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Protozoan Fauna and Abundance in Aeration Tanks of Three Municipal Wastewater Treatment Plants in the Eastern Cape Province of South Africa

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Abstract: This study focuses on the assessment of the protozoan fauna and abundance in the mixed liquors of aeration tanks of the three municipal wastewater treatment plants located in Fort Beaufort, Dimbaza and East London in the Eastern Cape Province of South Africa and their implication to the production of effluents of good quality. The samples were collected between September and December 2005 and protozoa species were identified by direct microscopic observations at x400 magnification by comparison with existing protozoa gallery collections. A total of 68 protozoan genera made up of 44 ciliates, 16 flagellates and 8 others were identified in wastewater treatment plants. Although in all aerobic zones the average density of ciliates was 10^4 cells mL^{-1}, which indicated that these plants were able to produce clear effluent of good quality, a better performance was found in Dimbaza and East London, which had total protozoan genera of 27 and 26, respectively.

Key words: Ciliates, flagellates, protozoa, wastewater treatment plant, aeration tank, South Africa

INTRODUCTION

South Africa is a water-scarce country and the demands on this resource are growing as the economy expands and the population increases. For the country to continue to develop economically, while meeting the wide-ranging needs for water, urgent steps must be taken to protect the quality of the resource. It is well known that water sources are subjected to frequent dramatic changes in microbial and chemical qualities as a result of the variety of activities on the watershed. Discharges of untreated or inappropriately treated municipal wastewaters or treated effluent at a specific point-source into the receiving waters such as streams, rivers, lakes, ponds etc. have been identified amongst the activities responsible for these changes (Momba and Mfenyana, 2005; Momba *et al*., 2006), thus resulting in the introduction of a wide range of potentially infectious agents to waters that may be supplied to many rural and urban communities, with the potential of culminating in incidences of waterborne diseases with far reaching socio-economic implications (Craun, 1991). It becomes imperative therefore, that municipal wastewaters must be properly treated before the effluents are discharged into receiving water bodies.

The wastewater treatment systems are extensively used for the stabilisation of diluted organic wastes. The efficiency of wastewater systems is linked to bacteria, fungi, rotifers, higher animals and protozoa population. According to their means of locomotion, protozoa are commonly placed in five groups which include amoebae, flagellates, free-swimming ciliates, crawling ciliates and stalked ciliates. Under normal conditions their concentrations are larger than 10^6 protozoa L^{-1} and the concentration of 10^7 protozoa L^{-1} corresponds to very good pollution abatement. On the contrary, concentrations lower than 10^5 protozoa L^{-1} are indicative of the low efficiency of the plant (Drakides, 1978). In terms of biomass, protozoa represent between 0.17 and 0.44% of the sludge during the colonization phase but can represent up to 9% at steady-state (Madoni, 1994a, b). Most of the protozoa found in the sludge are ciliated and they can be classified in four main groups: free-swimming, crawling, attached and carnivorous. Ciliated protozoa are currently used as biotic indicators due to the fact that they reduce the concentration of bacteria and suspended particles in the treatment process resulting in the production of effluents of good qualities (Curds and Cockburn, 1970a, b; Al-Shahwani and Horan, 1991; Madoni *et al*., 1993; Salvadó *et al*., 1995). It is also important to note that operational performance of biological wastewater processes is usually managed by the reduction of the Biological Oxygen Demand (BOD_5) and it is recognised that highly-purified effluent are those with BOD_5<25 mg L^{-1} (Curds, 1993).

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Up to date, no study has been done in identifying at protozoan fauna and determining their abundance in industrial and domestic sewage treatment plants in rural, semi urban and urban areas of South Africa. Hence, this study aimed at assessing the potential of municipal wastewater treatment plants for the production of good quality effluent through documentation of their protozoan fauna and abundance as well as the level of the BOD in aeration tanks of some wastewater treatment facilities in the Eastern Cape province of South Africa.

MATERIALS AND METHODS

Study sites and sample collection: Three wastewater treatment plants that serve the Buffalo City and Nkonkobe Municipal areas in the Eastern Cape Province of South Africa were used in this study. These plants are located in the rural areas of Fort Beaufort, semi-urban town of Dimbaza and in urban city of East London. Although the number of various zones depends on the capacity of each plant, all three wastewater treatment plants had the influent, anaerobic, aerobic and clarifier zones, followed by chlorination of the final aqueous effluent prior to discharge in the environment.

The final effluent from the Fort Beaufort sewage works is discharged into the Kat River. The Dimbaza wastewater treatment plant discharges its final effluent into a stream that empties into the Tembisa sewerage dam. The final effluent from the East bank reclamation works is discharged into the Indian Ocean between Nahoon and Eastern Beach at Bats Cave and into a pond for the irrigation of a nearby golf course. Supernatant liquor from the sedimentation tanks is channeled into a fishpond located within the plant premises. Mixed liquors from the aeration tanks of the wastewater treatment plants were used during this study and these were collected bimonthly during September and November 2005 and once a month in October and December 2005. Data for the months of September and November 2005 were used for quantitative reporting, while samples for the whole four months were pooled together for the qualitative protozoan diversity reporting.

Protozoan abundance and fauna analyses: Samples for protozoan analyses were aseptically collected in sterile 2 L glass bottles and transported to the laboratory in ice packs. Aliquots of the samples were fixed with an iodine solution and malachite green as described by Simunek *et al.* (2002) and the protozoan densities in the fixed and unfixed samples were estimated by direct microscopy under 400x magnification (Axioplan Carl Zeiss GmbH equipped with phase contrast, bright field and epifluorescence, HBO 50 illuminator and a digital imaging system) using an improved Neubauer hemocytometer. Protozoan strains were tentatively identified by direct microscopy under x400 magnification by comparison with existing protozoan gallery collections (Rothkiewicz, 2006). Protozoan counts were calculated using the following formula:

$$\text{Number of cells mL}^{-1} = (C \times V)/(A \times D \times F)$$

where, C is the number of organisms counted, F is the number of fields counted, D (mm) is the depth of the counting chamber, A (mm^2) is the area of a field and V (mm^3) is the volume of the counting chamber (APHA, 1998).

Physico-chemical analyses: Temperature, pH, conductivity and salinity were determined on-site. Temperature and pH were determined using a mercury thermometer and a pH meter, model 2000 (Crisson Instruments) respectively, while conductivity and salinity were determined using a conductivity meter (CRISON CM35, Crison instrument). Biochemical oxygen demand (BOD_5) was determined by using the Oxitop WTW BOD meter (Merck Pty Ltd.) with an incubation period of 5 days.

Statistical analysis: In order to show the role of the protozoan communities in wastewater treatment system and which of the variables measured at the wastewater treatment plants affect the structure of the protozoan communities, correlation coefficients (Pearson) were performed and this was also done to further enhance understanding of the relationships between protozoa population and operational parameters. In comparison of the three wastewater treatment plants, the General Linear Model (GLM) form SAS (Statistical Analysis Software) was used. An independent variable was considered a significant predictor when its Pearson correlation coefficient was statistically significant ($p = 0.05$). All parameters, except pH for its scarce variation, were included in this statistical analysis

RESULTS AND DISCUSSION

This study revealed a robust protozoan diversity in the three wastewater treatment plants assessed, with the mean protozoan counts in the order of 10^4 cells mL^{-1} in all cases and with the range of protozoan densities between 2.3×10^4 to 7.6×10^4 cells mL^{-1} (Fig. 1). The highest protozoan density (7.6×10^4 cells mL^{-1}) was observed in the Fort Beaufort treatment plants in September 2005. Also, the lowest protozoan density was again observed at this plant in the month of November. However, during the month of November, protozoan density was higher in the East London (6.4×10^4 cells mL^{-1}) treatment plant

Table 1: Ciliated protozoa diversity observed in the aeration tank mixed liquors of some wastewater treatment plants in the Eastern Cape Province of South Africa

	Wastewater treatment plants		
Ciliates identity	Dimbaza	East London	Fort Beaufort
Aspidisca	√	√	√
Euploites	√	√	√
Peridinium	√	√	
Paruloleptus	√		
Vorticella	√	√	√
Chilodonella	√	√	√
Paramecium	√	√	
Strombidium		√	
Litonotus	√	√	
Trachelophyllum	√	√	
Nassulla			√
Suctoreans		√	
Colpoda		√	
Podophrya		√	√
Tetrahymena	√	√	
Opercularia		√	√
Amphileptus	√	√	
Stentor		√	
Loxophyllum		√	
Carchesium	√	√	√
Epistylis	√	√	
Stylonichia	√	√	
Blepharisma		√	
Didinium	√		
Total	15	21	8

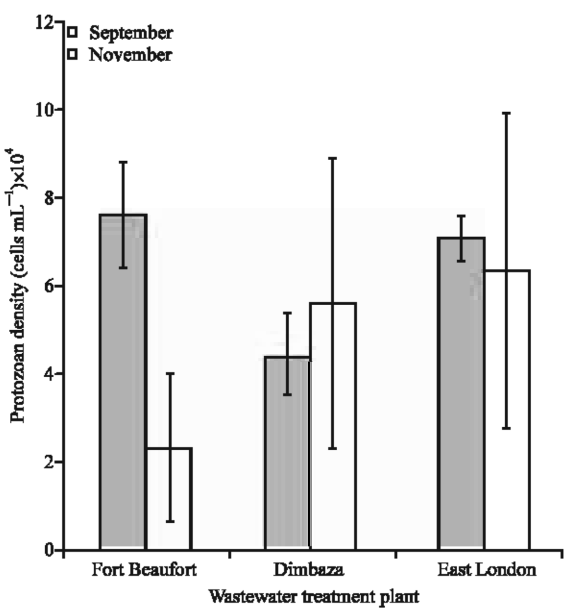

Fig. 1: Protozoan densities of mixed liquors of the aeration tanks of three wastewater treatment plants in the Eastern Cape province of South Africa

compared to the other two plants. This observation was further corroborated by the quality of the ciliated protozoan profile (Table 1). Twenty-one ciliated protozoa were identified in the East London plant, while the

Table 2: Flagellated and other non-ciliated protozoa diversity observed in the aeration tank mixed liquors of some wastewater treatment plants in the Eastern Cape province of South Africa

	Wastewater treatment plants		
Flagellates and others identity	Dimbaza	East London	Fort Beaufort
Flagellates			
Anthophysis	√		√
Trepomonas	√	√	√
Peranema	√		
Bodo	√	√	
Pleuromonas		√	√
Monosiga	√	√	√
Chilomonas			
Hexamitus	√		√
Unidentified	√		
Total	7	4	5
Others			
Ameaba	√	√	
Trichameaba	√		
Arcella	√		
Euglypha			√
Actinophrys	√		√
Hexastylus	√		
Total	5	1	2

Dimbaza and Fort Beaufort plants yielded 15 and 8, respectively. However, an almost reverse trend was observed in terms of the non-ciliated protozoa diversity (flagellates and others) (Table 2) where 12 strains were observed in the Dimbaza plant and 5 and 7 strains were observed in the East London and Fort Beaufort plants, respectively. In the wastewater treatment plant such as activated sludge process, protozoa are placed commonly in five groups according to their means of locomotion. According to the predominance in the wastewater treatment plants under the present study, these groups were stalked ciliates (Fig. 2a, b), crawling ciliates, free-swimming ciliates (Fig. 2d), flagellates (Fig. 2e) and amoebae (Fig. 2f).

Present observation corroborates the findings of previous workers (Wheale and Williamson, 1980; Curds *et al*., 1968) who had variously reported that most of the protozoan species in wastewater treatment plants such as the activated sludge systems are ciliates and they are commonly found in densities of about 10^4 cells mL^{-1}. Abundances of protozoan communities in mixed liquor suspensions were obtained through light microscope counts, confirming that the density of the peritrich ciliates were higher than that of the flagellates (Fig. 2). Other ciliate groups (Order) represented in the mixed liquor were scuticociliates, cyrtophorids, pleurostomatids, hypotrichs, peniculins and heterotrichs, but their densities were extremely lower than that of the peritrich group (Fig. 2a, e). Representatives of tetrahymenids, suctorians gymnostomids, nassulids and prostomids were also occasionally found in mixed liquor from the aeration tanks and they were accounted together

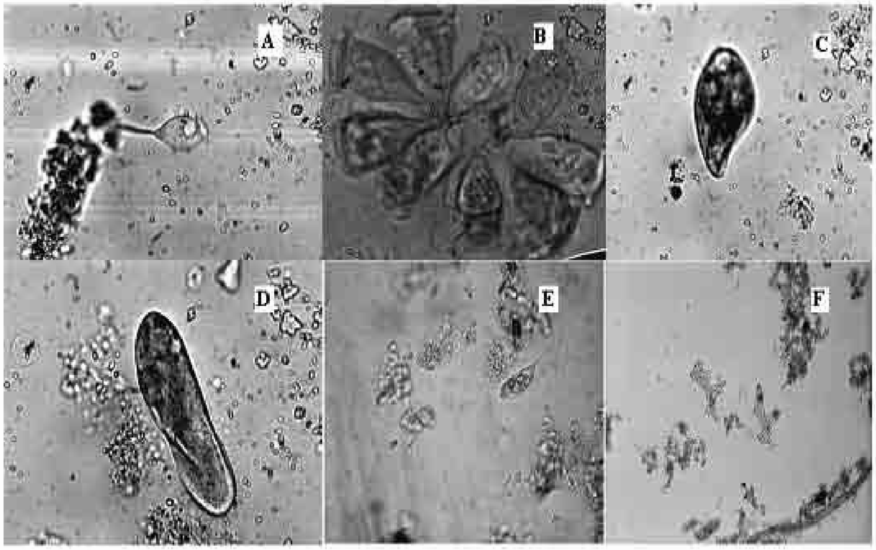

Fig. 2a-f: Light microscope microphotographs of representative species of protozoa in the aeration tank of wastewater treatment plants. (a) Peritrichs: *Vorticella convallaria* attached to a flock (x 400), (b) Colony of *Epistylis entzii* (x400), (c) Pleurostomatids: Free swimming ciliate *Blepharisma* sp. (x 400), (d) Peniculin: Free swimming ciliate *Paramecium caudatum* (x 400), (e) Euglenida: Flagellate *Peranema* sp. (x 400) and (f) Amoebida: *Amoeba* sp. (x 400)

for less than 1% of total ciliate abundance. The peritrich ciliates were mainly represented by *Vorticella convallaria*, *Epistylis entzii*, *Carchesium* spp. and *Opercularia* spp. (Table 1, Fig. 2a, e). Sessile and crawling ciliates species were present in all the aeration tanks of the three plants except for the *Trachelophyllum* species which was not observed in the aeration tank of Fort Beaufort wastewater treatment plant. Free living ciliates were observed in particular plants as in the case of *Blepharisma* species which were only observed in the East London wastewater treatment plant. Some free living ciliates (*Litonotus* spp.) were only observed in the two wastewater treatment plants (Table 1). Flagellates also showed the same pattern as the ciliates, with some species such as *Peranema* spp. (Table 2, Fig. 2c) which were only observed in the East London wastewater treatment plant and species of *Monosiga* spp. were observed in all the three wastewater treatment plants in this study (Table 2). Other representative species of ciliates, flagellates and amoeba in the aeration tanks of activated sludge studied are shown in Table 1 and Table 2.

Protozoa are known to produce clear effluents of good quality because of their ability to feed on bacteria and suspended particles and to induce flocculation. It has also been well documented that the community structure of protozoan species is an effective biological indicator of functional conditions of wastewater treatment plants (Madoni *et al*., 1993; Madoni, 1994a, b). Hence, based on the densities of protozoan fauna of the three wastewater treatment plants under investigation, we conclude that the plants should be highly efficient. These results are consistent with the findings of previous investigators such as Drakides (1978) and Madoni (1994b) who reported that under normal conditions the concentrations of protozoa are larger than 10^6 protozoa L^{-1} and that 10^7 protozoa L^{-1} corresponds to a very good pollution abatement.

Table 3: Status of some physicochemical parameters of mixed liquor samples from some wastewater treatment facilities in the Eastern Cape province

	Range		
Parameters	DWTP	FBWTP	ELWTP
pH	6.76-7.5	7.35-7.52	7.42-7.53
Temp (°C)	15.20-18.1	18.00-20.3	20.70-20.9
Conductivity (µS)	198.80-578	748.00-1322	383.00-459
TDS (mg L^{-1})	94.30-279	363.00-651	184.00-221
BOD_5 (mg L^{-1})	16.15-25.5	28.80-36.8	22.10-26.8

The physicochemical qualities of the mixed liquor samples varied throughout the sampling period. The pH was observed to range between 6.04 and 7.63, while the temperature varied from 16.8 to 24.3°C (Table 3). The conductivity values were between 362.5 and 1056 µS cm^{-1} (Table 1) corresponding to the Total Dissolved Solids (TDS) regimes of 173.2-523 mg L^{-1} (Table 3). There were significant difference between the

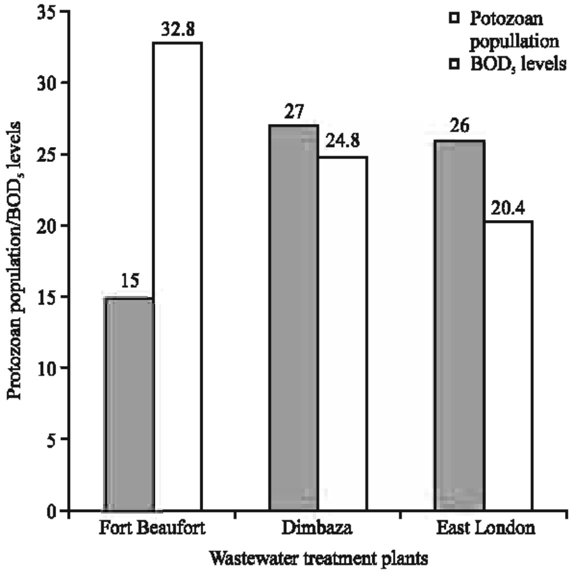

Fig. 3: Relationship between the protozoan population group and the BOD_5 level in the aeration thank of wastewater treatment plants

urban (including semi-urban) wastewater treatment plants and the rural wastewater treatment plants in terms of TDS and conductivity ($p<0.05$). This clearly demonstrated that the rural plant produced the effluent of lower quality compared that of urban plants. This was also confirmed by the BOD_5 concentrations in these plants. As previously stated, the operational performance of biological wastewater processes is usually managed by the reduction of Biological Oxygen Demand (BOD_5) and it is recognised that highly-purified effluents are those with $BOD_5<25$ mg L^{-1} (Curds, 1993). The Fort Beaufort wastewater (28.8-36.8 mg L^{-1}) showed the highest range levels of BOD_5 and the Dimbaza showed the lowest range of BOD_5 (16.15-25.5 mg L^{-1}) and from the results in Table 3 it can be seen that the semi- urban plant of Dimbaza and the urban plant of East London performed better than the rural Fort Beaufort wastewater treatment plant. These results followed similar trends as found for the protozoan population in the plants. The highest population of protozoa were recorded in Dimbaza (27) and East London (26) and the lowest population in Fort Beaufort (15). Statistically, in terms of the protozoan population diversity, significant differences were found between the rural Fort Beaufort plant and the urban/semi-urban plants (East London/Fort Beaufort) ($p<0.05$). This clearly demonstrated that the efficiency of the plants to produce highly-purified effluent was associated with the abundance of the protozoan population in the aerobic zone (Fig. 3).

CONCLUSION

Protozoa are known to be an important indicator of the efficiency of a wastewater treatment plant. The findings of this study have showed that there is high diversity of protozoan population in all the plants with ciliates being the dominant species out of all the other groups. Although the density of protozoa in the aeration tank of the three plants was similar (10^4 cells mL^{-1}), a better performance was statistically noted in plants that had higher protozoan population group.

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REFERENCES

Al-Shahwani, S.M. and N.J. Horan, 1991. The use of protozoa to indicate changes in the performance of activated sludge plants. Water Res., 25: 633-638.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn. American Public Health Association, Washington DC.

Craun, G.F., 1991. Causes of waterborne outbreaks in the United States. Water Sci. Technol., 24: 17-20.

Curds, C.R., A. Cockburn and J.M. Vandyke, 1968. An experimental study of the role of the ciliated protozoa in the activated-sludge process. Water Pollut. Control, 67: 312-329.

Curds, C.R. and A. Cockburn, 1970a. Protozoa in biological sewage-treatment Process-II. A survey of the protozoan fauna of British percolating filters and activated-sludge plants. Water Res., 4: 225-236.

Curds, C.R. and A. Cockburn, 1970b. Protozoa in biological sewage-treatment process-ii. Protozoa as indicator in the activated-sludge process. Water Res., 4: 237-249.

Curds, C.R., 1993. Interactions protozoaires-bactéries dans les methods aérobies de traitement des eaux useés. Ann. Biol., 1: 1-12.

Drakides, C., 1978. L'Observation Microscopique des Boues Activées Appliquée à la Surveillance des Installations d'Epurations: Technique d'étude et Interprétation, TSM-L'EAU, 2: 85-98.

Madoni, P., D. Davoli and M. Chierici, 1993. Comparative analysis of the activated sludge microfauna in several sewage treatment works. Water Res., 27: 1485-1491.

Madoni, P., 1994a. Estimates of ciliated protozoa biomass in activated sludge and biofilm. Bioresour. Technol., 48: 249-254.

Madoni, P., 1994b. A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. Water Res., 28: 67-75.

Momba, M.N.B. and C. Mfenyana, 2005. Inadequate Treatment of Wastewater: A Source of Coliform Bacteria in Receiving Surface Water Bodies in Developing Countries-Case Study: Eastern Cape Province of South Africa. In: Water Encyclopedia-Domestic, Municipality and Industrial Water Supply and Waste Disposal, Lehr, J.H. and J. Keeley (Eds.). A.J. Wiley and Son Inc. Publication, New Jersey, pp: 661-667.

Momba, M.N.B., A.N. Osode and M. Sibewu, 2006. The impact of inadequate wastewater treatment on the receiving water bodies case study. Buffalo City and Nkonkobe Municipalities of the Eastern Cape Province, WISA.

Rothkiewicz, P., 2006. Droplet. Microscopy of the protozoa. http://www.pirx.com/droplet/about.html.

Salvadó, H., M.P. Gracia and J.M. Amigó, 1995. Capability of ciliated protozoa as indicators of effluent quality in activated sludge plants. Water Res., 29: 1041-1050.

Simunek, J., A. Wójcik-Gladysz, M. Wakowska, P. Krejâí and J. Polkowska, 2002. Effects of short and long-term dietary protein restriction on the rumen fermentation activity in growing lambs. Acta Vet. Brno, 71: 457-462.

Wheale, G. and D.J. Williamson, 1980. Unusual behavior of ciliated protozoa in a secondary settlement tank. Water Pollut. Control, 80: 496-500.