10-16-2006

Microbial Content of Abattoir Wastewater and Its Contaminated Soil in Lagos, Nigeria

A. O. Adesemoye
Adekunle Ajasin University, tony.adesemoye@unl.edu

B. O. Opere
Lagos State University

S. C. O. Makinde
Lagos State University, olubunmi.makinde@lasu.edu.ng

African Journal of Biotechnology Vol. 5 (20), pp. 1963-1968, 16 October 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Papers

Microbial content of abattoir wastewater and its contaminated soil in Lagos, Nigeria

Adesemoye A. O.[1]*, Opere B. O.[2] and Makinde S. C. O.[3]

[1]Microbiology Department, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.
[2]Microbiology Department, Lagos State University, Ojo, P. M. B. 1087, Apapa, Lagos, Nigeria.
[3]Department of Botany and Centre for Environment and Science Education, Lagos State University, Ojo, P. M. B. 1087, Apapa, Lagos, Nigeria.

Accepted 25 August, 2006

Microbial content of wastewater in two abattoirs and the impact on microbial population of receiving soil was studied in Agege and Ojo Local Government Areas in Lagos State, Nigeria. Wastewater samples were collected from each of the abattoirs over three months period and examined for microbial content. Soil samples contaminated with the wastewaters were also collected and analysed for microbial content as compared to soil without wastewater contamination in the neighbourhood (control). Some physico-chemical parameters of the samples such as total dissolved solid, chemical oxygen demand etc were examined. The wastewater samples from both abattoirs were highly contaminated; Agege abattoir showed mean bacterial count of $3.32x10^7$ cfu/ml and Odo abattoir showed mean count of $2.7x10^7$ cfu/ml. The mean fungal populations were $1.6x\ 10^5$ and $1.2x0^5$ cfu/ml for Agege and Odo abattoirs respectively. In the contaminated soil sample, mean bacterial count was $3.36x10^7$ cfu/ml compared to the $1.74x10^6$ cfu/ml of the control sample. High microbial load in abattoir wastewater with negative effects on microbial population in soil, in this study, further confirmed the need to treat wastewater rather than discharging it to the environment.

Key words: Abattoir, bacteria, environmental pollution, fungi, microbial population, soil, wastewater.

INTRODUCTION

Efforts have been geared towards curbing the menace of pollution around the world, particularly by the United Nations organs e.g., United Nations Environmental Programme. There are many international conferences and protocols to this effect. Rio de Janeiro Conference of 1992 was a major effort, collating previous environmental issues and bringing them to the fore (Oyesola, 1998). Nevertheless, in many parts of the world, human activeties e. g., animal production, still impact negatively on the environment and biodiversity. Some of the consequences of man-made pollution are–transmission of diseases by water borne pathogens, eutrophication of natural water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health (McLaughlin and Mineau, 1995; Sinha, 1997; Bridges et al., 2000; Boadi and Kuitunen, 2003; Amisu et al., 2003).

The continuous drive to increase meat production for the protein needs of the ever increasing world population has some pollution problems attached. In many countries, pollution arises from activities in meat production as a result of failure in adhering to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP). Consideration is hardly given to safety practices during animal transport to the abattoir, during slaughter and during dressing. For example, during dressing, the oesophagus of cattle and sheep should be sealed to prevent leakage of animal contents. These ineptitudes often lead to contaminations from hides, hooves and content of alimentary tract during evisceration and negatively impact on the environment, including microbes in the soil and surface and ground water (Hinton et al., 2000; Laukova et al., 2002; Amisu et al., 2003).

A specific example of what happens is logging of contaminated water in the soil. In that situation, oxygen becomes less available as an electron acceptor, prompting denitrifying bacteria to reduce available nitrate into

*Corresponding author. E-mail: semoyet@yahoo.co.uk.

gaseous nitrogen which enters the atmosphere with resultant negative effects. Also, anaerobic archaea (methanogens), may produce excessive methane at a higher rate than aerobic methane oxidizing bacteria (methanotrophs) could cope with, thus contributing to green house effect and global warming. Increase in methane is a concern because it is five times more effective as a green house gas than CO_2 (Madigan et al., 2003; Rusanov et al., 2002; Tourova et al., 2002; Tortora, 1997). Leaching into groundwater is a major part of the concern, especially due to the recalcitrant nature of some contaminants (Lapygina et al., 2002; Shah and Thakur, 2002; Tortora et al., 1997; Federov et al., 1993; Bitton and Harvey, 1992). The processes of adsorption and trapping by fine sandy materials, clays and organic matter can remove pathogenic organisms and some dissolved organic matter during passage of polluted water through the soil, thus reducing the microbial load. However, if there is too high departure of conditions from normalcy, beyond the carrying capacity of the natural process, diversity of autochthonous species could diminish while count of individual species that are able to survive may increase with possibility of grave consequences on groundwater (Baker and Herson, 1994; Atlas and Bartha, 1998; Lapygina et al., 2002).

Different methods of waste treatment have been developed, for reasons of public health and conservation, which results in the destruction of pathogens and the mineralization of the organic components of sewage prior to discharge. Anaerobic wastewater treatment using granular sludge reactor is one of such methods (Liu et al., 2002; Boadi and Kuitunen, 2003). However, in Nigeria, like many developing countries, the discharge of untreated wastes into the environment is still a problem, despite the establishment of Federal Environmental Protection Agency (FEPA) since 1998 (Adeyemo, 2003). Better inspection of abattoir and strict enforcement of the law are needed to be able to reduce environmental contamination and related diseases especially zoonotic diseases. Attempts to control the hygiene of slaughter house should include visual assessment of premises and animals themselves, and those that are "visibly unacceptably dirty" or are affected by diseases should not be allowed for slaughter (Salami, 1998; Hinton et al., 2000; Inglis and Cohen, 2002; Amisu et al., 2003).

This study aims to examine the extent of contamination in untreated wastewater of two abattoirs in Lagos, Nigeria and the impact on the ecology of microorganisms in the soil receiving part of the wastewater. In the two abattoirs examined in this study, different species of cattle are usually slaughtered with their blood, part of the dung and abdominal content washed on cemented pavements. The wastewaters run through open drainage of the abattoirs to bigger adjoining drainages in the neighborhood without any treatment. Part of the wastewater get washed directly to the ground within the neighborhood and may affect the whole biological community, including species diversity and contaminant accumulation in the food chain. Previously, some authors have reported different contaminants in soil and aquatic environments in different parts of Nigeria (Nwachukwu et al., 2001; Adeyemo, 2003; Akpan, 2004; Adewoye and Lateef, 2004; Efe, 2005). Besides contributing to knowledge, hopefully, this report will re-awaken concerned government agencies and other stakeholders.

METHODS

Collection of wastewater and soil sample

Wastewater samples were collected from two abattoirs with Bijou bottles. The abattoirs were located in Agege (Agege Local Government) and Odo (Nigerian Army Cantonment Ojo, Ojo Local Government), both within Lagos State, Nigeria. The Bijou bottles were used to aseptically draw part of the wastewater running off the drainage system just as it was leaving the slaughter pavements. Sample bottles were placed on ice during transport to the laboratory. Soil samples were collected from Agege abattoir contaminated area and the neighbourhood without wastewater contamination to serve as control. Agege abattoir was chosen for soil sample collection because slaughtering activities was relatively higher and the abattoir was well demarcated with a fence. Whatever contamination observed from the soil samples was therefore attributed to the wastewater. Samples were collected at 10 days interval over a period of three months. All samples were transported to the laboratory for analyses immediately after collection. Samples were collected three times per month at an interval of 10 days over a period of three months from each abattoir and labeled appropriately. There were a total of nine replicates for each sample.

Analyses of wastewater samples for physico-chemical properties

Samples were analysed for the following physico-chemical parameters: hydrogen ion concentration, temperature, turbidity, total suspended solid, total dissolved solid, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and conductivity. The pH value of the samples were determined with a pH meter (Unicam 9450, Orion model No. 91-02). Temperature was measured with mercury thermometer immediately after sample collection. Turbidity was determined with Milton Roy (USA) Spectronic 20D meter. Gravimetric method involving filtration and evaporation were used to measure total suspended solids and total dissolved solids. Methods recommended by APHA (1998) were followed for the measurement of BOD and COD. Wastewater sample was drawn into a 250 ml bottle, incubated in the dark for five days at 20°C and at the end of five days, the final dissolved oxygen (DO) content was determined. Decrease in DO between the final DO reading and the initial DO reading was corrected for sample dilution and recorded as the BOD of the sample. The COD was estimated by determining equivalent amount of oxygen required to oxidize organic matter in the samples. Conductivity was determined using a conductivity meter (Metrohm 640, Switzerland).

Preparation of media and total viable count

All the media used in this study were prepared and sterilized according to manufacturer's instructions. The media used include

Table 1. Physico-chemical properties of wastewater from two abattoirs in Lagos, Nigeria

Parameters measured	Agege	Odo
Colour	**Ox-blood to yellowish red**	**Ox-blood to yellowish red**
Appearance	Turbid	Turbid
Temperature	27±1.1°C	26±0.72°C
pH value	4.6±0.3	4.7±0.45
Conductivity (μScm^{-1})	34.0±2.1	34.6±1.2
Turbidity (NTU)	7.6±0.6	7.1±0.3
Total suspended solids (mgl^{-1})	1800±20	1750±25
Total dissolved solids (mgl^{-1})	630±8.3	610±5.0
Biological oxygen demand (mgl^{-1})	35±1.5	30±2.0
Chemical oxygen demand (mgl^{-1})	142±6.2	140±2.8

Values are means of three repeated sampling of three replicates each.

potato dextrose agar (PDA), Nutrient agar (International Diagnostic Group, UK), centrimide agar (Schleicher and Schuell, UK), McConkey agar, No.3 (Oxoid, UK), Robertson's cooked meat medium, malt extract agar, Man, Rogosa and Sharpe (MRS) medium and Eosin methylene blue (EMB) agar (Fisher Scientific, USA). In estimating total fungi, potato dextrose agar (PDA) plates which had been supplemented with streptomycin (100 µg/ml), meant to inhibit the growth of bacteria, were aseptically inoculated with serial dilutions (10^{-2} to 10^{-6}) of samples by spread plate technique using a glass spreader (hockey stick) and incubated at 30°C for 72 h (Adesemoye and Adedire, 2005). The numbers of organisms on the plates with distinct growth were counted after incubation; fungal population was then estimated and recorded as colony per ml. In estimating total bacteria, method similar to Boulter et al. (2002) was used. Sterile nutrient agar plates were aseptically inoculated with aliquot of serial dilutions (10^{-4} to 10^{-9}) of the samples and incubated at 30°C for 24 h. After incubation, plates with distinct colonies were counted, total bacteria was estimated and recorded as colony forming units per ml.

Preparation of diluents, isolation and identification of isolates

A measure of 10 g of soil sample, crushed and slightly heated (or 10 ml in the case of wastewater) was diluted in 90 ml of sterile distilled water, followed by serial dilution. Then, the serial diluents were aseptically inoculated onto different plates of melted sterile medium after cooling to 45°C and glass spreader was used to spread the inoculum. Sub-culturing was done until distinct colonies (pure cultures) were obtained. In identifying fungi, microscopic and macroscopic examinations including staining for morphological characteristics were carried out on fungal isolates and identification was done based on the characteristics. For bacteria, pure cultures were isolated followed by biochemical tests to identify the isolates. Biochemical tests done using standard methods include; Gram stain, motility, urease activity, carbohydrate utilization, starch hydrolysis, gelatin hydrolysis, oxidase, catalase, indole production, citrate utilization, nitrate reduction and hydrogen sulphide production (Anon, 1994; Cappuccino and Sherman, 1998).

Data analysis

Data obtained were analyzed using SAS 9.1 software (SAS Institute, Cary, USA) and means were separated by least significant differences ($P \leq 0.05$).

RESULTS

Summary of the physico-chemical properties of analyzed wastewater

The results of the physico-chemical analyses are presented in Table 1. Physico-chemical parameters analyzed were not statistically different for the waste-waters from both abattoirs (Agege and Odo). For example, mean temperature was 27±1.1°C in Agege abattoir and 26±0.72°C in Odo abattoir. The pH value was 4.6±0.3 and 4.7±0.45 for Agege and Odo abattoirs respectively.

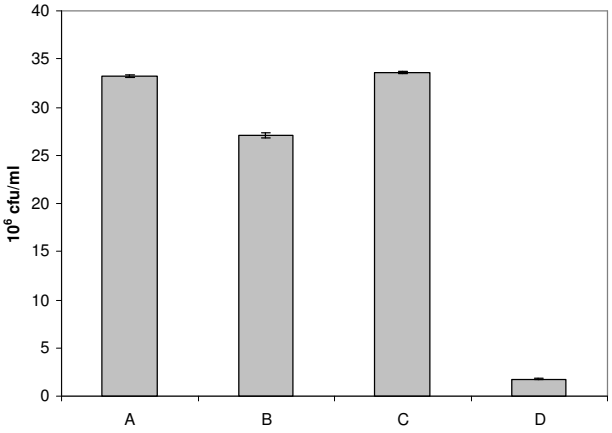

Figure 1. Population of bacteria in (A) Agege abattoir, (B) Odo Abattoir, (C) contaminated soil and (D) soil without abattoir contamination. Populations are means of three repeated sampling of three replicates each.

Total viable count of bacteria in wastewater and soil samples

The mean bacterial counts from Agege and Odo abattoirs were not statistically different. The mean total bacterial

Table 2. Microbial isolates from two abattoir wastewater samples.

Organism	Agege abattoir	Odo abattoir
Bacteria	*Bacillus* sp. *Clostridium welchii (C. perfringes)* *Pseudomonas aeruginosa* *Micrococcus luteus* *Vibrio* sp. *Lactobacillus plantarum*	*Bacillus* sp. *Clostridium welchii (C. perfringes)* *Pseudomonas aeruginosa* *Micrococcus luteus* *Vibrio* sp. *Lactobacillus plantarum*
Fungi	*Aspergillus niger* *Mucor* sp. *Saccarhomyces sp.* *Penicillium* sp. *Fusarium* sp.	*Aspergillus niger* *Mucor* sp. *Saccarhomyces* sp. *Penicillium* sp.

Table 3. Microbial isolates from Agege abattoir soil samples.

Organism	Contaminated soil	Uncontaminated soil
Bacteria	*Lactobacillus plantarum* *Pseudomonas aeruginosa* *Bacillus* sp. *Vibrio sp.*	*Bacillus* sp *Pseudomonas aeruginosa* *Bacillus subtilis* *Pseudomonas putida*

count of the replicates taken from Agege abattoir was $3.32x10^7$ cfu/ml while that of the Odo abattoir was $2.7x10^7$ cfu/ml. Meanwhile, mean bacterial population of the wastewater contaminated soil samples from Agege abattoir was $3.36x10^7$ cfu/g, which was statistically greater at $p \leq 0.05$ than $1.74x10^6$ cfu/g counted from the uncontaminated soil (soil without wastewater contamination) in the neighborhood (Figure 1).

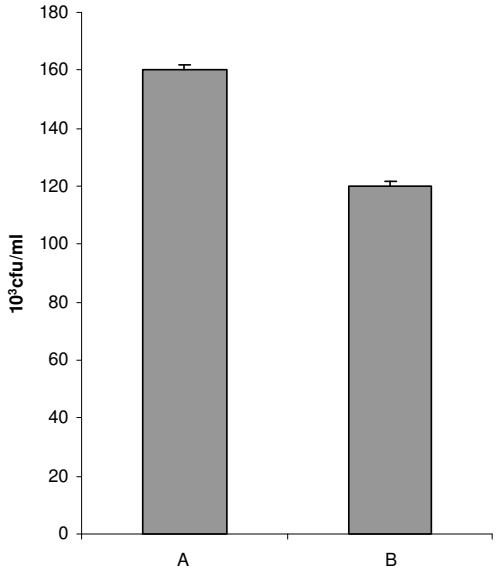

Figure 2. Population of fungi in (A) Agege abattoir (B) Odo Abattoir. Populations are means of three repeated sampling of three replicates each.

Total viable count and microbial isolates in analyzed soil

Mean total fungi/yeast counted from the Agege abattoir was $1.60x10^5$ cfu/ml and the mean count from Odo abattoir was $1.20x10^5$ cfu/ml (Fig. 2). Bacterial and fungal/yeast isolates are shown in Table 2. Bacterial isolates from soil samples with or without wastewater contamination are shown in Table 3.

DISCUSSION

The mean total bacterial count and fungi/yeast were high for samples from the two studied abattoirs (Figures 1 and 2). Going by international standard, any water contaminated to this level is neither good for domestic use nor is it supposed to be discharged directly into the environment without treatment. *Clostridium welchii* (*C. perfringens*), a common cause of gas gangrene and food poisoning as well as bowel disease called necrotizing colitis (Revis, 2004), was isolated from the wastewaters. Ogunseitan (2002) reported that animal excrement can be positive to tests on chemical indicators which will focus on compounds that would complement information based on indicators of pathogenic microorganisms present in feacal materials. The pH of the wastewaters was acidic, ranging from 4.3 to 5.1. However, a pH near 7.0 (neutral) plays a part in determining both the qualitative and quantitative abundance of microflora (Federov et al., 1993, Edward, 1990). It could be inferred then, that more hydrogen ion became available; lowering

the pH value of contaminated soil and affecting the pattern of microbial population. This can be corroborated by the report of Nazina et al., (2002) that abundance and activity of microflora in soil strata are controlled by the availability of water, nutrients, pH, concentration of metal ions, hydrodynamic communication with the ground surface, the lithology of bearing rocks, and so on.

Total bacterial population obtained from the contaminated abattoir soil was more than that in the soil without wastewater contamination. This could be regarded as destabilization of the soil ecological balance arising from contamination. Environmental stresses brought about by the contamination could be adduced for the reduction in microbial species diversity but increasing the population of few surviving species. Previous reports have proposed extensive microbial diversity (including species richness and species evenness) with population estimated between approximately $4x10^3$ to 10^4 species per g of uncontaminated soil (Borneman et al., 1996). A possible explanation on what transpired leading to the change in population pattern is that the organisms in the wastewater and organisms autochthonous to the soil engaged in competition and other negative microbial interactions such as antibiosis, after the water was discharged into the soil. Guided by the law of survival of the fittest (Madigan et al., 2003), those that were not able to survive the new conditions were probably excluded.

Similar to a previous study (Laukova et al., 2002), less diversity of bacteria but increase in population of surviving species was observed in this study. Higher population of bacteria observed in the contaminated soil (Figure 1) possibly had more of bacteria that were able to withstand acidic conditions. Changes in the ecology of soil have been observed by different authors but one germane question is how long such changes can persist. We believe the duration depends on many factors but in agreement with Hill et al. (1996), the type, quantity or concentration of the contaminant and the level of toxicity are very important. High level of contamination of the abattoir wastewater as revealed in this study, further confirmed the dangers associated with discharging untreated wastewater to the environment, thus the need for adequate treatment to ensure decontamination. We use some of the words of Ogunseitan (2003) to submit that sustainability in food production (in this case – meat production) should be given priority of place since it intertwines with public health and economic development. Another related question (outside this study) is the relationship of wastewater contamination to soil fertility. This area is recommended for further studies.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Poju Akinyanju of the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria and Prof. Henry Fadamiro, Department of Entomology and Plant Pathology, Auburn University, USA, for their helpful criticisms of the initial manuscript.

REFERENCES

Adesemoye AO, Adedire CO (2005). Use of cereals as basal medium for the formulation of alternative culture media for fungi. World J. Microbiol. Biotechnol. 21: 329-336.

Adewoye SO, Lateef A (2004). Assessment of the microbiological quality of *Clarias gariepinus* exposed to an industrial effluent in Nigeria. The Environmentalist 24: 249-254.

Adeyemo OK (2003). Consequences of pollution and degradation of Nigerian aquatic environment on fisheries resources. The Environmentalist 23: 297-306.

Akpan AW (2004). The water quality of some tropical freshwater bodies in Uyo (Nigeria) receiving municipal effluents, slaughter-house washings and agricultural land drainage. The Environmentalist 24: 49-55.

American Public Health Association (APHA) (1998). Standard methods for examination of water and wastewater. American Public Health Association, American Water Works Association and Water Pollution Control Federation. 20th edn. Washington DC, USA, pp 5-17.

Amisu KO, Coker AO, On SLW, Isokpehi RD (2003). *Arcobacter butzlieri* strains from poultry abattoir effluent in Nigeria. East Afri. Medical J. 80: 218-221.

Anon (1994). Bergey's manual of determinative bacteriology. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (ed). Williams and Wilkins, Baltimore, USA.

Atlas RM, Bartha R (1998). Biogeochemical cycling: carbon, nitrogen and oxygen. In: Microbial ecology: fundamentals and applications. 4th edn. Benjamin/Cummings science publishing, California, pp 386-410.

Baker KH, Herson DS (1994). Principles of bioremediation. In: Bioremediation. McGraw-Hill, Inc. USA, pp 24-28.

Bitton G, Harvey RW (1992). Transport of pathogens through soils and aquifers. In: Environ. Microbiol. Mitchell, R. (ed). John Willey & sons, Inc, New York, pp 103-127.

Boadi KO, Kuitunen M (2003). Municipal solid waste management in the Accra metropolitan area, Ghana. The Environmentalist 23 :211-218.

Borneman J, Skroch PW, O'Sullivan KM, Palus JA, Rumjanek NG, Jansen JI, Neinhuis J, Triplett EW (1996). Molecular microbial diversity of an agricultural soil in Wiscosin. Appl. Environ. Microbiol. 62:1935-1943.

Boulter JI, Trevor JT, Boland GJ (2002). Microbial studies of compost: bacterial identification and their potential for turfgrass pathogen suppression. World J. Microbiol. Biotechnol. 18: 661-671.

Bridges O, Bridges JW, Potter JF (2000). A generic comparison of the airborne risks to human health from landfill and incinerator disposal of municipal solid waste. The Environmentalist 20:325-334.

Cappuccino JG, Sherma N (1998). Standard qualitative analysis of water. In: Microbiology: A laboratory manual. 5th ed. Benjamin/Cummings Publishing Co, England, pp 297-303.

Edward C (1990). Microbiology of extreme environments. Open University Press, Milton Keynes, pp 245-250.

Efe SI (2005). Quality of water from hand dug wells in Onitsha metropolitan areas of Nigeria. The Environmentalist 25: 5-12.

Federov AY, Volchenko EV, Korzhenevich VI, Singirtsev IN, Krest'yaninov VY (1993). A polysubstrate strain that degrades wastewater components of phenol production. J. Appl. Biochem. Microbiol. 29: 532-536.

Hill RT, Straube WL, Palmisano AC, Gibson SL, Colwell RR (1996). Distribution of sewage indicated by *Clostridium perfringens* at a deep-water disposal site after cessation of sewage disposal. Appl. Environ. Microbiol. 62:1741-1746.

Hinton MH, Mead GC, Rowlings C (2000). Microbiology control in the meat industry. Flair flow Europe technical manual. F-Fe 339A/00May 2000, pp. 4-12 (www.exp.ie/flair.html).

Inglis GD, Cohen AC (2002). Influence of anti-microbial agents on the spoilage of meat-based entomophage diet. J. Economic Entomol. 97: 235-250.

Lapygina EV, Lysak LV, Zvyaginstsev DG (2002). Tolerance of soil bacterial complexes to salt shock. Microbiol. 71:143-147.
Laukova A, Marekova M, Vasilkova Z, Papajova I, Juris P (2002). Selected microbial consortium of raw and digested slurry and its susceptibility to enterocins. World J. Microbiol. Biotechnol. 18:11-15.
Liu Y, Xu H, Show K, Tay J (2002). Anaerobic granulation technology free wastewater treatment. World J. Microbiol. Biotechnol. 18: 99-113.
Madigan MT, Martinko JM, Parker J (2003). Brock biology of microorganism. International edn. Prentice Hall Intern Ltd, London, pp 1020-1040.
McLaughlin A, Mineau P (1995). The impact of agricultural practices on biodiversity. Agric. Ecosystem Environ. 55:201-212.
Nazina TN, Grigor'yan AA, Xue Y, Sokolova DS, Novikova EV, Yourova TP, Poltaraus AB, Balyaev SS, Ivanov MV (2002). Phylogenetic diversity of aerobic saprotrophic bacteria isolated from the Daqing Oil Field. Microbiol. 71 :91-97.
Nwachukwu SCU, James P, Gurney TR (2001). Inorganic nutrient utilization by "adapted" *Pseudomonas putida* used in the bioremediation of agricultural soil polluted with crude petroleum. J. Environ. Biol. 22:153-162.
Ogunseitan OA (2002). Caffeine-induced enzyme activity in *Pseudomonas putida* ATCC 700097. World J. Microbiol. Biotechnol. 18: 423-428.
Ogunseitan OA (2003). Biotechnology and industrial ecology: new challenges for a changing global environment. Afr. J. Biotechnol. 12: 596-601.
Oyesola D (1998). Protection and control of the environment: major regimes. In: Politics of international environmental regulations. Daily Graphic Publications, Ibadan, Nigeria, pp 62-92.
Revis DR (2004). Clostridial gas gangrene. eMedicine. (www.emedicine.com/med/topic394.htm).
Rusanov II, Lein AY, Pimenov NV, Yusupov SK, Ivanov MV (2002). The biochemical cycle of methane on the northwestern shelf on the Black Sea. Microbiol. 71: 479-487.
Salami OA (1998). Statutory control of municipal and industrial water pollution. In: Environmental law and policy. Simpson S, Fagboun O (ed). Proceeding of the international conference on environment held at the Lagos State University (LASU), Lagos, Nigeria. LASU Law Centre publications, pp 321-324.
Shah S, Thakur IS (2002). Enrichment and characterization of a microbial community from tannery effluent for degradation of pentachlorophenol. World J. Microbiol. Biotechnol. 18: 693-698.
Sinha RK (1997). Fluorosis – A case study from the Sambher Salt Lake Region in Jaipur, Rajasthan, India. The Environmentalist 17: 259-262.
Tortora GJ, Funke BR, Case CL (1997). Environmental Microbiology. In: Microbiology: An introduction. 6th edn. Benjamin/Cummings Publishing Co, California, pp 714-725.
Tourova TP, Kolganova TV, Kuznetsov BB, Pimenov NV (2002). Phylogentic diversity of the archaeal component in microbial mats on coral-like structures associated with methane seeps in the Black Sea. Microbiol. 71:196-201.