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PHYSICOCHEMICAL AND MICROBIAL ASSESSMENT OF SEWAGE FROM OGWA AND EBELLE COMMUNITIES OF ESAN LAND, EDO STATE

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ABSTRACT
The increasing anthropogenic disposal of untreated sewage causes major damage to the ecosystem. This study assessed sewage from Ogwa and Ebelle communities for its physicochemical and microbial qualities. Standard methods were used for the analyses. The physicochemical parameters had low concentrations except pH (5.27±0.03 – 5.76±0.20), nitrate (15.42±0.01 – 62.30±0.11mg/l), phosphate (12.28±0.28 – 75.40±0.46mg/l), Chemical oxygen demand (542.87±0.18 – 1850.11±0.14mg/l), Biochemical oxygen demand (198.63±0.18 – 497.62±0.03mg/l), zinc (4.12±0.12 – 10.38±0.02mg/l), nickel (5.82±0.21 – 25.22±0.12mg/l), chromium (1.45±0.10 – 4.12±0.01mg/l) and lead (1.11±0.02 – 2.43±0.03mg/l) which were higher than FEPA recommended limit. The concentrations of heavy metals in the sewage samples were statistically significantly different compared to the control ($p<0.05$). The heterotrophic bacterial, coliform and fungal counts ranged from $1.0\times10^6 \pm 0.02$ - $8.57\times10^6 \pm 0.12$cfu/ml, $0.00 \times10^6 \pm 0.00$ - $5.82 \times10^6 \pm 0.04$cfu/ml and $0.10\times10^6 \pm 0.00$ - $4.94\times10^6 \pm 0.07$cfu/ml respectively. Among the bacterial identified Staphylococcus aureus had the highest (18.21%) while Rhizopus sp. had the highest (3.89%) of all fungal isolates. The parasites identified belonged to eight genera with Ascaris lumbridoides present in all samples with the highest at Eguare, Ogwa. There is need to construct good sewers to allow for discriminate disposal thereby promoting safety of the environment and it's teeming population.
Keywords: Ascaris lumbridoides, Ecotoxicity, Heavy metals, Microorganisms, Proteus sp., Public Health, Sewage.

INTRODUCTION

The volume of sewage generated is increasing with the ever increasing population, urbanization as well as economic development. Sewage comprises of liquid wastes discharged by domestic, commercial, industrial and agricultural activities which plays host to lots of potential contaminants with varying concentrations (Nielsen *et al*., 2006). It may in risky cases, take the form of diluted raw sewage, still it is considered outlawed (Odjadjare *et al*., 2011). Untreated sewage results in major damage to environmental quality and human health. A large number of microorganisms are associated with sewage sludge and other wastes of biological origin. These causative agents of many infectious diseases are excreted by the faecal - oral route (WHO, 2006). Although wastewater is usually 99.9 % water and 0.1 % solids, discharges from industrial and domestic sources also add solids to the plant influent (Akpor and Muchie, 2011; FEPA, 1991). An increasing amount of sewage requires reasonable management, whereas its storage might be environmentally hazardous.

The high concentration of nitrogen and phosphorus content in sewage provides it with its ability to serve as organic manure as well as beneficial soil conditioning properties. However, one of the limitations associated with the use of sewage may be the input of contaminants into the soil and the food chains associated with arable land as well as causing several chronic diseases to man and animals within the environment (Onuoha *et al*., 2016). It could also contain high concentrations of potentially toxic heavy metals like cadmium, copper and lead which are considered the most important constituents of pollution from the terrestrial environment due to their accumulation, toxicity and subsequent transfer into food chain (Storelli, 2008; Gryta *et al*., 2014). The aim of this study was to access the physicochemical and microbial qualities of sewage from Ogwa and Ebelle Communities of Esan Land in Edo State.

MATERIALS AND METHODS

STUDY AREA

The studied areas were Eguare and Ukpogo in Ogwa as well as Okpujie and Idumogho in Ebelle Communities. Ogwa is located in Esan West local Government Area while Ebelle is of Igueben Local Government Area, both in Edo State, Nigeria. They lie approximately on latitude 6^0 30' 0N and longitude 6^0 12' 0E and are among the constituent independent communities of Esan land. The soil type is precisely reddish-brown in colour and it's fertile for farming which is the main occupation of the people.

Sampling and Samples Collection

Sewage samples were collected from sewers in four different locations. Eguare and Ukpogo, in Ogwa community as well as Idumogho and Okpujie in Ebelle community in triplicates in the early hours of the day. Everwell bottled water served as control. A sterile sample bottle was dipped into the sewer to a depth of about 60cm and sewage samples were aseptically collected and poured into sterile labelled plastic bottles. They were transported in ice packed containers to the Samuel Adegboyega University laboratory for analyses.

Determination of Physicochemical Parameters

The Physiochemical parameters were determined following the methods of APHA (2005) and Enerijiofi *et al*. (2017). The parameters were pH, electrical conductivity, total dissolved solids, turbidity, total suspended solid, chlorine, sulphate, nitrate, phosphate, Chemical oxygen demand, Biochemical oxygen demand, , total soluble solid, dissolved oxygen,. For heavy metals determination, the soil samples were air dried in the laboratory to constant weight and grounded with a ceramic mortar and pistle. The samples were sieved with 2mm mesh to exclude debris. Two grammes (2g) of the samples were digested with HNO_3: HCl in 1: 10. Twenty five milliliters (25mls) of distilled water was added to the mixture and placed in a water – bath followed by heating for 20min to obtain a milky colour. Thereafter, they were removed from heat, allowed to cool and transferred into 50ml sample bottles and made up to the 25ml mark with distilled water. The concentrations of the metals, Sodium, Potassium, Calcium and Magnesium were determined directly using flame photometer (Jenway, model PFP7) while the concentrations of the heavy metals were also determined directly using Shimadzu Atomic Absorption Spectrophotometer (model PG 550).

Microbiological analyses of sewage samples

Determination of total heterotrophic bacterial, coliform and fungal counts

Inoculation and enumeration: Nutrient agar was used for determining total heterotrophic bacterial counts. Eosine Methylene Blue, MacConkey and Salmonella - Shigella agars were used for coliform counts while Potato Dextrose Agar was used for determining fungal counts. One ml of appropriate ten-fold serial dilution (10^{-3}, 10^{-6} and 10^{-9}) of the sewage samples were inoculated into plates containing the different agars in triplicate using the pour plate method (Cheesbrough, 2005). The inoculated plates were incubated at 37^{o}C for 24hrs for the enumeration of the total heterotrophic bacterial and coliform counts while total fungal counts was determined at room temperature of 28^{o}C for 72hrs. Visible discrete colonies on inoculated plates were counted using colony counter (Model- Labtech) and expressed in cfu/ml of the sewage sample.

Characterization and Identification of Bacterial And Fungal Isolates

Discrete colonies were purified by subculturing into freshly prepared Nutrient agar and Potato dextrose agar plates for bacterial and fungal respectively. Pure cultures of bacterial isolates were identified based on cultural, morphological and biochemical characteristics (Holt *et al*., 1994). The fungal isolates were also examined macroscopically and microscopically using Lactophenol blue and identified based on the scheme of Barnett and Hunter (1972).

Determination of Parasites

The wet preparation method according to Ochei and Kolhatkar, (2000) was used. The sewage samples were thoroughly mixed and a 10ml syringe was used to draw the sewage sample into a centrifuge tube and centrifuged at 3,000 rpm for 5 min. The supernatant was decanted while the sediment was re-mixed by vortexing. A drop was placed on a grease free slide, covered with a cover slip and examined microscopically using ×10 and ×40 objectives and the total counts were calculated.

Statistical Analysis of Data

Data were presented as mean ± standard error of means (mean ± S.E.M) of the respective replicate. One way ANOVA was done to compare means of the groups as well as Duncan multiple range test to analyze differences among different means. The differences at $p<0.05$ were statistically significant.

RESULTS

The sewage colour ranged from pale yellow grey. The odour ranged from earthy to faeces - like smell and there were lots of mosquitoes larva in the sewage samples (Table 1). The results of the physicochemical parameters analysed from the study as shown in Table 2 revealed that electrical conductivity (1065 µS/cm) in Okpujie and nitrates (15.42±0.09 – 62.30±0.11 mg/l), phosphates (12.28±0.28 – 75.40±0.46 mg/l), chemical oxygen demand (542.87±0.18 – 1850.11±0.14 mg/l) and biochemical oxygen demand (198.63±0.18 – 497.62±0.03 mg/l) from all samples were higher than the FEPA (1991) regulatory standards. The macronutrients (sodium, calcium and magnesium) from all samples were within the limits of set by FEPA (1991) for discharge of industrial effluent, however, Zinc (4.12±0.12 – 10.38±0.02mg/l), Nickel (5.82±0.21 – 25.22±0.12 mg/l), Chromium (1.45±0.10 – 4.12±0.01 mg/l) and Lead (1.11±0.02 – 2.43±0.03 mg/l) were above the permissible limit set by FEPA (1991). It was revealed that samples form Eguare had the highest heterotrophic, coliform and fungal counts of 8.57×10^{6} ± 0.12cfu/ml, 5.82×10^{6} ± 0.04cfu/ml and 4.94×10^{6} ± 0.04cfu/ml while the control had the least (Table 3). *Staphylococcus aureus* (18.21%) was the most frequently isolated bacterial isolate while *Rhizopus* sp. (3.89%) had the highest among the fungal isolates (Table 4). *Balantidium coli* (30.80%) from Eguare was the highest of all parasites. Also, samples form Eguare had the highest parasites counts of 182 parasites while the least was found in sample from Ukpogo with 63 counts. However, no parasite was present in the control (Table 5). *Ascaris lumbricoides* was present in samples from all locations. However, all parasites were absent in the control (Table 6).

Table 1: Physical Characteristics of Sewage Samples

Sampling Points	Particles	Odour	Colour
Eguare	Numerous dark particles containing few mosquito larva	Earthy	Brown
Ukpogo	Particles containing dead insects	Sewage-like odour	Grey
Idumogho	Particles with numerous mosquito larva	Pungent Septic smell	Yellow
Okpujie	Particles with few mosquito larva	Faeces - like smell	Pale yellow
Control	Free of particles	Odourless	Colourless

Table 2: Physicochemical parameters of sewage samples from Ogwa and Ebelle Communities

Heavy metals	Eguare	Ukpogo	Idumogho	Okpujie	Control	FEPA (1991) Effluent limit
pH	5.76±0.10^{a}	5.33±0.02^{a}	5.27±0.03^{a}	5.45±0.01^{a}	6.69±0.03^{a}	6 – 9
EC (µS/cm)	987±0.02^{d}	856±0.00^{c}	589±0.21^{b}	1065±0.06^{e}	89±0.12^{a}	1000
TDS (mg/l)	493.5±0.21^{d}	428±0.01^{c}	294.5±0.07^{b}	523.5±0.10^{e}	44.5±0.29^{a}	2000
Turbidity (NTU)	6.45±0.07^{b}	4.24±0.12^{b}	10.35±0.12^{c}	21.35±0.21^{d}	1.67±0.41^{a}	300
TSS(mg/l)	23.87±0.11^{d}	11.78±0.11^{b}	15.73±0.18^{c}	18.78±0.22^{c}	0.88±0.11^{a}	30
Chlorine (mg/l)	28.84±0.01^{b}	35.78±0.29^{c}	19.57±0.14^{a}	21.85±0.02^{a}	26.7±0.01^{b}	600
Sulphate (mg/l)	4.15±0.16^{b}	2.67±0.02^{b}	6.52±0.12^{c}	13.73±0.03^{d}	0.28±0.21^{a}	50
Nitrate (mg/l)	15.42±0.09^{b}	59.91±0.41^{d}	62.30±0.11^{d}	51.04±0.21^{c}	0.2±0.06^{a}	1.0
Phosphate (mg/l)	22.78±0.06^{c}	12.28±0.28^{b}	29.96±0.11^{d}	75.40±0.46^{e}	0.08±0.04^{a}	5.0
COD (mg/l)	542.87±0.18^{b}	758.240.41^{d}	1850.11±0.14^{e}	694.89±0.02^{c}	25.72±0.10^{a}	40
DO (mg/l)	0.76±0.02^{a}	0.01±0.00^{a}	0.01±0.00^{a}	1.52±0.03^{b}	4.56±0.21^{c}	40
BOD (mg/l)	243.53±0.06^{c}	375.79±0.04^{d}	497.62±0.03^{e}	198.63±0.18^{b}	2.33±0.11^{a}	10
Sodium (mg/l)	96.15±0.18^{d}	84.55±0.29^{c}	87.71±0.03^{c}	65.02±0.01^{b}	0.30±0.10^{a}	200
Potassium (mg/l)	79.75±0.16^{e}	25.06±0.42^{b}	30.41±0.18^{b}	48.76±0.06^{d}	0.23±0.00^{a}	NI
Calcium (mg/l)	15.01±0.11^{d}	9.20±0.22^{c}	9.50±0.04^{c}	5.20±0.18^{b}	1.42±0.13^{a}	100
Magnesium (mg/l)	41.44±0.01^{c}	29.42±0.11^{b}	73.67±0.14^{d}	47.32±0.11^{c}	0.35±0.15^{a}	100
Iron (mg/l)	36.22±0.10^{d}	28.49±0.18^{c}	24.80±0.11^{c}	13.83±0.01^{b}	0.31±0.10^{a}	20
Zinc (mg/l)	7.46±0.28^{c}	10.38±0.02^{c}	4.12±0.12^{b}	9.44±0.10^{c}	0.23±0.21^{a}	1.0
Manganese(mg/l)	2.79±0.02^{c}	3.18±0.29^{c}	1.71±0.00^{b}	1.53±0.14^{b}	0.06±0.24^{a}	NI
Copper (mg/l)	1.69±0.02^{c}	2.23±0.41^{c}	0.86±0.01^{b}	1.90±0.03^{c}	0.04±0.41^{a}	1.5
Nickel (mg/l)	25.22±0.12^{c}	7.77±0.00^{b}	7.71±0.46^{b}	5.82±0.21^{b}	0.05±0.11^{a}	1.0
Cadmium (mg/l)	1.56±0.14^{a}	2.09±0.01^{a}	0.88±0.02^{a}	2.00±0.11^{a}	ND	1.0
Vanadium (mg/l)	5.93±0.01^{b}	3.45±0.00^{b}	4.91±0.00^{b}	3.91±0.01^{b}	0.05±0.07^{a}	NI
Chromium (mg/l)	2.95±0.06^{b}	4.12±0.01^{b}	1.45±0.10^{a}	2.39±0.09^{b}	0.09±0.02^{a}	0.5
Lead (mg/l)	2.43±0.03^{a}	1.34±0.02^{a}	1.35±0.01^{a}	1.11±0.02^{a}	ND	0.5
Arsenic (mg/l)	0.32±0.01^{a}	0.11±0.01^{a}	0.15±0.12^{a}	0.24±0.21^{a}	ND	NI

Values represent the Means ± Standard error of triplicate samples.
NB: Mean with different superscript on the same row are significantly different (P<0.05).
Legend: EC: electrical conductivity, TDS; total dissolved solid, TSS: total suspended solid COD: chemical oxygen demand DO: dissolved oxygen, BOD: biochemical oxygen demand, ND: Not detected, NI: Not indicated

Table 3: Mean Bacterial, Coliform and Fungal Counts n x 10^{6} cfu/ml

Sampling Points	THC	TCC	TFC
Eguare	8.57 ± 0.12^{b}	5.82 ± 0.04^{c}	4.94 ±0.04^{c}
Ukpogo	1.10 ± 0.02^{a}	1.28± 0.01^{b}	1.83 ± 0.00^{b}
Idumogho	1.40 ±0.10^{a}	1.52± 0.09^{b}	1.23 ± 0.01^{b}
Okpujie	8.34 ±0.11^{b}	2.27± 0.04^{b}	4.93 ± 0.07^{c}
Control	1.00±0.00^{a}	0.00 ±0.00^{a}	0.10 ±0.00^{a}

Values represent the Means ± Standard error of triplicate samples.
NB: Mean with different superscript on the same column are significantly different (P<0.05).
Legend: THC = total heterotrophic count, TCC = total coliform count, TFC = total fungal count

Table 4: Percentage Frequency of Occurrence of Bacterial and Fungal Isolates

Bacterial Isolates	Sewage (%)	Control (%)
Staphylococcus aureus	18.21	12.70
Pseudomonas aeruginosa	8.22	7.30
Escherichia coli	17.53	19.10
Bacillus sp.	14.93	16.40
Klebsiella sp.	11.03	12.70
Proteus sp.	18.18	17.30
Fungal Isolates		
Penicillium sp.	2.38	3.60
Aspergillus sp.	3.25	4.10
Rhizopus sp.	3.89	4.10
Yeast	1.29	1.40
Mucor sp.	1.08	1.40
Total	**462**	**220**

Table 5: Total Parasitic Counts (Count per 100ml)

Parasite	Eguare (%)	Ukpogo (%)	Idumogho (%)	Okpujie (%)	Control
Balantidium coli	30.80	0.00	20.65	12.73	0
Cyclospora cayetanensis	11.50	17.46	0.00	14.55	0
Entamoeba histolytica	24.70	0.00	22.83	23.64	0
Schistosoma haematobium	0.00	7.94	14.13	0.00	0
Schistosoma mansoni	4.40	25.39	0.00	10.91	0
Faxciola hepatica	0.00	12.69	2.17	5.45	0
Taenia solium	9.90	0.00	28.26	10.91	0
Ascaris lumbricoides	18.70	36.51	11.96	21.82	0
Total	**182**	**63**	**92**	**110**	**0**

Table 6: Distribution of the Parasites

Parasite	Eguare	Ukpogo	Idumogho	Okpujie	Control
Balantidium coli	+	-	+	+	-
Cyclospora cayetanensis	+	+	-	-	-
Entamoeba histolytica	+	-	+	+	-
Schistosoma haematobium	-	+	+	+	-
Schistosoma mansoni	+	+	-	-	-
Faxciola hepatica	-	+	+	-	-
Taenia solium	+	-	+	+	-
Ascaris lumbricoides	+	+	+	+	-

Legend: + = Organism Present. - = Organism Absent

DISCUSSION

The physical characteristics as observed in the sewage samples showed that they were contaminated and not fit for disposal without proper purification. The sewage samples were acidic as observed in the pH range reported from all studied sites in this study. This range was lower than the ranges (7.38-781) and (8.94 -10.34) reported by Das and Acharya (2003) and Akan *et al*. (2008) respectively. This acidic pH range is known to influence the availability of micro-nutrients, trace metals and high number of fungi which are more able to tolerate acidic environment than bacteria (Kirkham, 2006). The electrical conductivity recorded in this study fell within the range, 850 - 1524 µS/cm reported by Endamana *et al*. (2003) as well as 760 µS/cm reported by Katoria *et al.* (2013) on raw sewage samples from sewers except at Idumogho which recorded 589±0.21 µS/cm. The electrical conductivity being a measure of dissolved salts in solution implies that the sewage played host to lots of dissolved solids. The high concentration of the Biochemical oxygen demand resulting from the easily decomposable solid organic component may have accounted for the very low dissolved oxygen value recorded particularly at Ukpogo and Idumogho. The results from this study showed that most concentrations of the physicochemical parameters were low except for pH, nitrate, phosphate, chemical oxygen demand and Biochemical oxygen demand which were higher than FEPA (1991) recommended limit and the control.The study revealed the presence of Sodium, Potassium and Magnesium whose concentrations were higher than that of Calcium concentration. This report agreed with the findings of Enerijiofi *et al.* (2017) that the excess of one macronutrient leads to the deficiency of another.

Generally, the levels of the heavy metals concentrations recorded were above the FEPA (1991) standard for effluent discharge and also the control. This implied that the sewage had heavy metals enrichments. Iron and Zinc recorded the highest concentration of all heavy metals recorded at all sites however, Mgbemena *et al*. (2012) reported that they are essential for the growth of microorganisms by acting as catalysts in enzymatic reactions, hence zinc and iron are less toxic to the microorganisms compared to other heavy metals.

The presence of lead could have resulted from either incomplete combustion from cooking stoves, fire woods and or generating sets used by individuals in the studied area. Momodu and Anyakora (2010) reported that lead poisoning could cause severe kidney pain, loss of appetite, insomnia constipation and brain damage resulting from prolong exposure. It is much more worrisome that these heavy metals can substitute for calcium in bones causing skeletal anomalies especially in children (Enerijiofi and Ajuzie, 2012).

This sewage samples had higher microbial loads at all sites with Eguare and Okpujie communities leading in this trend. Also, Sewage from Eguare was the most contaminated because it had the highest microbial load compared to sewage samples from other locations. This could be due to the increased population that may have resulted in higher volume of sewage been disposed into the sewers and also because sewer from this location is the oldest of all studied sewers. This may be attributed to high quantity of organic matter available in sewers at Eguare and Okpujie. The bacterial and fungal reported in this study are in consonance with those of Odjadjare *et al.* (2011) where they identified similar microorganisms. These microorganisms especially *Pseudomonas aeruginosa* is known to cause several debilitating diseases especially in immunocompronised individuals while *Aspergillus* species*.* is known for Aflatoxin production that destroys the liver by inducing fatty acid metamorphosis of its cells (Enerijiofi and Ajuzie, 2012). *Bacillus* species are known versatile aerobic spore formers, capable of respiration using a variety of simple organic compounds (sugars, amino acids, organic acids). *Bacillus* species such as *Bacillus cereus* is a pathogen of humans and other animals causing food borne illness such as diarrhoeal-type and emetic-type syndromes as well as opportunistic infections (Imarhiagbe and Obayagbona, 2018). The parasites identified in sewage samples from this study are of public health concern. It should be noted the parasites were completely absent in the control sample. *Ascaris lumbricoides* are intestinal parasites transmitted through contaminated food and water. Almost all microbes isolated in this study are capable of causing diseases such as urinary tract infections, diarrhoea, gastrointestinal diseases and respiratory tract infections (Enerijiofi and Ajuzie, 2012). The presence of these microorganisms isolated from sewage samples in this study has revealed that sewage consists of opportunistic microbial species which are important to human and public health. Individuals who discriminately or indiscriminately come in contact with raw sewage stand the risk of contracting opportunistic infections and diseases.

CONCLUSION

The study showed that most of the concentrations recorded for some physiochemical parameters especially nickel, cadmium, chromium, lead and arsenic were above the permissible limits recommended by Federal Environmental Protection Agency. Also, the microorganisms identified are capable of leading to disease outbreak. These facts stated above possess serious threat to public health.

Recommendation

There is need for construction of proper sewage tanks to prevent indiscriminate disposal. Also, the Ministries of Environment and Health should wake up to their responsibilities of monitoring and enforcing sanitation regulations so as to minimize health and environmental risks associated with discharge of untreated sewage into the receiving environment.

Contributions of Authors

This work was carried out in collaboration between all Authors. Enerijiofi, K. E. and Fakeye, O. D. designed and managed the analyses of the study. Oziegbe, O. S. did the laboratory analyses. Enerijiofi, K. E. wrote the first draft of the manuscript. All authors managed the literature searches, read and approved the final manuscript.

Conflict of Interest

There are no conflict of interests arising from this work.

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