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Listeria abundance and physicochemical quality of a RW used for irrigation and fish farming in a typical urban settlement in South Africa

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We evaluated the microbial (listerial) and physicochemical quality of a reclaimed municipal wastewater (RW) used for irrigation and aquaculture in South Africa between August 2007 and July 2008. Listerial density in RW ranged between 9.6 × 10^3 and 2.8 × 10^5 cfu/100 ml. pH varied from 6.7 to 7.75 while temperature ranged between 18 and 27°C. Turbidity varied between 1.6 and 19 NTU whereas chemical oxygen demand (COD) ranged from 10 to 965 mg/l. Total dissolved solids (TDS) for RW varied between 288 and 715 mg/l while dissolved oxygen (DO) ranged between 0.14 and 6.1 mg/l. Other parameters recorded the following values after wastewater reclamation: Nitrate (0.27 – 6.8 mg NO_3^-N/l); Nitrite (0.12 - 6.3 mg NO_2^- N/l); and Orthophosphate (PO_4^{3-}) (0.08 – 2.17 mg PO_4^{3-} P/l). Although the physicochemical quality of the RW was generally compliant with recommended standards, its microbial quality disqualifies it for use in agriculture and aquaculture in lieu of the public health implication for farm workers and consumers of the farm produce.

Key words: Reclaimed wastewater, *Listeria,* physicochemical, aquaculture, public health, environment.

INTRODUCTION

Growing economic and physical scarcity of water, made worse by global climatic changes and increasing demands for freshwater, calls for innovative ways of water use and development (Inocencio et al., 2003). The Southern African region is predicted to experience more and longer droughts over the next 70 years (Palitza, 2009); according to the report the impending water-shortage will result in more strain on available freshwater resources and in turn lead to increased crop failures, less pasture for livestock and ultimately less food for the growing population. The United Nations Environment Program (UNEP, 2009) also predicted that the situation may get so bad in the coming years that wastewater may account for 25-75% of the total available irrigation water in the region, especially in the very dry zones. The bleak future of freshwater availability is thus forcing planners and stakeholders to consider any sources of water which might be useful economically to promote food security and further development (FAO, 1992).

Innovative approaches to agricultural water use have

Abbreviations: ANOVA, Analysis of variance; **AEMREG,** applied and environmental microbiology research group; **CCME,** Canadian council of ministers of the environment; **COD,** chemical oxygen demand; **DO,** dissolved oxygen; **DWAF,** department of water affairs and forestry; **LCA,** listeria chromogenic agar; **RS,** raw sewage influent; **RW,** reclaimed wastewater; **SPSS,** statistical package for the social sciences; **TDS,** total dissolved solids; **UNEP,** united nations environment program; **FAO,** food and agricultural organization; **WHO,** world health organizations.

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been reported to have the capacity not only to raise agricultural productivity and food security in sub- Saharan Africa, but also lead to the general improvement of living standard of the poor (Inocencio et al., 2003). It is little wonder therefore that wastewater reuse for agriculture is increasingly becoming an attractive option to many stakeholders in the Southern Africa region due to its potential to efficiently conserve water resources, recycle nutrients, and minimize pollution of surface water bodies (Al-Sa'ed, 2007). UNEP (2009) reported the use of sewage in the cultivation of fishes in Malawi, South Africa and Zimbabwe with fish yields in Malawi reaching 4-5 tons/ha/growth period as against yields of 0.8-1.2 tons/ha/year in South Africa. The report also indicated that South Africa recycles about 8% of her total sewage output as against up to 50% in Namibia, and 65% or less in Botswana. Ironically, there is dearth of information on the quality of these reclaimed wastewaters (RW), thereby leaving stakeholders with little or no means of verifying the true usefulness of this water resource to the Southern African polity.

While it is necessary to encourage the reuse of wastewater especially in the dry zones of the world such as South Africa, conscious steps must be taken to ensure acceptable quality of this water resource in order to preserve the public health and protect the environment. Central to the preservation of public health is the monitoring of relevant contaminants including pathogens in RW.

The Food and Agricultural Organization (FAO, 1992) disqualified the use of coliforms and Faecal Streptococci as indicators in monitoring the quality of RW meant for agricultural uses; while on the other hand, FAO recommended *Salmonella* for the same purpose due to their presence in good numbers in urban sewage. However, reports in the literature, Watkins and Sleath (1981), Paillard et al. (2005) and Odjadjare and Okoh (2010) suggest that *Listeria* species might be more abundant in urban municipal sewage than the Salmonellas, due to their relative resistance to adverse environmental conditions including wastewater treatment. *Listeria* survives wide ranges of temperature (-7-45°C), pH (4.3-9.6), and salt concentrations (up to 10%) (Roberts and Wiedmann, 2003), and is capable of saprophytic existence on plant and in soil for years (Al-Ghazali and Al-Azawi, 1986; Beuchat, 1996).

Although the literature is replete with reports on aspects of wastewater in agriculture, including health impacts and risks, and the environmental fate of organics (Hamilton et al., 2007), not much has been done in South Africa to monitor the quality and public health significance of applying this water resource in agriculture. This paper therefore reports the *Listeria* abundance and physicochemical quality of a RW used for irrigation and fish farming in a typical urban settlement in South Africa, with a view to ascertaining its suitability for the intended purposes viz-a-viz its public health and environmental significance.

MATERIALS AND METHODS

Description of study site

The wastewater treatment plant (Figure 1) is located in East London, a large and highly populated urban community in the Eastern Cape Province of South Africa, with the geographical coordinates: 32.97°S and 27.87°E. The plant receives municipal domestic sewage and a heavy industrial effluent and comprise of four screens, a grit channel, two anaerobic tanks, two anoxic tanks and two aerobic tanks (each equipped with three vertically mounted mechanical aerators). The plant has six sedimentation tanks (clarifiers) with the return activated sludge pumped from the bottom of the clarifiers via the screens with raw sewage to the aeration tanks. Supernatant liquor from the sedimentation tanks (RW) was used for irrigation and watering of a fish farm located within the treatment plant premises. The average daily inflow of raw sewage during the period of study was 32 000 m^3/day, while the plant has a designed capacity of 40 000 m^3/day.

Sample collection

Wastewater samples were collected on a monthly basis from the raw sewage influent (RS) and RW between August 2007 and July 2008. Samples were collected in duplicates from the surface of each site in clean sterile one litre Nalgene bottles and transported in cooler boxes containing ice packs to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice, South Africa for analyses. Analysis of samples was done within 6 h of sample collection.

Estimation of *Listeria* abundance

The isolation of *Listeria* species were done according to the description of Hitchins (2001) with modifications. Briefly, aliquots of samples were directly inoculated onto *Listeria* chromogenic agar (LCA agar) (Pronadisa® Madrid, Spain) following standard spread plate technique and incubated for 24-48 h at 35^{o}C. Typical *Listeria* colonies appeared blue-green on LCA agar plates while pathogenic strains (*L. monocytogenes* and *L. ivanovii*) were surrounded by an opaque halo in addition to their blue-green colour. Total presumptive *Listeria* counts were recorded and the isolates purified and stored on nutrient agar slants at 4°C for further analyses. The presumptive *Listeria* pathogens were randomly confirmed by standard cultural characteristics and biochemical reactions (Hitchins, 2001) and using the API *Listeria* kits (10 300, bioMerieux, South Africa). *Listeria monocytogenes* (ATCC 19115) and *Staphylococcus aureus* (ATCC 25923) were used as positive and negative controls, respectively.

Physicochemical analyses

All field meters and equipment were checked and appropriately calibrated according to the manufacturers' instructions. pH, temperature, total dissolve solid (TDS), and dissolved oxygen (DO), were all determined on site using the multi- parameter ion specific meter (Hanna-BDH laboratory supplies). Turbidity was also determined on site using a microprocessor turbidity meter (HACH Company, model 2100P) while concentrations of orthophosphate (PO_4^{3-}) as P, Nitrate (NO_3^-), Nitrite (NO_2^-), and chemical oxygen demand (COD) were determined in the laboratory by the standard photometric method (DWAF, 1992) using the spectroquant NOVA 60 photometer (Merck Pty Ltd). Samples for COD analyses were digested with a thermoreactor model TR 300 (Merck Pty Ltd) prior to analysis using the spectroquant NOVA 60 photometer.

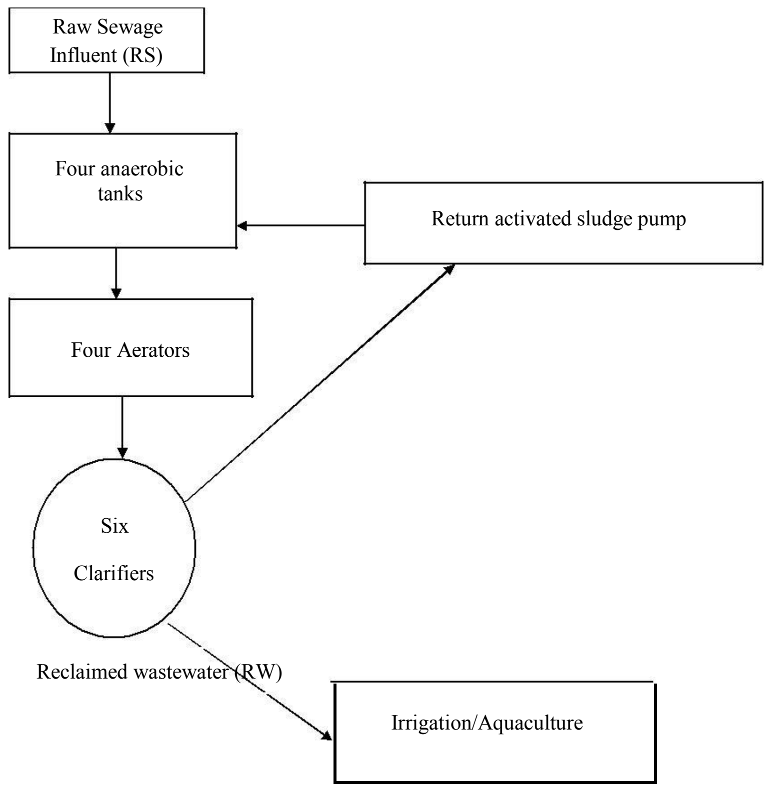

Figure 1. Schematic representation of the wastewater treatment plant.

Statistical analyses

Calculation of means and standard deviations were performed using Microsoft Excel office 2007 version. Correlations (paired T-test) and test of significance (ANOVA) were performed using SPSS 17.0 version for Windows program (SPSS Inc.). All tests of significance and correlations were considered statistically significant at P values < 0.05 or < 0.01.

RESULTS

Tables 1, 2, and 3 show results of *Listeria* abundance and physicochemical quality of the RS and RW as well as the correlation matrix of the parameters evaluated.

Listeria abundance

Table 1 shows the average listerial densities of the wastewater before and after treatment. Listerial density ranged between 1.3×10^5 to 1.4×10^7 cfu/100 ml in RS and 9.6×10^3 to 2.8×10^5 cfu/100 ml in RW. The highest listerial density was recorded in April 2008 in RS while the lowest density was observed in the RW in November, 2007. The annual mean listerial density was 3.9×10^6 cfu/100 ml for RS and 6.1×10^4 cfu/100 ml for RW. The percentage reduction achieved by the secondary treatment ranged from 77.8 to 99.5% with the highest percentage reduction observed in the months of November and December, 2007 and the lowest recorded in January, 2008. Listerial density varied significantly with sampling site (P < 0.05) but not with season. *Listeria* abundance showed significant positive correlation with TDS (r = 0.670, P < 0.01), PO_4^{3-} (r = 0.652, P < 0.01) and pH (r = 0.376, P < 0.05); and negatively correlated with DO (r = -0.461, P < 0.01) and NO_3^- (r = -0.389, P < 0.05).

pH

pH in the RS varied from 6.31 to 7.75 while that of the RW ranged from 6.70 to 7.75 (Table 2). Values of pH for spring varied significantly (P < 0.05) with those of autumn and winter but not with summer. pH in winter also varied significantly with those of summer (P < 0.05) and autumn (P < 0.01). There was no significant difference in pH with sampling site. pH correlated significantly (positive) with

Temperature

Temperature ranged between 18°C (July 2007) and 26°C (March 2008) for RS and varied from 18°C (July 2007) to 27°C (March 2008) in RW. Temperatures during spring and winter differ significantly (P < 0.01) from those of summer and autumn. Temperature did not vary significantly with sampling site, and it showed significant negative correlations with DO (r = -0.311, P < 0.05) and

Table 1. *Listeria* density in raw sewage and reclaimed wastewater.

Season	Month	*Listeria* density (cfu/100 ml)		
		Raw sewage (RS)	Reclaimed wastewater (RW)	Reduction (%)
Spring	August 2007	3.5×10^{6}	6.4×10^{4}	98.2
	September 2007	1.2×10^{6}	1.6×10^{4}	98.6
	October 2007	ND[a]	ND[a]	ND[a]
Summer	November 2007	1.9×10^{6}	9.6×10^{3}	99.5
	December 2007	5.0×10^{6}	2.3×10^{4}	99.5
	January 2008	1.3×10^{5}	2.9×10^{4}	77.8
Autumn	February 2008	3.1×10^{6}	4.0×10^{4}	98.7
	March 2008	4.9×10^{6}	9.7×10^{4}	98.0
	April 2008	1.4×10^{7}	2.8×10^{5}	98.0
Winter	May 2008	6.1×10^{6}	4.1×10^{4}	99.3
	June 2008	1.6×10^{6}	6.2×10^{4}	96.1
	July 2008	2.1×10^{6}	1.4×10^{4}	99.3
Annual Average		3.9×10^{6}	6.1×10^{4}	96.6
Range		1.3×10^{5} - 1.4×10^{7}	9.6×10^{3} - 2.8×10^{5}	77.8 -99.5

[a] Not determined.

nitrite (r = -0.355, P < 0.05).

Turbidity

Turbidity was in the range of 95 NTU - 1000 NTU (RS) and 1.6 NTU - 19 NTU (RW) during the study. The values varied significantly with sampling site (P < 0.01) but not with season. Turbidity negatively correlated with DO (r = - 0.615, P < 0.01) and positively correlated with COD (r = 0.411, P < 0.05) and PO_4^{3-} (r = 0.646, P < 0.01).

Total dissolved solids (TDS)

TDS varied between 320 - 907 mg/l (RS) and 288 - 715 mg/l (RW); concentrations in autumn were significantly different (P < 0.05) from those of spring and summer, but not with winter. TDS did not vary significantly with sampling site; but positively correlated with PO_4^{3-} (r = 0.305, P < 0.05) and negatively correlated with DO (r = - 0.434, P < 0.01).

Dissolved oxygen (DO)

DO was in the range of 0.14 – 6.1 mg/l (RS) and 1.5 – 7.4 mg/l (RW). There were significant differences in DO values for spring with those of summer and winter (P < 0.05) and autumn (P < 0.01). DO also varied significantly with sampling site (P < 0.05) and showed significant negative correlation with COD (r = - 0.339, P < 0.05) and PO_4^{3-} (r = -0.473, P < 0.01); while positively correlating with nitrate (r = 0.324, P < 0.05).

Chemical oxygen demand (COD)

COD varied between 10 - 1956 mg/l in the RS and 10 - 956 mg/l in the RW. COD did not show significant difference with regards to season and sampling site. There was also no significant correlation between COD and other parameters except as cited previously for turbidity and DO.

Nitrate

Concentration of nitrate ranged between 0.09 - 4.8 mg NO_3^-N/l (RS) and 0.27 - 6.8 mg NO_3^-N/l (RW) and varied significantly with sampling site (P < 0.05) but not with season. Nitrate showed significant negative correlations with PO_4^{3-} (r = -0.334, P <0.05) and nitrite (r = -0.602, P <0.01).

Nitrite

Nitrite concentration varied from 0.10 - 3.4 mg NO_2^-N/l (RS) and 0.12 - 6.3 mg NO_2^-N/l (RW) and with reference to temperature and may not significantly offset the homeostatic balance of the receiving ecosystems vis-à-vis its environmental implication.

The turbidity of the RW during this study was generally compliant with target limits (<1 - <5 NTU) for reuse wastewater for irrigation (Lazarova et al., 2008) in lieu of public health and environmental concerns except in October 2007 (10 NTU) and December 2007 (19 NTU). Based on the USEPA (2004) recommended standard (<20 - 90 mg/l) for COD in reuse wastewater, the RW quality during this study could also be adjudged fit for application in agriculture except for values recorded in November 2007 and April 2008 (Table 2).

The RW under study was compliant with target limit for TDS (<500 and 2000 mg/l) (FAO, 1992; Abu- Zeid, 1998; WHO, 2006a) and suggests that it was fit for application in agriculture in lieu of environmental and public health concerns. Although there are no recommended limits for TDS concentration in waters meant for aquaculture, Morrison et al. (2001) reported that high salt concentration in wastewater can result in adverse ecological effects on aquatic biota. TDS concentration did not vary significantly with sampling site in this study, suggesting that the secondary treatment did not significantly remove dissolved salts from the raw sewage (Table 2). The strong positive correlation between TDS and listerial density is consistent with previous reports (Al- Ghazali and Al-Azawi, 1986; Czeszejko et al., 2003) on the capacity of the bacteria to tolerate high salt concentrations.

DO levels in this study fell short of the acceptable limit (5 mg/l) of no risk for the support of aquatic life (Fatoki et al., 2003) except in the month of September 2007 when the RW was compliant with the stipulated standard at 6.1 mg/l (Table 2). This is an indication that the RW may not be fit for aquaculture purposes except in the growth of oxygen tolerant fish species (WHO, 2006b). The nitrate concentration observed during this study fell within recommended limits (< 30 mg NO_3^-N/l) that may increase productivity in agriculture (WHO, 2006a). Although there are no recommended standards for nitrate in aquaculture, high nitrate levels in water systems is reported to result in eutrophication leading to loss of diversity in the aquatic biota and overall ecosystem degradation through algal blooms, excessive plant growth, oxygen depletion, reduced sunlight penetration and ultimately, death of aquatic life (CCME, 2006).

Nitrite concentration during this study fell within acceptable limits for agriculture (< 30 mg NO_2^-N/l; WHO, 2006a) but not for the preservation of the aquatic ecosystem (<0.5 mg NO_2^-N/l) as recommended by the South African government (DWAF, 1996). This therefore implies that whilst the RW may be suitable for agriculture it may not be beneficial for aquaculture in lieu of its public health and environmental implications. Phosphate levels similar to those observed in this study had been previously reported (Igbinosa and Okoh, 2009). Conversely, Fatoki et al. (2003) reported lower PO_4^{3-} levels, whereas Ogunfowokan et al. (2005) reported higher levels in their studies. The phosphate concentrations observed during this study complied with recommended limits for agriculture (< 20 mg PO_4^{3-}P/l) but fell short of aquaculture target limits (5 µg/l or 0.005 mg PO_4^{3-}P/l) in lieu of risk of eutrophication (DWAF, 1996; WHO, 2006a). The observation suggests that the RW is suitable for agriculture but not for aquaculture with reference to orthophosphate, and in view of its environmental and public health significance.

The RW under study was generally of good quality by physicochemical standards; however, its microbial quality fell short of recommended target limits for application in irrigation and aquaculture in lieu of public health concerns. We therefore recommend the need for relevant authorities to regularly monitor the indiscriminate and unsupervised use of RW in agriculture in order to preserve the public health and ensure maximum benefits from the use of this important water resource.

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