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Clostridia as Surrogate Indicator of Water Quality: Two Wastewater Treatment Plants in South Africa as Case Study

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The microbiological qualities of the final effluents of two wastewater treatment facilities in the Eastern Cape Province of South Africa were investigated using clostridia as surrogate indicators. Relevant physicochemical parameters were also assessed. Temperature (15-20°C), pH (5.3-8.1), salinity (0.15-0.25 psu), conductivity (278-464 μS/cm) and total dissolved solids (TDS) (139-232 ppm) of the final effluents were well within the recommended limits of no risks. However, dissolved oxygen (DO) (0.94-6.60 mg/l) and the turbidity (2.04 - 310 NTU) did not comply with the standard guidelines for the protection of aquatic ecosystems. High levels of vegetative cell counts (0-2.3×10^5 cfu/100ml) and spore counts (0-2.9×10^4 cfu/100ml) were obtained for sulphite reducing clostridia (SRC) in most samples collected from the final effluents of the two treatment plants which exceeded the set No Observed Adverse Effect Levels (NOAELs). The need for regular monitoring of the activities of wastewater treatment facilities in rural communities of the Eastern Cape Province for compliance to regulatory standards is therefore advocated.

Key words: Clostridium, Surrogate indicators, Wastewater treatment plant, Pollution.

Domestic wastewater, collected by the drainage system, is piped to the sewage treatment plants where it is depurated from organic and inorganic residues and subsequently disinfected. However, no system is able to completely eradicate all microorganisms present in sewage and detection of significant concentrations of human enteric pathogens from sewage effluents remains a challenge[1]. The problem is further compounded in areas where wastewater treatment systems are rather simple with inefficient performance indices[2].

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The Eastern Cape Province is the second poorest province in South Africa with restricted resources, forced settlement, lack of democratic control of development after and poor education. Its population is largely non-urban with inadequate water-supply infrastructure[3]. Its rural communities rely mainly on river, stream, well and pond water sources for their daily water needs. Water from these sources is used directly by the inhabitants and in many cases the waters are faecal contaminated and devoid of treatment. Consequently, a significant proportion of residents in these communities are exposed to waterborne diseases and their complications[4]. The old South African Water Act (Act 54 of 1956) made it mandatory that effluents be treated to acceptable standards before being discharged into watercourses. Thus, effluent discharge investigation is one of the water quality management tools being used by the Department of Water Affairs and Forestry (DWAF) in the management of point-source pollution[5].

Indicator microorganisms are used to predict the presence of and/or minimize the potential risk of discharged effluents. Indicator organisms are useful in that they circumvent the need to assay for every pathogen that may be present in water. Total and faecal coliforms have been used extensively for many years as indicators for determining the sanitary quality of surface, recreational, and shellfish growing waters[6]. It has been widely demonstrated that coliforms do not adequately reflect the occurrence of pathogens in disinfected wastewater effluents due to their relatively high susceptibility to chemical disinfection[7], and failure to correlate with protozoan parasites such as *Cryptosporidium*[8], and enteric viruses[9]. Alternative microbiological indicators like; *Enterococcus* spp.[7-10], sulphite reducing clostridia including *Clostridium perfringens*[11,12], and coliphages[13,14] have been suggested for evaluation of wastewater, drinking water and environmental waters. The sulphite-reducing clostridium (SRC) group, including *Clostridium perfringens*, has been shown to be of value in assessing faecal pollution of aquatic environments[15]. Also, SRC spores have been demonstrated to be useful surrogate indicators for monitoring water treatment processes for the removal of viruses, *Giardia* cysts, and *Cryptosporidium* oocysts[11]. The performance indices using clostridia as a surrogate indicator in wastewater treatment plants in the Eastern Cape Province of South Africa have not been reported. In this paper, we assess the efficiencies of two wastewater treatment plants in typical peri-urban and rural communities of the Eastern Cape Province for the removal of protozoan parasites using sulphite reducing clostridia as the surrogate indicator of microbiological water quality against the backdrop of the alarming increase in the incidences of waterborne diseases in the country.

METHODS

Study sites and sampling

The sampling sites include two wastewater treatment plants (SU-WWTP and R-WWTP) situated in typical peri-urban and rural communities in the Eastern Cape Province of South Africa. The activated sludge system is used in the plants, followed by chlorination of the final effluent. Both plants empty their final effluents into nearby rivers. Wastewater samples were collected monthly between August 2007 and October 2007 from the raw influents, mixed liquor and chlorinated final effluents.

Physicochemical analysis

On site physicochemical analyses were done for each sampling point according to the procedure of APHA (16). The chlorine residual of the final effluents was measured using an ion specific meter (Hanna instruments, version HI 93711), while turbidity of the samples was determined using a microprocessor turbidometer (HACH company, model 2100P). The pH, temperature, conductivity, total dissolved solids (TDS), salinity and dissolved oxygen were determined using a multiparameter (Hanna instruments, version HI9828).

Microbial analysis

Sample collection and processing

All samples were collected aseptically using sterile 500 ml Nalgene bottles and transported on ice from the sampling sites to the laboratory for analysis. Water samples from the final effluents were dechlorinated by adding 0.5 mL of sterile concentrated sodium thiosulphate solution to give a final concentration of 100 mg/L. At the laboratory, all samples were stored at 4°C until analysis was complete usually within 24 h of collection[16].

Determination of vegetative clostridial cell count

One hundred millilitres of water sample were filtered through a 0.45-µm-pore-size cellulose filter (Millipore), and the filter was then placed[17] on Tryptose sulphite-cycloserine (TSC) agar. Plates were then transferred to anaerobic jars containing a gas pack and incubated at 37°C for 24h [18]. For samples with high turbidity, clostridial counts were determined by standard spread plate methods after serially diluting the samples. One millilitre of the serially diluted samples were aseptically inoculated onto TSC agar and spread to dryness using a sterile glass spreader. The plates were then incubated as described above. In both cases, plates were examined and typical clostridial black colonies were enumerated.

Determination of clostridial spore count

Determination of *Clostridium* spore

densities was carried out by heat treatment of water samples at 75°C for 20 min to kill vegetative cells as described by Payment and Franco[11]. Heat treated samples were then filtered through a 0.45-µm cellulose filter (Millipore). Membranes were then transferred onto TSC agar plates, and incubated as described earlier after which plates were examined and typical clostridial black colonies were enumerated.

Statistical Analysis

The data obtained was subjected to descriptive statistics using Mean, Range and Standard Deviation. Also, analysis of variance (ANOVA) test under Completely Randomized Design (CRD) was carried out while Duncan Multiple Range Test (DMRT) was used to test for the means that are significantly different from each other and were presented by alphabets in superscripts in the tables presented.

RESULTS

Physicochemical characteristics of the wastewater samples

The results of the pH, temperature, turbidity, residual chlorine, dissolved oxygen (DO), salinity, conductivity and total dissolved solids (TDS) are shown in Tables 1 and 2. For both wastewater treatment plants, the values obtained for temperature, pH, salinity, conductivity, turbidity and TDS in the final effluents were well within the recommended limits of no risks with the exception of turbidity which was above the limits for the peri-urban community plant. The ranges of the physicochemical parameters in all samples were as follows: DO (0.94-6.60 mg/L), pH (5.3-8.1), temperature (15-20°C), conductivity (278-464 µS/cm), TDS (139-232 ppm), salinity (0.15-0.25 psu) and turbidity (2.04-310 NTU). There was no significant difference in the pH, temperature, conductivity and salinity of the final effluents compared to the influents entering both treatment facilities, except for conductivity that was significantly different at the rural community treatment plant. However, there was no significant difference in TDS between the final effluent and the prechlorinated water in the peri-urban community facility. Residual chlorine concentration ranged between 0.07 and 3.85 mg/L throughout the sampling period with pronounced overdosing

Table 1. Physicochemical parameters of samples from the peri-urban community located wastewater treatment plant

Sampling point	DO (mg/L)	pH	Temp (°C)	Cond (µS/cm)	TDS (PPM)	Salinity (psu)	DO % (NTU)	Turbidity (mg/L)	CR
Influent	1.23-2.15^{a}	7.1-7.7^{a}	16.8-19.2^{a}	299-407^{a}	150-204^{a}	0.16-0.22^{a}	14.2-23.7^{a}	83-122^{a}	
ML	0.94-4.10^{a}	6.9-8.1^{a}	15.9-19.3^{a}	357-401^{a}	179-200^{a}	0.19-0.21^{a}	10.8-46.5^{a}	100-172^{a}	
FE	5.02-5.08^{b}	6.8-7.7^{a}	18.63-20.0^{a}	278-306^{a}	139-156^{b}	0.15-0.17^{a}	57.3-69.5^{b}	6-35.7^{b}	0.07-3.85

Legend: ML- Mixed Liquor, FE- Final Effluent, CR- Chlorine Residual.
Values are presented as Mean range (n=3). All sampling points are compared to each other at p<0.05. Values with different superscripts along the same column are statistically different from each other.
Limits for final effluents and aquatic environments: DO (8-10mg/L) (Pearce *et al.*, 1999); DO% (80-120%), pH (5.5-9.5), Temp (No limit set), conductivity (<1500 µS/cm), TDS (0-450 ppm), Salinity (No limit set), Turbidity (0-3 NTU), Chlorine residual (0.25mg/L) (DWAF, 1996).

Table 2. Physicochemical parameters of samples from the rural community located wastewater treatment plant

Sampling point	DO (mg/L)	pH	Temp (°C)	Cond (µS/cm)	TDS (PPM)	Salinity (psu)	DO % (NTU)	Turbidity (mg/L)	CR
Influent	2.25-4.35^{a}	7.4-7.9^{a}	16.4-19.6^{a}	319-464^{b}	160-232^{b}	0.17-0.25^{a}	25.8-47.3 a	93.4-259^{b}	
ML	1.35-4.73^{a}	5.3-6.9^{a}	15.0-19.0^{a}	295-328^{a}	148-164^{a}	0.16-0.18^{a}	15.4-49.8 a	99.4 -310^{b}	
FE	3.83-6.60^{a}	6.5-7.1^{a}	17.6-20.3^{a}	287-313^{a}	144-156^{a}	0.15-0.17^{a}	44.6-69.5 b	2.04-6.0^{a}	0.18-0.26

Legend: ML- Mixed Liquor, FE- Final Effluent, CR- Chlorine Residual
Values are presented as Mean range (n=3). All sampling points are compared to each other at $p<0.05$. Values with different superscripts along the same column are statistically different from each other.
Limits for final effluents and aquatic environments: DO (8-10mg/L) (Pearce et al., 1999); DO% (80-120%), pH (5.5-9.5), Temp (No limit set), conductivity (<1500 µS/cm), TDS (0-450 ppm), Salinity (No limit set), Turbidity (0-3 NTU), Chlorine residual (0.25mg/L) (DWAF, 1996).

observed in October at the peri-urban community plant (Table 1).

Microbiological characteristics of the wastewater samples

Results obtained from monitoring both wastewater treatment plants for SRC vegetative cell counts and SRC spore counts are represented in logarithmic scales as shown in Figs. 1 to 4. Generally, an increase was observed in the enumeration of the vegetative cells and spores of SRC in samples collected from the influent point to

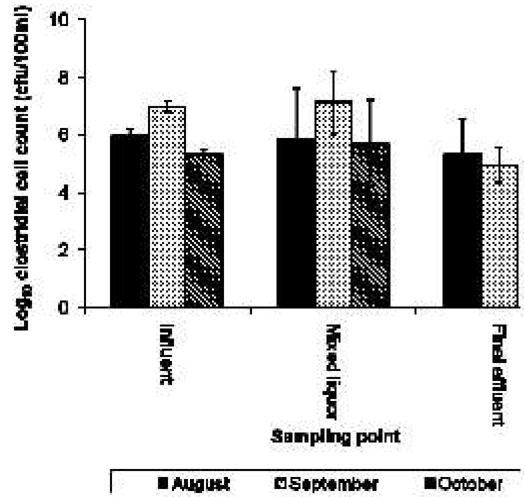

Fig. 1. Profile of vegetative clostridial cell densities from the peri-urban community located wastewater treatment facility

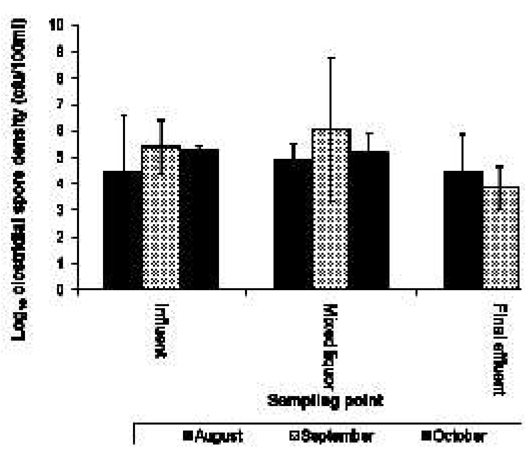

Fig. 2. Profile of clostridial spore densities from the peri-urban community located wastewater treatment facility

the mixed liquor point of the two treatment facilities (Figs. 1 to 4) with the exception of clostridial cell counts in August from the peri-urban community treatment plant and clostridial spore count in August from the rural community treatment plant.

However, it is interesting to note that without exception, there was a drastic reduction in the vegetative cell and spore counts of SRC from the mixed liquor point to the final effluent stage of the two treatment plants throughout the sampling period with the ranges of real values of 0-1.40×10^7 cfu/100ml and 0-1.14×10^6 cfu/100ml for vegetative cell and spore counts respectively. Results obtained across the three-month sampling period were not significantly different ($p < 0.05$). Besides the October and August samples in the peri-urban and rural communities' plants respectively (Figures 1 and 4), all the samples contained high count levels of both vegetative cells and spore forms of SRC in the final effluents (3.0×10^3 – 2.3×10^5cfu/100ml) throughout the sampling period (Figs. 1 to 4). The highest count of SRC in the final effluent was found in the peri-urban plant samples of August where 2.3 × 10^5 cfu/100ml was recorded for vegetative cells for the final effluent (Fig. 1). The highest spore form counts of 2.9 × 10^4 cfu/100ml for the final effluent was also obtained in the same month from this peri-urban plant samples (Fig. 2).

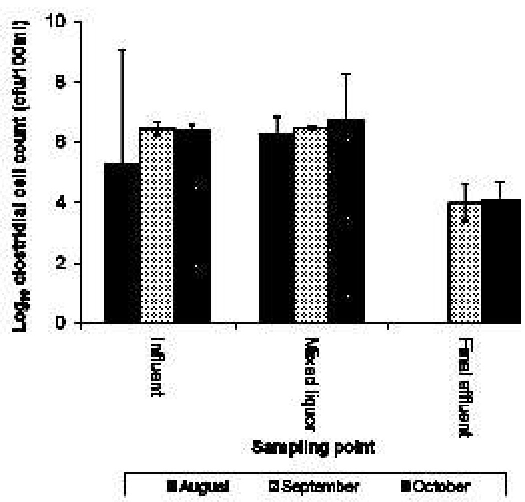

Fig. 3. Profile of vegetative clostridial cell densities from the rural community located wastewater treatment facility

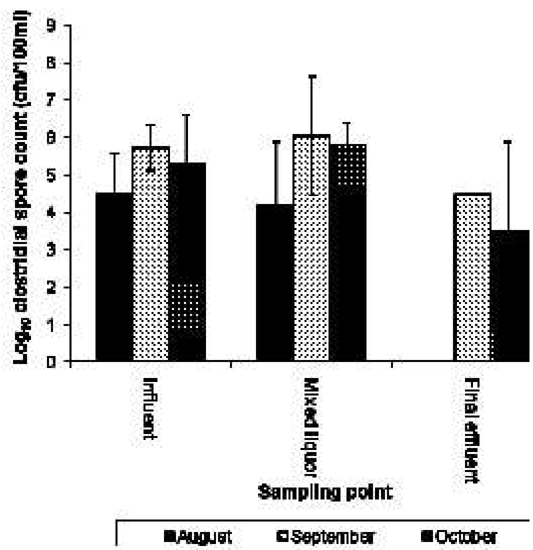

Fig. 4. Profile of clostridial spore densities from the rural community located wastewater treatment facility

DISCUSSION

Classical indicator bacteria have proved to be useful in studies describing the environmental distribution of wastewater discharges. Sulphite reducing clostridia have been used successfully as an indicator of faecal contamination in aquatic systems and have been shown to be an ideal indicator organism under conditions of which extended survival is critical[19]. The notable increase observed in most cases of the enumeration of the vegetative cells and spores of SRC in samples collected from the influent point to the mixed liquor (activated sludge) point of the two treatment facilities (Figs. 1 to 4) could be linked to the contribution of reintroduced activated sludge leading to the increment of mass of microorganisms in the wastewater for the purpose of stabilising the organic contents to achieve secondary biological treatment. A good mixed liquor will consist of light brown masses of filamentous and non-filamentous microorganisms which tend to clump together as flocs and settle at a uniform rate[20]. However, a drastic removal of SRC was observed within stages of the wastewater treatment process from the mixed liquor point to the final effluent, though, in most of the cases, the SRC counts in the final effluent exceeded the No

Observed Adverse Effect Levels (NOAELs) for various uses of water as reported by Jagals[21]. For domestic water use, a limit within which only an insignificant chance of infection could be expected was determined to be 1 cfu/100ml whilst that for recreational water use was given as 5.0×10^1 cfu/100ml[22]. Hence generally, the microbial qualities of the effluents exceeded the NOAELs, with the exception of the October and August samples of peri-urban and rural communities respectively which both recorded zero values (Figures 1 to 4).

The results of this study showed an improvement in the microbial water quality vis-à-vis the wastewater treatment process. It was observed that the microbial load significantly decreased with increasing chlorine residual levels, and decreasing turbidity. For both wastewater treatment plants, the temperature, pH, salinity, conductivity and total dissolved solids (TDS) observed in the final effluents were well within the recommended limits of no risk (Tables 1 and 2) and thus could not affect the biological health of the receiving aquatic environments and/or humans and animals. Salinity refers to the dissolved salt content in water. Mallin *et al.*[23] indicated a strong inverse relationship between microbial counts and salinity. Due to the high resistance of the SRC to environmental stress, changes in these parameters could not have significant effects on the SRC microbial load[24].

The total dissolved solid (TDS) is a measure of the amount of various inorganic salts dissolved in water. The TDS concentration is directly proportional to the electrical conductivity (EC) of water. Electrical conductivity (EC) is a measure of the ability of water to conduct an electrical current. This ability is a result of the presence of ions in water such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium, all of which carry an electrical charge. The results showed that all the sampling points were in compliance with the required levels of conductivity[25]. Most organic compounds dissolved in water do not dissociate into ions, consequently they do not affect the EC. Virtually all natural waters contain varying concentrations of TDS as a consequence of the dissolution of minerals in rocks, soils and decomposing plant material. The TDS of natural waters is therefore often dependent on the characteristics of the geological formations the water was, or is, in contact with. Low concentrations of particularly calcium and magnesium salts have nutritional value, although water with an extremely low TDS concentration may be objectionable because of its flat, insipid taste[25]. The TDS concentrations observed during this research were in compliance with water quality standards set by DWAF[25].

Gaseous oxygen (O_2) from the atmosphere dissolves in water and is also generated during photosynthesis by aquatic plants and phytoplankton. Oxygen is moderately soluble in water. Equilibrium solubility, termed the saturation solubility, varies non-linearly with temperature, salinity and atmospheric pressure, and with other site-specific chemical and physical factors. The maintenance of adequate dissolved oxygen (DO) concentrations is critical for the survival and functioning of the aquatic biota because it is required for the respiration of all aerobic organisms. Therefore, the DO concentration provides a useful measure of the health of an aquatic ecosystem. The effect of waste discharge on a surface water source is largely determined by the oxygen balance of the system, and its presence is essential to maintaining life within a system. Dissolved oxygen concentrations in unpolluted water normally range between 8 and 10 mg/L[26]. The DO con-centrations of all the effluents studied were less than 8 mg/L (Tables 1 and 2). Thus, the final effluents studied were polluted and the discharge of such would not be suitable for supporting healthy receiving aquatic ecosystems. Saturation concentration at the time and place of sampling must be calculated and reported, in order to assess the severity of the possible effects of oxygen depletion on biota. The concentrations of less than 100% saturation observed for all samples indicate that dissolved oxygen was depleted from the theoretical equilibrium concentration[25]. Continuous exposure to concentrations of final effluents of less than 80% dissolved oxygen saturation can be harmful, and could have acute effects; repeated exposure to reduced concentrations may lead to physiological and behavioural stress[25]. All the results for the peri-urban and rural communities based plants showed dissolved oxygen concentrations of less than 80% meaning the final effluents would not be safe for discharge as this may bring the ecosystems in the receiving aquatic environments under immense stress and consequently have adverse effects on aquatic organisms. Aerobic organisms are dependent on the presence of dissolved oxygen in water for respiration. Anoxic or hypoxic conditions may be lethal within short time scales (minutes to hours). Cold-water-adapted species such as salmonds (e.g., trout) are especially sensitive to depletion of dissolved oxygen. Reproduction and growth in these species is reduced under continuous exposure to oxygen concentrations less than 100% saturation[25].

Generally, the peri-urban community plant had the poorest effluent quality with regards to both physicochemical parameters and microbial load, and during the August sampling period, it was noted that one of the clarifiers was not functional which could have compounded the poor effluent quality. The problem of non- operational clarifiers was also observed at the rural WWTP for the entire sampling period. The poor operational state and inadequate maintenance of most of the Eastern Cape Province of South Africa municipalities' wastewater treatment works, i.e. design weaknesses, overloaded capacity, faulty equipment and machinery, has resulted in major pollution problems and impacts on the quality of water resources, with marine water quality standards consequently not meeting regulatory standards[3]. The peri-urban community based plant sample of October showed that, owing to chlorine overdosing, there was no SRC in the final effluent (Figs. 1 and 2).

CONCLUSION

Although the treatment plants succeeded in removing some sulphite reducing clostridia from the influents, effluent discharges were only occasionally devoid of the organisms, thus constituting a potential threat of incidences of infectious diseases due to robust pathogens. Pearson and Idema[27] reported that in many cases in developing countries, a high level of reliability of water supply schemes, particularly the treatment process, is the exception rather than the rule, and that various factors such as cost, operator training and problems with maintenance of infrastructure could contribute to these problems. The current disinfection practices and guidelines in terms of chlorine residuals were found not to be sufficient for the removal of SRC since high levels could still be detected in the final effluent. The inefficiency of both wastewater treatment plants for the removal of SRC and maintenance of acceptable levels of physicochemical parameters in their final effluents would have a negative impact on the microbial and chemical quality of the receiving water bodies, although there might be other sources of faecal pollution. Thus from the results obtained, the Eastern Cape WWTP effluents may be implicated as probable sources of parasites (e.g. Giardia and Cryptosporidium) in the receiving aquatic environments. More stringent surveillance of the performances of wastewater treatment facilities in the Eastern Cape Province of South Africa is needed at local government level, in order to ensure compliance with regulatory standards. This study has indeed verified the value and usability of the sulfite-reducing clostridia (SRC) as a surrogate indicator of water quality in the assessment of efficiency of wastewater treatment plants.

Ethical approval

None declared

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