9% of overflow polluted water samples and 83% of stream water samples collected from pools were positive for *Salmonella* presence. An overall positivity rate of 56% on all the samples tested, was observed. Significantly, no *Salmonella* positive samples were observed when water samples were collected from the one borehole which serviced the villagers living around the oxidation ponds. It may be concluded from these results that the treatment of sewage and semi-urban waste water, using the oxidation pond methods at the University of the North, did not efficiently remove pathogenic bacteria such as *Salmonella* from treated effluent. The direct or indirect use of the pond oxidation treated effluent may constitute a major source of contamination for human salmonellosis. It is recommended that the use of the pond oxidation treated effluent for the irrigation of the sports fields at the University of the North not be practised as it may be a source of *Salmonella* infection among the students that use the sports fields. It may also be concluded that the borehole water used by the Mamotintane Villagers is safe potable water since no *Salmonella* contamination was observed. Furthermore, the overflow water and the stream water, used by the villagers' livestock could be a major source of *Salmonella* contamination to their livestock. The University of the North should use a pond oxidation system that has a high pathogen removal efficiency in order to improve on the health of the people and their livestock.

Introduction

The study was carried out to determine the incidence of *Salmonella* spp. in sewage and semi-urban waste water treated by the pond-oxidation methods at the University of the North, South Africa. The four oxidation ponds receive sewage and waste water from the University of the North which has a total population of 20 000 people and from the Mankaweng residential township, with a population of about 15 000. The overflow waters from these ponds, flow over a surrounding grazing area before being collected into the Matangwaneng Stream which flows through Mamotintane Village. The villagers use the stream water for washing their clothes and for watering their livestock. Potable drinking water and water for other domestic uses for the Mamotintane Villagers comes from a borehole situated near the Mantangwaneng Stream. Most of the sewage and semi-urban waste water from the ponds' oxidation treatment system is recycled and used for irrigating the main sports fields at the University of the North, thus increasing the probability that the grass lawn on the main sports fields was contaminated with pathogens such as *Salmonella*. *Salmonella* spp. were targeted in this study because they are causative agents of human salmonellosis which results in enterocolitis, typhoid fever, paratyphoid fever and septicemia (Al-Qarawi et al., 1995; Petit and Wamola, 1994).

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The diseases can either be symptomatic or asymptomatic (Raphael et al., 1983). *Salmonella* spp. are harboured in and are transmitted by sewage and other polluted waters (Shuval, 1991). *Salmonella* spp. are also water-and food-borne pathogens that cause food-borne human salmonellosis (Cano et al.; Cheng, 1992; Falcao et al., 1993; and Ryan, 1990).

Methods which have been employed in the detection of *Salmonella* spp. in polluted and recycled water are: coliphage test (Maniwego et al., 1993), monoclonal antibody-based ELISA (Brigmon et al., 1992), and nucleic acid-based tests (Fitts et al., 1983; Gopo et al., 1988; Cheng et al., 1992; and Way et al., 1993). The nucleic acid probe assays are based on the principle of DNA-DNA hybridisation methods using either radioisotopes or non-isotopic materials for the labelling system (Olsen et al., 1995). In developing countries, the use of radioactively labelled DNA probes is not safe and also such labelled DNA probes have a short shelf-life when compared to non-isotopically labelled probes (Rubin, 1990). Short non-isotopically labelled oligonucleotides may be associated with false positive and false negative results (Olsen et al., 1995; and Tsen et al., 1991). In this study, a *Salmonella* genomic DNA probe (1.8 Kb) was used (Gopo and Chingobe, 1995).

Study area

The study area for this research project was made up of two primary and two secondary oxidation ponds of the University of the North, the irrigation water for the main campus sport-field, the area surrounding the four oxidation ponds, Matangwaneng

Stream that collects overflows from the oxidation ponds and a drinking-water source borehole for the Mamotintane Village. The study area was divided into oxidation ponds A, B, C and D; the overflow area which flooded the grazing area for the Mamotintane villagers' livestock; the Matangwaneng Stream, the Mamotintane Village borehole and the University of the North's sports fields (Fig. 1). The pond layout treatment flow process was arranged in such a way that raw sewage and waste water were piped into Pond A for the initial treatment process. The outflow from A went into Pond B and that from B to C and from C to D, for subsequent treatments (Fig. 1).

Materials and methods

Materials

A *Salmonella*-specific DNA probe (Gopo et al., 1988) was used in this study. Restriction endonuclease Hpal and the DIG labelling and the DIG detection calorimetric and chemiluminescent systems were used, following the manufacturers' conditions (Boehringer Mannheim, 1994).

Methods

Sample collection

A total of 803 sewage and semi-urban waste-water samples were collected from the University of the North's main campus sports fields irrigation water, the oxidation ponds A, B, C and D, the oxidation ponds' overflow areas, the Matangwaneng Stream and the Mamotintane Village borehole, in 100 mℓ volumes. One hundred and ten samples were collected from Pond A at about 2 m distance, 1 m into the pond, alongside the pond, 100 samples from Pond B at 1.5 m distance, 100 samples from Pond C at 1 m distance, 100 samples from Pond D at 0.8 m distance. All samples were transported to the laboratory for processing.

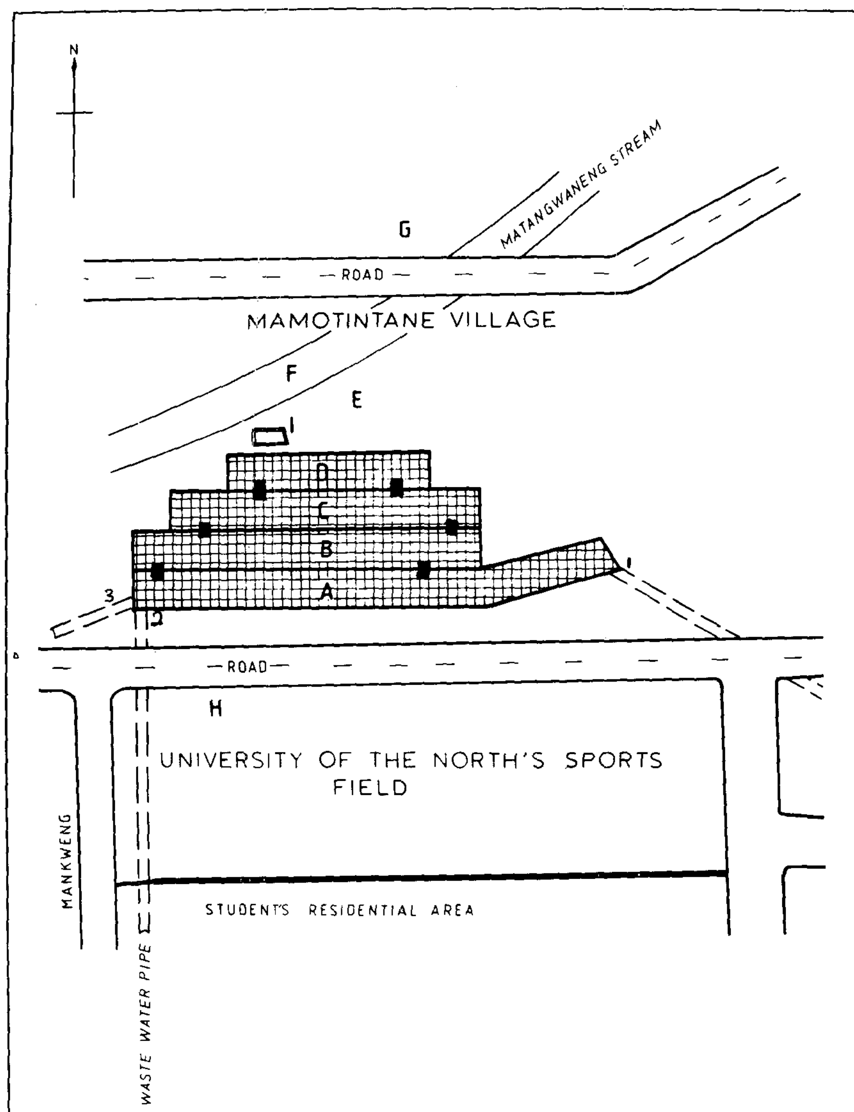

Figure 1
The study area

The map represents the study area, showing the layout of oxidation ponds A, B, C, and D, the pump station I, the overflow area E, the Matangwaneng Stream F, water boreholes G, the University of the North's sports fields H., raw sewage pipe inlets 1, 2 (UNIN) and 3 Mankaweng residential area. ■ = pond-overflow points. Raw sewage and urban waste water are initially collected and processed in Pond A, then overflows into Pond B for the next stage of the treatment process, then into Pond C and lastly into Pond D.

Sample processing

The oxidation ponds water samples were filtered through two layers of cheesecloth, to remove suspended matter and algae. Each 100 mℓ volume sample was vacuum filtered through 0.45 µM Sartorius cellulose filter to trap the bacteria. The entrapped bacteria were resuspended by shaking the filters vigorously in 10 mℓ of distilled water in screw-cap centrifuge tubes. The bacteria-free cellulose filters were removed from the centrifuge tubes and the 10 mℓ bacterial suspensions were each centrifuged at 6 000 $r \cdot min^{-1}$ in a Beckman bench centrifuge for 15 min to pellet the bacteria. The supernatants were carefully discarded and the bacterial pellets were resuspended in 2 mℓ volumes of distilled water. The suspensions were further centrifuged at 12 000 $r \cdot min^{-1}$ for 15 min. using a microphage to pellet and concentrate the bacteria. After carefully discarding the supernatants, each bacterial pellet was resuspended in 20 µℓ of distilled water. Each whole 20 µℓ bacterial suspension was dot-spotted onto a nylon membrane to form dotblots for filter colony hybridisation. The bacteria on the colonised filters were lysed by transferring the membranes, colony side up, onto Whatman 3 MM filter papers, pre-soaked in lysing solution [0.5N NaOH, 1.5 M NaCl and 0.1% sodium dodecyl sulfate (SDS)], and incubated for 10 min at room temperature. The denatured bacterial chromosomal DNAs were fixed to the nylon membranes by

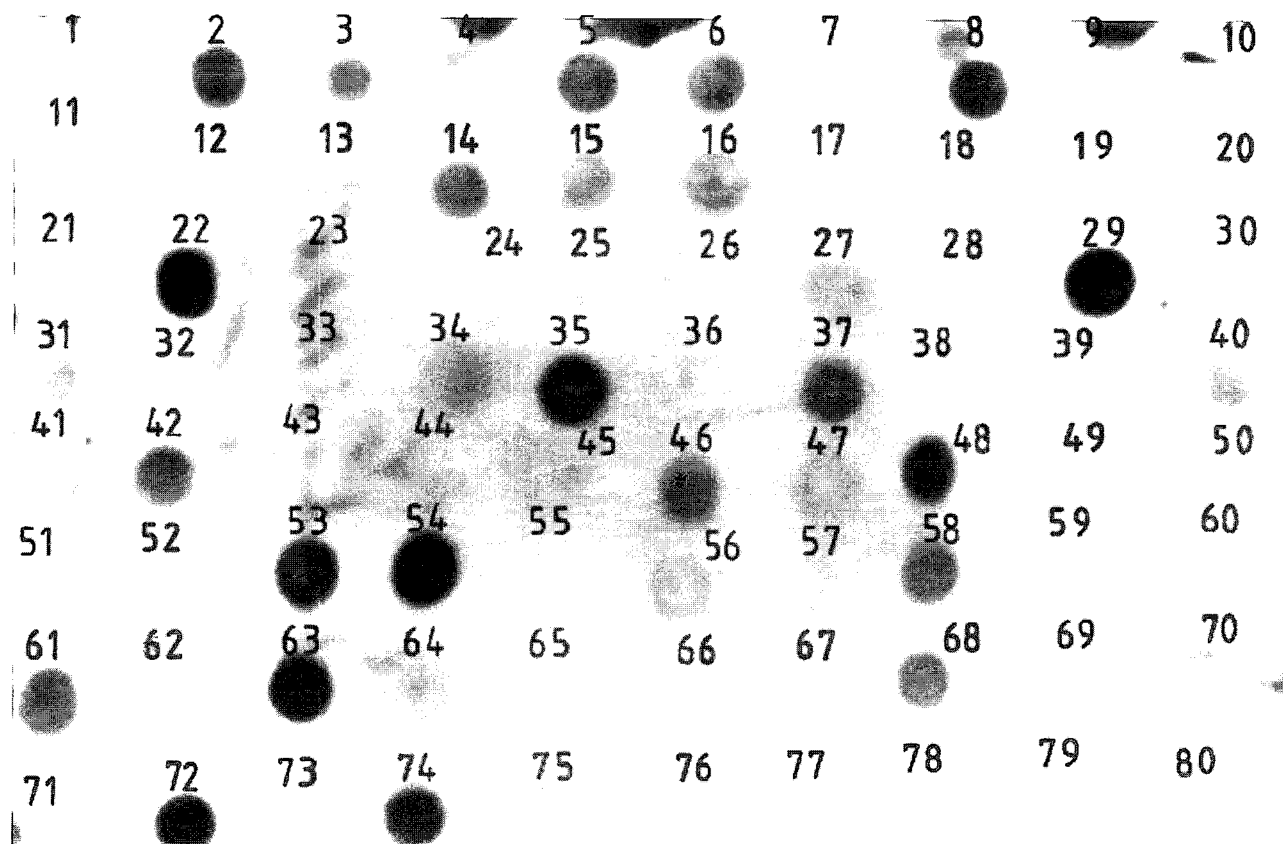

Figure 2
An example in which 80 water samples from oxidation pond B were screened using the Salmonella*-specific DNA probe*

ultraviolet cross-linking. Bacterial cellular debris were removed from the membranes by soaking in 3 x sodium chloride and sodium citrate (3 x SS) made from 20 x SSC stock solution (3M NaCl, 300 mM sodium citrate, pH 7.0); for 3 h at 65°C, with gentle shaking. The membranes were wiped with moistened 3 MM filter papers and air-dried.

Probe DNA-DIG-labelling

The *Salmonella*-specific DNA probe was DIG-labelled with the Digoxigenin-11-dUTP, following the standard random primed DNA labelling procedure (Boehringer Mannheim, 1994).

Colony hybridisation and detection

The hybridisations were carried out using a Techne DNA Hybridiser HBID model, following the colony hybridisation methods outlined in the DIG-System Users Guide for Filter Hybridisation. Both the calorimetric detection system with NBTX phosphate and the chemiluminescent lumigen ppD detection system were carried out according to the Boehringer manual (Boehringer Mannheim, 1994).

Results

This study was conducted to determine whether the use of the oxidation methods is efficient in the removal of pathogens such as *Salmonella* in recycled sewage effluent and semi-urban waste water at the University of the North sewage treatment system. When a total of 803 water samples collected from the oxidation ponds A, B, C and D, the irrigation water, the overflow water from the ponds, the Mantangwaneng Stream and the Mamotintane Village borehole, were screened using a *Salmonella*-specific DNA probe, the results showed that 96.4% of the samples from Pond A; 51% from Pond B; 25% from Pond C; and 71% from Pond D were positive for *Salmonella* presence (Fig.1). Further analysis of the results also showed that 44.1% of the water samples from the irrigation water, 69.9% of the overflow water used to irrigate the Mamotintane Village livestock, and 83% of the Mantangwaneng Stream water samples were positive for *Salmonella*. The positive and negative samples were determined as shown in Fig. 2. The study also showed that 450/803 (56%) of all the samples tested, were positive for *Salmonella* presence. The results significantly showed that no *Salmonella*-positive samples from the Mamotintane Village borehole were observed out of 60 samples tested. These results are summarised in Table 1. It may be concluded from these results that the treatment of sewage and semi-urban waste water, using the pond oxidation methods, at the University of the North was not efficient in the removal of pathogens such as *Salmonella* from treated effluent. The University of the North should use a pond oxidation sewage and urban waste-water treatment system that has a high pathogen removal efficiency. These results may also indicate that the direct use of treated effluent from these ponds to irrigate the University's sports fields may be a major source of *Salmonella* contamination to the students who use the fields.

TABLE 1
***SALMONELLA* INCIDENCE AT THE VARIOUS STAGES OF THE POND OXIDATION SEWAGE AND SEMI-URBAN WASTE WATER TREATMENT PROCESS**

Sample type	No. tested	No. positive	Percentage positive
Oxidation Pond A	110	106	96.4
Oxidation Pond B	100	51	51.0
Oxidation Pond C	100	25	25.0
Oxidation Pond D	100	71	71.0
Irrigation water	170	75	44.1
Overflow polluted water	103	72	69.9
Stream	60	50	83.3
Borehole	60	0	0.0
Total	803	450	56.0

Discussion

The determination of the presence of pathogens such as *Salmonella* in sewage and urban waste water is subject to many factors such as water temperature, pH, sewage chemical composition, dissolved oxygen and organic matter. Domestic waters (sewage and waste) are known to carry a full spectrum of enteric pathogens that cause such diseases as enteritis, salmonellosis, shigellosis, cholera and yersiniosis (Cowen and Johnson, 1985). Most of these water- and excreta-related diseases are prevalent in developing countries because of poor sanitation and lack of adequate sewage and urban waste-water treatment regimes (Lewis-Jones and Winkler, 1991). Conventional sewage and urban waste-water treatment which involves screening, sludge formation, sludge digestion and disposal of the effluent supernatants was shown to reduce the incidence of *Salmonella* and other pathogens by as much as 90% or greater (Feachem et al., 1983). The treatment methods which were found to have the above effects were: the primary sedimentation method, the trickling filter method and the activated sludge method (Feachem et al., 1983 and Lewis-Jones and Winkler, 1991). The above-mentioned sewage treatment methods do not achieve total removal of pathogens such as *Salmonella* from the sewage effluent. Studies have shown that a 100% elimination of *Salmonella* from the sewage effluent using the sludge treatment methods is unachievable (Lewis-Jones and Winkler, 1991).

In the pond oxidation sewage and urban waste-water treatment system used at the University of the North, the sludge formed from the sediment by simple settlement was not subjected to any further treatment. The sludge and the supernatants were not further separated. The supernatant was not chlorinated before use in the irrigation of the sports fields at the University of the North. The results of this research work have shown that the pond oxidation treatment of sewage and urban waste water does not significantly reduce the incidence and presence of pathogens such as *Salmonella*. The use of the pond-oxidation treated effluent for irrigating the sports fields at the University may be a major source of contamination with enteric pathogens since 69.9% of the water samples were positive for *Salmonella*. It may be concluded from this study that the pond oxidation system used at the University of the North for treatment of sewage and semi-urban waste water does not remove sewage and water-borne enteric pathogens such as *Salmonella* from the treated effluent. The use, direct or indirect, of such treated water effluent may cause a major source of contamination with *Salmonella*, leading to human salmonellosis among the users of the sports fields at the University of the North. The overflow polluted water from the ponds which floods the Mamotintane Village grazing area is a source of *Salmonella* infection to the villagers' livestock. The Mamotintane Village children are exposed to *Salmonella* when they play, bath and swim in the polluted Matangwaneng Stream. The polluting effects of these ponds to the area can only grow worse with the increasing student population at the University of the North, worsened by the impending growth of the residential areas of Mankweng and Mamotintane Villages. A more effective method of sewage disposal and treatment must be put in place.

Table 1 shows the status of *Salmonella* at the various stages of treatment by the pond oxidation method. An effective reduction in *Salmonella* incidence is shown from Pond A (96.4%), Pond B (50%) and Pond C (25%). Pond D shows an increase in *Salmonella* incidence at 71%, while the incidence increases to 70% in the overflow area and 83% in the stream.

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