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Preliminary evaluation of wastewater effluents from two food companies in Nigeria

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Effluent samples from two top Nigerian food and beverage industries, swords food industry and 7-Up Bottling Company, Ibadan, Oyo State, Nigeria were investigated for microbial loads, physiochemical properties and presence of heavy metals. While repeated sampling revealed two bacteria and a fungus from the effluents of 7-Up bottling company, 15 bacteria and 5 fungi were isolated from Swords foods Industry. The biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were equally higher in the effluent of sword food industry than that of 7-Up bottling company. Heavy metals analyses revealed Lead (4.83 and 6.75 mgl^{-1}) Copper (3.80 and 3.93 mgl^{-1}), Iron (3.10 and 3.45 mgl^{-1}), Cadmium (7.20 and 8.10 mgl^{-1}) and magnesium (5.68 and 9.38 mgl^{-1}) in sword food industry while Lead (0.12 and 0.14 mgl^{-1}), copper (1.20 and 1.22 mgl^{-1}), iron (1.60 and 1.63 mgl^{-1}), cadmium (0.10 and 0.09 mgl^{-1}) and magnesium (1.10 and 1.20 mgl^{-1}) were the concentrations in 7-Up bottling company. The conclusion was that, there is a high probability of polluting the environment by sword food industry as a result of discharge of untreated wastewater into the water body or soil that may lead to death of crops or reduction in crops yield, contamination of drinking water supplies and/or accumulation and dissemination of toxic chemicals that may further endanger ecosystems and threaten public health.

Key words: Microbial loads, heavy metals, physicochemical properties.

INTRODUCTION AND LITERATURE REVIEW

Wastewater contains offensive and potentially dangerous substances which cause pollution and contamination of receiving water bodies (Shaw and Schrudam, 2000). One of the most important factors of water pollution is the microbial contamination, especially with pathogenic microorganisms. Enteric pathogens are typically responsible for several waterborne sicknesses (Nieiwlak, 1998; Sabae, 2004). Contamination of water is a serious environmental problem as it adversely affects the human health and the bio-diversity in the aquatic ecosystem. The use of indicator bacteria such as feacal coliforms (FC) in water quality determination on fresh water source is widely used (You-Joe et al., 2003). Currently, coliforms and *Escherichia coli* are of great importance among bacterial indicators used in water quality definition and health risk (Giannoulis et al., 2005). However, operational evaluation of microbial load of waste water (biologically) is often complicated because of variation in raw waste water composition, strength and flow rate owing to the changing and complex nature of the treatment processes (Akarinwor and Gwin, 2006). Moreover, a lack of suitable processing variable limits the effective control of effluent quality (Agedengbe et al., 2003). Pathogens are a serious concern for managers of waste water because excessive amount of faecal bacteria in sewage and urban run-off have been known to indicate risk of pathogen-induced illnesses in humans (Fleisher et al., 1998). Thus, identification of these pathogenic agents in water resources is beneficial for controlling and preventing planning of the infectious diseases. In recent years, the efficacy of industrial wastewater evaluation has focused on new technology rather than conventional method such as ion-exchange, chemical precipitation and solvent extraction amongst others which are prohibitively expensive and inefficient, thus, the need for the use of potential microorganism in their treatment or in some cases, recycling is done (Technologous et al., 2003). The

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average daily amount of waste in the sewage produced by individual industry is customarily expressed by a related Biochemical Oxygen Demand (BOD) (Yang, 1996) . Effluents generally have an adverse effect on water bodies such as lakes, rivers, oceans and groundwater, and this is caused by human activities (Abel, 1996). Pollutants in water include a wide spectrum of chemicals, pathogens and physical chemistry or sensory changes. Alterations of water's physical chemistry include acidity, conductivity, temperature and eutrophication (Abel, 1996; Nascimento et al., 2000; Sabae, 2004).

Alterations are often caused by changes in characteristic and operation conditions (David, 2002). Many problems found in wastewater treatment that perform biological removal of pollutants are due to alteration in the microbial communities involved. Plate-counting and most probable number (MPN) techniques have been used for the study of microbial communities in mixed culture systems. However, less than 1% of microorganism in the environment can be cultivated by standard techniques because culture techniques fail to reproduce in artificial media, the niche of many microorganisms found in high diversity environment such as activated sludges (David, 2002). The use of heat activated or dead biomass in industrial application may offer some advantages over living cells such as less sensitivity to heavy metals together with excellent mechanical property (Sabae et al., 2006). Sword Food Industry produces foods like sausage roll, spicy meat, meat and fish pies and so many appetizers, while 7-Up Bottling Company is widely known for bottling and marketing of soft drinks such as 7-Up, Pepsi, Mirinda, Mountain Dew etc. The objective of this study is to comparatively analyze the microbial loads and heavy metal concentrations from the effluents of a food and a beverage company in Nigeria, so as to determine the degree of compliance of these industries to environmental laws.

MATERIALS AND METHOD

Samples collection and preparation

Composite wastewater effluent samples (24-h) were collected weekly from two food industries: 7-Up Bottling Company and Sword Food Industry, for a period of six months. Biological and chemical assessments were carried out via microbial analysis, chemical and physical properties in the months of January, 2007 to July, 2007. Sterile containers were used to collect four samples each from the industries at four different points tagged A, B, C and D. Point A was taken to be the immediate point where the finally treated wastewater mix with the receiving water body while point D was several meters away from point A. However, two points equidistant away from points A and D were designated points C and D. The same method of collection of samples was applied in Sword Food Industry designated E, F, G and H. Points F and G were equidistant away from E and H. These samples were transported immediately to the laboratory for analyses.

Microbial analysis

Serial dilutions were carried out on each of the samples and were cultured using the spread plate method. Nutrient Agar was inoculated with 10^{-4} or more dilution of the sample(s) for bacteria and Potato Dextrose Agar was inoculated with 10^{-2} or more dilution of the sample(s) for fungi. The NA plates were incubated at 35°C for 24 h and PDA plates were incubated at 25°C for 48 - 72 h. For each sample cultured, observation was made on at least one plate of the series whose bacteria or fungi numbers were sufficiently low which allowed the development of well separated colonies. The colonies were sub- cultured until pure cultures of the isolates were obtained and were identified according to Cowan and Steel (1985). These were then stocked for further biochemical analysis. MacConkey broth was used for the determination of coliform counts.

Biochemical characterization

The bacterial isolates were subjected to Gram Staining as described by (Holdeang and Collee, 1997). After staining, various biochemical tests were carried out on the bacterial isolates for possible identification (Holdeang and Collee, 1997).

Determination of pH

The pH of the collected samples was determined using the methods of Hewitt (2001).

Total solids and moisture determination

2 g of the sample was weighed in a previously weighed crucible. The crucible plus sample was then transported into the oven set at 100°C to dry to a constant weight for 24 h overnight, and this was later transferred to the dessicator, cooled for ten minutes and weighed (Hewitt, 2001).

Measurement of turbidity

The instrument, Spectrophotometer was used to determine the turbidity of the effluent samples. The entire visible spectrum (white light) was used, and consequently, the complementary colour of the one absorbed was observed as transmitted light (U.S. Clean water Act, 1992).

Chloride content

50 ml of the sample was pipetted into a 25 ml volumetric flask and 1 ml of KCr_2O_4 was added to it as a reagent; titrated with 0.1 M of $AgNO_3$ in the burette with the addition of 1ml of KCr_2O_4, the solution turned brick red (Fergusson, 2000).

Measurement of total titrable acidity (TTA)

Total titratable acidity (expressed as lactic acid) was determined for each sample collected aseptically by titrating 20 ml of the decanted much homogenized sample against 0.1 M NaOH to a pH of 6.8 using phenolphthalein as an indicator. The titre volume for each homogenates was multiplied by 0.09 to give the percentage TTA as lactic acid (Manly, 2000).

Table 1. The bacterial and fungal isolates and their level of occurrence at various sampling points from 7-Up Bottling Company.

Bacterial and fungal isolates	Sampling points and level of occurrence[a] (cfu/ml)			
	Point A	Point B	Point C	Point D
B. cereus	0.01×10^{2}	ND	ND	ND
S. faecium	ND	0.02×10^{2}	ND	ND
Saccharomyces spp.	0.02×10^{2}	ND	ND	ND

*ND: Not detected; a: mean reading. This shows two bacterial isolates and one fungal isolate.

Biochemical oxygen demand

The organic matter in water was determined in terms of the oxygen required to oxidize it by treatment with potassium permanganate. In contact with oxidizable organic matter, potassium permanganate readily gave up its oxygen. The iodine formed dissolved in excess of potassium iodide and was estimated by titration with sodium thiosulphate using starch as an indicator (Geely and van Demark, 1992; Volesky and Holaz, 2005).

Chemical oxygen demand

A predetermined amount of the reference substance dispersed in water was oxidized by potassium dichromate in a strong sulphuric acid medium with silver sulphate as a catalyst, under reflux for two hours. The residual dichromate was determined by titration with standardized ferrous ammonium sulphate. In case of chlorine-containing substances, mercuric sulphate was added to reduce chloride interference (Gerike, 1984; Volesky and Holaz, 2005).

RESULTS

The results of this study are illustrated in Tables 1 - 6.

DISCUSSION AND CONCLUSION

7-Up Bottling Company's wastewater did not show much microorganisms; only two microorganisms were isolated from Point A (*Bacillus cereus* and *Saccharomyces spp*) while just one was isolated from Point B (*Streptococcus faecium*) (Table 1). However, none was isolated from points C and D (Table 1). From the results, it is evident that the effluent of Sword Food Industry was not effectively treated, as series of microorganisms were isolated from the four points of samples collection (Table 2). Sword Food Industry employed one of the secondary wastewater treatments known as the oxidation pond while 7-Up Bottling Company combines both secondary and advanced wastewater treatments. Also, no coliform and *Staphylococcus aureus* counts were detected from the samples obtained from 7-Up bottling company (Table 3). This indicated that the quality control units of the industry were effective. *Bacillus spp* detected in this study may have contributory effect as it may be employed in the bioremoval of heavy metals from tropical aquatic environments impacted with heavy metals (Ponamareva, 1994; Odokuna, 2003). Nevertheless, significant progress should be made in upgrading wastewater treatment processes in this industry as the *Bacillus cereus* isolated from point A can cause two distinct types of illnesses (an emetic and diarrheal types) depending on the types of toxin produced (Prescott et al., 2008). Accumulation of industrial food processing effluent from Sword Food samples (codes E, F, G and H) supported the growth of bacteria but randomly decreased the fungal counts (Table 4). The total viable and coliform counts increased. Each point harbored one or more of *Bacillus* spp and *Pseudomonas* spp*,* which are putrefying bacteria.

Numbers of heavy metals was high in all the points because the industrial food waste is organic in nature (Table 5). Lead (Pb) is known to produce neurotoxicity and it has been shown that infant and children may be differentially sensitive to environmental Pb exposure (Zaydab, 1996; Odokuna, 2003). Exposure during development can result in a spectrum of defects including structural abnormalities, altered growth and functional defects, sexual immaturity and death (Needham, 2001; Shaw and Schrudam, 2000). People exposed to toxic chemicals especially Pb manifest similar symptoms of neurotoxicity (Odokuna, 2003). If food crops are planted near a river contaminated with heavy metals, it is possible for the metal contaminants to be transferred to the food crops (WHO, 2001; Sabae et al., 2006). Hg, Zn and Pb have posed a high level of threat to Public health (Needham, 2001; Sabae et al., 2006). Organic pollutant can be removed by activated carbon filters (Yang, 1996; Prescott et al., 2008; USEPA, 2006).

The conclusion of this study is that, the level of occurrence of bacteria and fungi at various sampling points from Sword Food Industry is higher than that of 7-Up Bottling Company which is at its minimal. The nutrients produced from the inadequately treated waste water have been found to lead to greater dissemination of pathogenic microorganisms, increased danger in using natural bodies of water for drinking supplies, contamination of oysters and other shellfish by the pollution, making them unsafe for human consumption, depletion of oxygen supply of the water by unstable organic matter in sewage, killing aquatic life and accumulation and dissemination of toxic chemicals that endanger ecosystems and threaten public health (Pelczar et al., 2002; Sabae, 2004). Despite the provisions for

Table 2. The bacterial and fungal isolates and their level of occurrence at various sampling points from sword food industry.

Bacterial and fungal isolates	Sampling points and level of occurrence[a] (cfu/ml)			
	Point E	Point F	Point G	Point H
Aerobacter aerogenes	0.24×10^5	0.94×10^5	2.62×10^5	2.99×10^5
B. cereus	0.18×10^5	ND	ND	3.67×10^5
Bacillus macerans	ND	ND	2.67×10^5	ND
E. coli	1.20×10^5	ND	0.27×10^5	0.32×10^5
Klebsiella aerogenes	1.87×10^5	ND	ND	ND
Micrococcus acidophilus	ND	0.27×10^5	ND	ND
Proteus mirabilis	ND	ND	1.74×10^5	2.20×10^5
Proteus morganii	ND	0.49×10^5	ND	ND
Proteus vulgaris	ND	1.34×10^5	ND	ND
Pseudomonas fluorescence	2.36×10^5	2.01×10^5	0.22×10^5	ND
Pseudomonas spp	ND	0.23×10^5	ND	0.39×10^5
Serratia marcencens	ND	ND	0.26×10^5	ND
S. aureus	0.75×10^8	1.40×10^8	1.08×10^8	6.80×10^8
Streptococcus faecalis	ND	ND	0.42×10^5	ND
S. faecium	2.76×10^5	2.01×10^5	ND	1.25×10^5
Aspergillus niger	1.47×10^5	1.23×10^5	1.47×10^5	1.32×10^5
Fusarium oxysporum	ND	ND	0.67×10^5	ND
Penicillium chrysogenum	0.67×10^5	0.55×10^5	ND	ND
Penicillum oxalicum	ND	0.46×10^5	0.37×10^5	0.33×10^5
Saccharomyces spp	1.44×10^5	1.24×10^5	1.30×10^5	0.57

*ND: Not detected; a: mean reading. This shows a total number of 15 bacterial isolates and 5 fungal isolates in the sample but most predominantly are *S. aureus, Aerobacter aerogenes* and *Aspergillus niger* at every point of collection.

Table 3. Results of microbial analysis of samples obtained from 7-Up Bottling Company.

Sample code	Total viable count cfumm^{-1}	Total coliform count cfumm^{-1}	*S. aureus* count × 10^6	Total fungal count
A	01	-	-	02
B	02	-	-	-
C	-	-	-	-
D	-	-	-	10

-: No detection.

Table 4. Results of microbial analysis of samples obtained from sword food industry.

Sample code	Total viable count cfumm^{-1}	Total coliform count cfumm^{-1}	*S. aureus* count × 10^6	Total fungal count
E	100	175	75	14
F	120	220	140	13
G	160	300	108	15
H	140	270	68	12

effective measure of waste water treatment by modern technology, many industries and municipalities do not employ adequate treatment procedures, and even worse, some perform no treatment, they dump raw waste water into the waterways.

Recommending that regulatory bodies be put in place, guiding the waste disposal in order to prevent health dangers for both aquatic and plant lives while preventing health dangers for human lives as well. Regulation and standard must be identified; this can help in eradicating environmental pollution, and hence, terminate the hazards caused so far by its effects. There is more need

Table 5. Physiochemical analysis of samples.

S/N	Sample code	pH	Turbidity	Odour	TTA	Alkalinity	B_2CO_3	Ts	Tss	Tds	Cl	Pb
1	7 up Pt. A	6.50	1.2	Not offensive	0.180	1552.30	3104.6	0.213	0.20	1.204	355	0.12
2	7 up Pt. B	6.30	1.20	Not offensive	0.010	1480.10	2960.2	1.279	0.90	1.238	365	0.14
3	Sword Pt. A	5.75	4.75	Not offensive	0.026	4368.10	8736.2	24.724	6.30	12.740	1171.5	4.83
4	Sword pt. B	5.60	5.68	Not offensive	0.019	4801.30	9602.6	26.912	5.20	14.830	1084.40	6.75

Table 5. Continued.

Cd	Cu	Na	Ca	K	PO_4	Mg	SO_4 mgl^{-1}	OC%	%OM	TN%	NL MgL^{-1}
0.10	1.20	230	75	350	9.75	1.10	7.10	1.24	2.14	0.13	3.40
0.09	1.22	245	83	360	10.24	1.20	6.40	1.37	2.36	0.15	3.70
7.20	3.80	1280	750	575	15.75	9.38	24.10	8.50	14.65	1.30	5.60
8.10	3.93	1475	814	583	17.40	5.68	28.30	9.10	15.69	1.57	6.80

for conservation together with more efficient wastewater treatment processes so that the cycle between 'used' water and its reuse can be shortened. It would be disastrous to nature, as well as to mankind if this thoughtless interference with water is continued.

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