Science World Journal Vol 5 (No 3) 2010
www.scienceworldjournal.org
ISSN 1597-6343

MICROBIOLOGICAL AND PHYSICO-CHEMICAL ASSESSMENT OF SOIL CONTAMINATED WITH ABATTOIR EFFLUENTS IN SOKOTO METROPOLIS, NIGERIA.

*RABAH, A. B[1].; OYELEKE, S. B. [2]; MANGA, S. B. [1]; HASSAN, L. G. [3]; & IJAH, U. J. J. [2]

[1]Department of Microbiology, Usmanu Danfodiyo University, Sokoto.
[2]Department of Microbiology, Federal University of Technology, Minna.
[3]Department of Chemistry, Usmanu Danfodiyo University, Sokoto.
*abrabah2009@gmail.com

ABSTRACT
The microbial population in soil contaminated with abattoir effluent was investigated using the spread plate method. The results revealed a high mean count of $3.70\pm0.01\times10^6$ cfu/g and $1.40\pm0.04\times10^4$ cfu/g for bacteria and fungi respectively. The counts were higher than that of the control soil sample with $2.40\pm0.02\times10^4$ cfu/g and $1.10\pm0.02\times10^3$ cfu/g for the bacteria and fungi respectively. Among the bacteria isolated, *Escherichia coli* had the highest frequency of occurrence of 21.0 %, followed by *Bacillus subtilis* and *Staphylococcus aureus* with 17.0 % each and *Bacillus anthracis* with 13.0 %. The lowest frequency of occurrence of 4 % was observed in *Bacillus polymyxa* and *Staphylococcus epidermidis* respectively. In terms of fungal isolates, *Aspergillus niger* had the highest frequency of occurrence of 27 %, followed by *Fusarium sporotrichioides* (22 %) while *Absidia* sp and *Mucor pusillus* with 6 % each. Results of the physico-chemical analysis showed that the contaminated soil samples had mean pH of 7.36, mean temperature of 33 ^oC, Cation Exchange Capacity (CEC) of 18.53 % and nitrogen concentration of 0.23 mg/g.

Keywords: Microbiological, Physico-chemical, Soil, Abattoir, Effluents, Sokoto

INTRODUCTION

The continuous drive to increase meat production to meet the protein needs of the population is usually associated with some pollution problems (Hinton *et al.,* 2000). The meat industry uses large quantity of wastewater that drains into surrounding soil environments (Amisu *et al.,* 2003). One of the effects of waste water source draining into the soil is making the soil oxygen to become less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate to gaseous nitrogen which enters the atmosphere with resultant negative effects. Also, the anaerobic archae (methanogens) may produce excessive methane at a higher rate than aerobic methane oxidizing bacteria (methanotrophs) could cope with, thus contributing to greenhouse effect and global warming. Similarly, the physicochemical properties of the soil may become altered, such as the pH, due to the uncontrolled discharge of untreated abattoir wastewater resulting in the loss of certain soil microbes (Edward, 1990).

Tortora *et al.*, (2007) reported that following the discharge of untreated wastewater into the soil, certain elements (for example, iron, lead, phosphorus, calcium, and zinc) previously absent or present in minute quantities will be introduce into the leading to the magnification of these chemicals and thus altering the physicochemical nature of the soil. Some of these chemicals may be toxic to the microbial, floral and faunal communities of the soil.

Some of the wastewater from Sokoto abattoir drains into the surrounding soil environment while the remaining is channeled through the abattoir drainages into River Rima. The resultant consequences could be the degradation of soil fertility due to the accumulation of certain nutrients and heavy metals that may lead to low productivity in the surrounding farmlands, in addition to damages and destructions of aquatic lives. Since the water from both the River as well as the soil are used for irrigation farming along the River banks, the possibility of zoonotic diseases amongst the consumers of produce from such irrigated fields cannot be ruled out. Therefore, the objective of this study is to investigate the diversity of microorganisms and the physico-chemical qualities of the soil surrounding the Sokoto abattoir contaminated with the discharged abattoir effluents.

MATERIALS AND METHODS

Study area: The study was conducted at the Microbiology laboratory, Faculty of Science, Usmanu Danfodiyo University, Sokoto, Nigeria. Sokoto town has a population of 669,413 people as projected in the 1991 National population census with a land mass of 364,122 km^2. It is situated between latitude of $12^o0'$ and $13^o58'$ N and longitude $4^o8'$ and $6^o54'$ E and lies 350 m above sea level. The area is within the Sudan-savanna agro-ecological zone of Nigeria whose climate is characterized by a long dry season (November to February) and and a short rainy period (June to September) (Mamman *et al*., 2000).

Sample Collection: This was carried out according to the methods described by Adesemoye *et al*., (2006). Twenty grammes (20 g) of soil contaminated with abattoir wastewater was collected from the Sokoto abattoir near Kara market three times in February, 2009. The samples were placed in sterile polythene bags and transported to the laboratory for processing. The samples used as control were collected from an area devoid of butchering activities.

Microbiological analysis of the soil samples: The microbiological analysis of the soil samples were carried out according to the methods of Oyeleke & Manga (2008a) and Rabah *et al*., (2008). The bacterial isolates were identified and characterized using standard biochemical tests (Cheesebrough, 2006). The tests employed include colonial, morphological characteristics, gram stain, motility, catalase, methyl red, Voges-Proskaeur, indole production, urease activity, H_2S and gas production, citrate utilization, glucose, sucrose, and lactose utilization tests. The fungal isolates were identified according to Oyeleke & Okusanmi (2008b) based on the colour of aerial hyphae and substrate mycelium, arrangement of hyphae, conidial arrangement as well as morphology.

Physico-chemical analysis of the soil samples: The physico-chemical qualities of the soil samples were determined using the methods of Udo & Ogunwale (1986) and the Association of Official Analytical Chemists (AOAC, 1990). The parameters determined were:

pH: This was determined using pH meter 3015 (Jenway, U. K.) Ten grams of the soil sample was placed in a beaker, then 10 ml of distilled water was added and the mixture was stirred. It was allowed to stand for 30 minutes. A 0.1 M phosphate buffer solution was used to standardize the pH meter. Then the electrode of the pH meter was inserted into the mixture and the pH readings were taken.

Temperature: This was determined at the point of sample collection by dipping the bulb of mercury-in-glass thermometer into the soil suspension and recording the readings.

Organic matter: Five grams of the sample was placed in a porcelain crucible and weighed. The crucible was heated in a muffle furnace at 400 ^{o}C for 3 hours until the sample became light tan. The sample was cooled and reweighed to determine the loss in weight. The organic matter was calculated using the formula:

$$\% \text{ Organic matter} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

Phosphorus: This was determined using Vanado-molybdo-phosphoric acid colometric method using ammonium molybdate which formed molybdo-phoshoric acid under acidic condition. The intensity of the yellow colour was measured using spectrophotometer at 490 nm.

Sulphide: This was done by adding 2 cm^3 of concentrated HCl to 100 cm^3 of the sample and the mixture heated to dryness. The residue was dissolved into 5 cm^3 of concentrated HCl and the insoluble portion was filtered off and washed with hot distilled water. It was further diluted to 100 cm^3 and adjusted to pH 4.5 using acetate buffer. The mixture was again heated to boiling until the precipitation was completed. The precipitate was digested at 80 ^{o}C for 3 hours, filtered, dried and weighed to constant weight in a pre-weight evaporating dish. Finally the value of sulphide was calculated.

Magnesium using Atomic absorption spectrophotometry: These were determined by preparing various dilutions from 1000 ppm of stock solutions of magnesium and chromium (1000 ppm). The dilutions were used for the preparation of standard calibration solutions. Then 100 cm^3 of the samples were digested with concentrated HCl and HNO_3 in a ratio of 3:1, filtered and diluted to 250 cm^3 with distilled water. A blank solution was prepared by treating 100 cm^3 of distilled water in the same manner. The elements magnesium and chromium were determined by aspirating the standard solutions, samples and blank at 285.2 nm and 425.4 nm respectively.

Potassium and calcium by Flame emission spectrophotometry: These were determined by preparing various dilutions from 1000 ppm of stock solutions of potassium and calcium. The diulutions were used for the preparation of the calibration standards. Then the standard solutions, samples and blank were aspirated using a flame photometer with the filters of potassium and calcium.

Cation exchange capacity: Five grams of the soil sample was placed in a beaker containing 25 ml of distilled water and allowed to stay overnight. It was then filtered with No 1 whatmann filter paper. The filterate was placed in a Kjeldahl flask and 20 ml of distilled water and 20 ml of 20% NaOH were added. To the mixture, 20 ml of boric acid indicator was placed into a seperate beaker and used to collect the ammonium gas. The solution was titrated with Hydrochloric acid and the titre values were recorded and used in the determination of % CEC.

Data analysis: The data generated was subjected to students' paired t-test to established significant difference at 5 % confidence limit using SPSS (version 14.0) statistical package.

RESULTS

The total mean viable counts for bacterial and fungal isolates are presented in Table 1.

TABLE 1. TOTAL VIABLE COUNTS* OF BACTERIA AND FUNGI ISOLATED FROM SOIL CONTAMINATED WITH ABATTOIR WASTEWATER AND UNCONTAMINATED SOIL (CONTROL).

Organisms	Soil	
	Contaminated cfu/g	Uncontaminated cfu/g
Bacteria	$3.70\pm0.01\times10^6$	$2.40\pm0.02\times10^4$
Fungi	$1.40\pm0.04\times10^4$	$1.10\pm0.02\times10^3$

*****Counts represnt means of triplicate samples,
cfu/g: coliform forming units per gramme

The results of the percentage frequency of occurrence of the microbial isolates were presented in Table 2. From the results *Escherichia coli* (21 %) had the highest frequency of occurrence. This was followed by *Bacillus subtilis* and *Staphylococcus aureus* (17 % each) and *Bacillus anthracis* (13 %). The lowest frequency of occurrence of 4 % was observed in *Bacillus polymyxa* and *Staphylococcus epidermis* respectively. In terms of fungal isolates, *Aspergillus niger* showed the highest percentage occurrence of 27 %. This was followed by *Fusarium sporotrichioides* (22 %) while *Absidia* sp and *Mucor pusillus* showed the lowest occurrence of 6 % each.

TABLE 2. FREQUENCY OF OCCURRENCE OF MICROORGANISMS ISOLATED FROM SOIL CONTAMINATED WITH ABATTOIR WASTEWATER

Isolates	Frequency of occurrence (%)
a) Bacteria	
Pseudomonas aeruginosa	8
Bacillus anthracis	13
Staphylococcus epidermidis	4
Bacillus polymyxa	4
Bacillus subtilis	17
Klebsiella pneumoniae	8
Escherichia coli	21
Streptococcus faecalis	8
Staphylococcus aureus	17
b) Fungi	
Aspergillus niger	27
Absidia sp	6
Penicillium echinulatum	11
Mucor pusillus	6
Aspergillus fumigates	11
Fusarium sporotrichiodes	22
Aspergillus flavus	17

The result of the physico-chemical qualities of the contaminated soil is presented in Table 3. The mean temperature value was 33 ^{o}C and the pH 7.36. The organic matter content as well as the cation exchange capacity were 12 % and 18.53 % respectively while the total nitrogen 0.42 %. The result revealed a concentration of 2.67 mg/g, 5.60 mg/g, 1960 ppm and 76 ppm for magnesium, phosphorus, potassium and calcium respectively.

DISCUSSION

The high counts of both bacteria and fungi obtained indicated that the contaminated soil had a high population density than the control soil. Also, it revealed a significant difference ($p \square 0.05$) between the counts in the contaminated soil compared to the uncontaminated soil.

TABLE 3. PHYSICOCHEMICAL PARAMETERS OF SOIL CONTAMINATED WITH ABATTOIR EFFLUENTS AND UNCONTAMINATED SOIL (CONTROL).

Parameters	Contaminated soil	Uncontaminated soil	*FMEnv limit
pH	7.36	8.20	6.00-9.00
Temperature (°C)	33.00	34.00	40.00
Nitrogen (mg/g)	0.23	0.42	NA
Magnesium(mg/g)	2.67	2.30	200.00
Phosphorus(mg/g)	5.60	5.20	5.00
Potassium (ppm)	1960	280	NA
Calcium (ppm)	76	63	200.00
Sulphide (mg/g)	-	-	0.20
Organic matter(%)	12	3.25	NA
CEC (%)	18.53	13.20	NA

KEY: CEC- Cation exchange capacity; °C: degree celcius; mg/g: milligrammes/grammes; ppm: parts per millions; %: percent; FMEnv: Federal Ministry of Environment; NA: not available; *FEPA (1991).

This was because the effluents may contain many growth factors that could be easily utilized by the organisms which are not available in the uncontaminated soil. Also, it may be attributable to the destabilization of the soil ecological balance as a result of the contamination due to the discharged of the abattoir wastewater into the soil ecosystem. This result was in conformity with that of Adesemoye *et al.,* (2006) who reported similar high counts of 3.36×10^7 cfu/g of microorganisms from soil samples contaminated with wastewater at Agege and Odo in Lagos, Nigeria abattoirs.

The presence and abundance of various species of *Bacillus* observed in the contaminated soil may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment (Atlas & Bartha, 2007). However, the presence of *E. coli* and *Streptococcus faecalis* in the contaminated soil may be attributable to the high load of animal excreta in the wastewater. It is also an indication of recent faecal pollution. Similar findings were reported (Ezeronye & Ubalua, 2005) from Aba River as a result of contamination with abattoir effluents where the researchers reported the isolation of *Streptococcus faecalis, Escherichia coli, Staphylococcus* sp, *Clostridium* sp, and *Salmonella* sp among other organisms. Bala (2006) reported the isolation of similar organisms from water sources in Jimeta-Yola that were faecally contaminated. Most of the fungal isolates were also soil-inhabiting microorganisms (Atlas & Bartha, 2007) as well as common spoilage organisms associated with beef industry (Alonge, 1991). The presence of these organisms is a pointer to possible pollution and may have an effect on the soil ecological balance. These findings were in conformity to that of Adesemoye *et al.,* (2006) as well as Ogbonna & Igbenijie (2006).

The temperature value obtained indicated that there was no great temperature fluctuation within the analyzed soils while the pH was near neutral. These two factors may play a role in determining both the qualitative and quantitative abundance of microorganisms in the contaminated soil. Although, there was no significant difference ($p>0.05$) in the values obtained for temperature, nitrogen, magnesium and phosphorus in the contaminated soil and the control, a significant difference ($p<0.05$) existed in the values obtained for organic matter, cation exchange capacity, potassium and calcium for the values obtained for the contaminated soil to that of the uncontaminated soil. Despite the fact that these values were higher than that of the control sample, they are below the limit set by the Federal Ministry of Environment. Similar low values of these chemicals were observed by Yusuff & Sonibare (2004) in tannery effluents. The present results revealed that in both the contaminated as well as the uncontaminated soils, sulphide was not detected. This may not be surprising as abattoir do not make use of sulphur containing chemicals in the handling and processing of meat. Also, the area where the uncontaminated soil was obtained is a place devoid of any tanning and mining activities and this may account for the non-detection of sulphide in such soils.

The result revealed a high counts and varieties of microorganisms most of which are pathogenic in the soil contaminated with the abattoir effluents. Also, it revealed a significant difference between the counts in the contaminated soil and the uncontaminated soil. The contaminated soil contained a number of chemicals which although in small quantities points to high microbial activities in such soil. Therefore, it is highly recommended that the abattoir effluents need to be treated before being discharged into the surrounding environments.

REFERENCES

Adesemoye, A. O.; Opere, B. O. & Makinde, S. C. O. (2006). Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria. *African Journal of Biotechnology* 5(20):1963-1968.

Alonge, D. O. (1991). *Textbook of Meat Hygiene in the Tropics.* Farm Coe Press, Ibadan, Nigeria.

Amisu, K.O.; Coker A.O. & Isokpehi, R. D. (2003). *Arcobacter butzlieri* strains from poultry abattoir effluent in Nigeria. *East African medical Journal* (80):218-220.

Association of Official Analytical Chemists (AOAC) (1990). Methods of Analysis, 12th Edition, AOAC, Washington D. C., USA.

Atlas, R. M., & Bartha, R. (2007). *Microbial Ecology: Fundamentals and Applications*, Benjamin/Cummings Publishing Company Inc, India.

Bala, J. D. (2006). Occurrence of faecal coliforms in water sources in Jimeta-Yola, Nigeria. *Journal of Environmental Sciences*, 10(2):64-69

Cheesebrough, M. (2006). *District laboratory practice in tropical countries.* Part 2. Low Price Edition. Cambridge University Press, London.

Edward, C. (1990). *Microbiology of Extreme Environments*, 2nd edition. Open University Press, Milton Keynes.

Ezeronye, O. U. & Ubalua, A. O. (2005). Studies in the effect of abattoir and industrial effluents on the heavy metals and microbial quality of Aba River in Nigeria. *African Journal of Biotechnology*, 4 (3):266-272

FEPA (Federal Environmental Protection Agency) (1991), Guidelines to standards for Environmental Pollution Control in Nigeria, Lagos, Nigeria.

Hinton, M. H.; Mead, G. C. & Rowlings, C. (2000). *Microbiology Control in meat industry*. Flair Flow Europe Technical Manual. F-Fe 339A/00 May 2000. (www.exp.ie/flair.html).

Mamman, A. B.; Oyebanje, J. O. & Peters, S. W. (eds) (2000). *Nigeria: A people united, A future assured.* (Survey of States), Vol 2. Gabumo Publishing Co. Ltd, London.

Ogbonna, D. N. & Igbenijie, M. (2006). Characteristics of microorganisms associated with waste collection sites in Port Harcourt City, Nigeria. *Nigerian Journal of Microbiology* 20 (3):1427-1434

Oyeleke, S. B. & Manga, S. B. (2008a). *Essentials of Laboratory Practicals in Microbiology*. First edition, Tobest Publishers, Minna, Nigeria.

Oyeleke S. B. & Okusanmi, T. A. (2008). Isolation and Characterization of Cellulose Hydrolyzing Microorganisms from the Rumen of Ruminants. *African Journal of Biotechnology*, 7(9):30.

Rabah, A. B.; Ijah, U. J. J.; Manga, S. B. & Ibrahim, M. L. (2008). Assessment of Physico-chemical and microbiological qualities of abattoir wastewater in Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Sciences.* 16 (2):145-150

Tortora, G. J.; Funke, B. R. & Case, C. L. (2007). *Microbiology: An Introduction.* 9th Edition. Benjamin/Cummings Publishing Company Inc, California.

Udo, E. J. & Ogunwale, J. A. (1986). Laboratory *Manual for the Analysis of Soil, Plant and Water Samples,* 2nd Edition., University of Ibadan, Nigeria.

Yusuff, R. O. & Sonibare, J. A. (2004). Characterization of textile industries effluents in Kaduna, Nigeria and pollution implications. *Global Nest: The International Journal,* 6:212-221.