ISSN 1996-3351

Asian Journal of Biological Sciences

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research
http://knowledgiascientific.com

Asian Journal of Biological Sciences 6 (2): 116-123, 2013
ISSN 1996-3351 / DOI: 10.3923/ajbs.2013.116.123

Bacterial Genera and Their Some Species of Nile Water

Arafat Mohammed Goja
Department of Food Sciences and Technology, Faculty of Agriculture and Natural Resources, University of Bakht Alruda, P.O. Box 1311, ED Dueim, Sudan

ABSTRACT

The aim of this study was to evaluate bacterial quality and pathogenic organism presence in the Nile water (Blue, White and Main River Nile) as sources of drinking water in Sudan. The bacterial genera were isolated and identified using microbiological and biochemical method of evaluation. The most predominant aerobic microflora genera and species found in the Nile waters were *Bacillus, Aerococcus, Micrococcus, Pseudomonas, Staphylococcus, Actinomycetes, Aeromonas* and *Streptococcus*. Only two genera were identified to their species, *Bacillus* (*B. cereus, B. subtilis, B. licheniformis* and *B. Penibacillus*) and *Streptococcus* (*S. faecalis, S. faecium*). The most frequent coliforms isolated were *Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter agglomerans, Klebsiella oxytoca, Klebsiella planticola, Serratia* spp. and *Citrobacter* spp. The results of bacterial genera isolated in this study indicate that, the Nile water was potentially contaminated with the pathogenic bacteria and therefore, presence of *Escherichia coli* could be public health risk.

Key words: Bacteria, *Enterobacter, Bacillus,* Nile water, Khartoum

INTRODUCTION

The Nile is probably the longest river of the world, although some sources say the Mississippi precedes it. The Nile, according to a popular wisdom in Sudan, is one of the cleanest rivers of the world, if not the cleanest. The Farmers lay on the Nile banks in north of Khartoum; they use irrigation water for bathing, food preparation of food, ablution and for drinking. If this water is not clean and free of microorganisms, it is almost certain to cause disease and the greatest microbial risks are associated with ingestion of water contaminated with human and animal excreta (Atiribom *et al.*, 2007). It has been suggested most of the mortality and morbidity associated with water related disease especially in developing countries are due directly or indirectly to infectious agents who infect man (Obasohan *et al.*, 2012).

Khartoum City, occupies a unique position in that it lies on the bank of the Nile at the junction of the Blue and the White Niles, the major tributaries of the most important river of the world. However, the growing city could have a negative effect on the pristine water of the Nile, because of increasing the urbanization on the Nile banks, a remarkable increase in industrial, agricultural and human activities and recreational activities. This situation increased the waste discharged into water bodies. Moreover, the wastes of buildings take their way into the Nile waters during the flood season. It has been observed that most factories located in Khartoum discharge their waste directly into Nile Rivers without any treatment. Therefore, microbiological monitoring is one of the most important methods to safeguard the river Nile. The microbiological quality of the water of the Blue Nile, White Nile and the Main Nile were provided in the mid-1980's. Abdel Magid *et al.* (1984), Mahgoub (1984), Mahgoub and Dirar (1986) and Dirar (1986) investigated the Nile water for faecal contamination and microbial pollution using samples from various points on the three rivers. The results of these studies indicated that the Nile water was contaminated with the indicators organisms, which reflected the contamination with human faeces. The aims of this study were to know the prevalence of coliform bacteria and bacterial genera in Nile water, so as to determine their risk in Nile water as one of the main sources of drinking water.

MATERIALS AND METHODS

Samples: A total number of 120 samples were collected from three Nile waters (Blue, White and Main River) from different locations on monthly basis for two years (2006-2007). Sterile screw-cap bottles were used to collect water samples from all sites. Then the samples were stored in ice containers and laboratory analysis began immediately after arrival.

Bacteriological analysis

Isolation and identification of dominant bacteria: The predominant bacterial colonies were isolated from plate count agar done by pour plate method according to Harrigan (1998); these isolates were purified by streaking twice on nutrient agar and stored in a refrigerator, then further the identification done through Biochemical tests Harrigan (1998). The genus of *Bacillus* was identified to species level using BD BBL crystal$_{TM}$ identification systems (Enteri/Nonfermenter ID Kit) according to the manufacturing procedures, Germany.

Isolation and identification of *Streptococci*: Positive tubes of Azide dextrose test were selected and faecal streptococci were isolated by using Brain heart infusion agar (Barrow and Feltham, 1993).

Isolation and identification of coliform bacteria: Total coliforms and faecal coliform were determined by the Most Probable Number (APHA, 1995). Coliform bacteria were isolated by taking a loopful of positive BGB (Brilliant Green lactose-bile Broth) medium tubes (Oxoid) and streaking onto MacConkey agar, which was incubated at 37°C. Primary isolates were identified by Barrow and Feltham (1993) methods and then followed by IMViC tests for differentiation.

Isolation and identification of *E. coli*: *Escherichia coli* was isolated by taking a loopful of positive E. C medium tubes (Oxoid) and streaking onto MacConkey agar, which was incubated at 37°C. Primary *E. coli* isolates from MacConkey agar (pink colonies) were examined by using Eosin Methylene Blue Agar plate (EMB) (Oxoid) as a selective media for *E. coli*. Colonies with metallic green sheen were recorded as positive *E. coli*. Then metallic green sheen colonies were cultured into Colic Brilliance *E. coli*/Coliform selective media (Oxoid) for more confirmation. Blue or violet colonies were recorded as positive *E. coli* (Barrow and Feltham, 1993).

RESULTS

The predominant aerobic microflora genera isolated from Nile water samples are shown in Table 1 and Fig. 1-3.

In the Blue Nile, Fig. 1 illustrates that, *Bacillus* is the genus of the greatest proportion of isolates (78%) of Nile water samples. *Aerococcus* ranks second (67%), followed by *Micrococcus* (64%), *Pseudomonas* (47%) and *Streptococcus* (42%). *Actinomycetes* and *Aeromonas* (22% each) represented the smallest proportion. In the White Nile, Fig. 2 illustrates that the genus which has

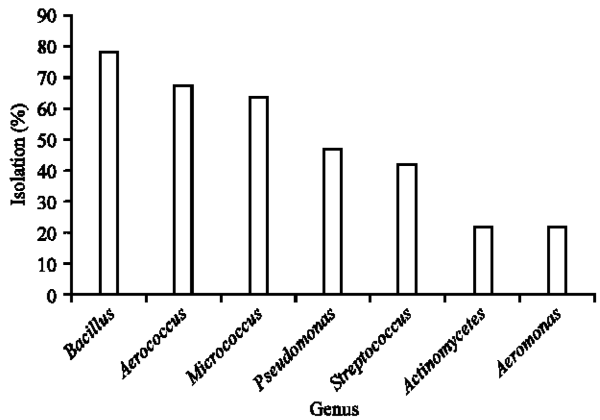

Fig. 1: Predominant microorganisms isolates of the blue Nile water

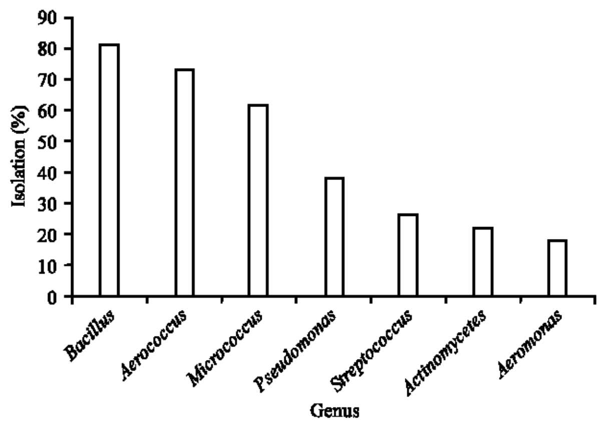

Fig. 2: Predominant microorganisms isolates of the white Nile water

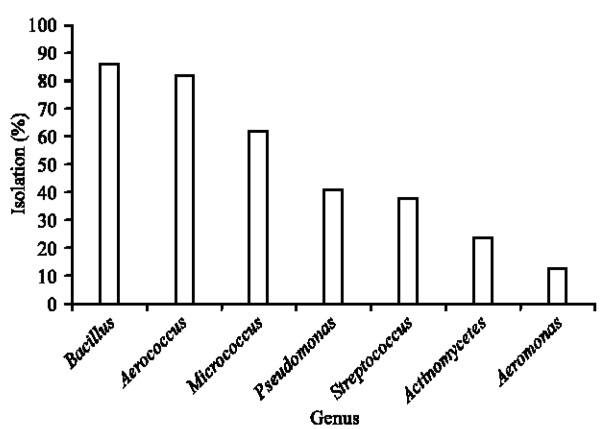

Fig. 3: Predominant microorganisms isolates of the main river Nile water

recorded the highest proportion was also *Bacillus* (81%), followed by *Aerococcus* (73%), *Micrococcus* (62%) and *Pseudomonas* (38%). The genera *Staphylococcus* (26%), *Streptococcus* (22%) and *Aeromonas* (18%) recorded the lowest proportion. In the Main River Nile, Fig. 3 illustrates that, *Bacillus* gave the greatest proportion of the isolates (86%). *Aerococcus* was the second largest

Table 1: Morphological and biochemical identification tests of the aerobic microflora genera in Nile water

Unknown isolates	Gram stain test	Shape	Oxidase test	Catalase test	O/F test	Motility test	Spore forming ability	Acid from glucose test	Genus
1	+	Rod	+	+	F	+	+	+	*Bacillus*
2	+	Cocci	-	-	O	-	-	+	*Aerococcus*
3	+	Cocci	-	+	O	-	-	+	*Micrococcus*
4	-	Rod	+	+	O	+	-	+	*Pseudomnas*
5	+	Cocci	-	-	F	-	-	+	*Streptococcus*
6	+	Cocci	-	+	F	-	-	+	*Staphylococcus*
7	-	Rode	-	+	F	+	-	+	*Aeromonas*
8	-	Rode	-	+	F	-	-	+	*Actinomycetes*

Table 2: Results of morphological and biochemical tests of coliforms bacteria

						IMViC tests				
Shape	Gram stain	Oxidase test	Catalase test	Motility	Growth in air	Indole	Methyl red	Citrate	V/p	Organism
Rod	-	-	+	+	+	-	-	+	+	*Enterobacter aerogenes*
Rod	-	-	+	+	+	-	-	+	+	*Enterobacter cloacae*
Rod	-	-	+	+	+	-	-	+	+	*Enterobacter agglomerans*
Rod	-	-	+	-	+	+	-	+	+	*Klebsiella oxytoca*
Rod	-	-	+	-	+	-	-	+	+	*klebsiella planticola*
Rod	-	-	+	+		+	-	+	+	*Citrobacter* spp.
Rod	-	-	+	+	+	-	-	+	+	*Serratia* spp.

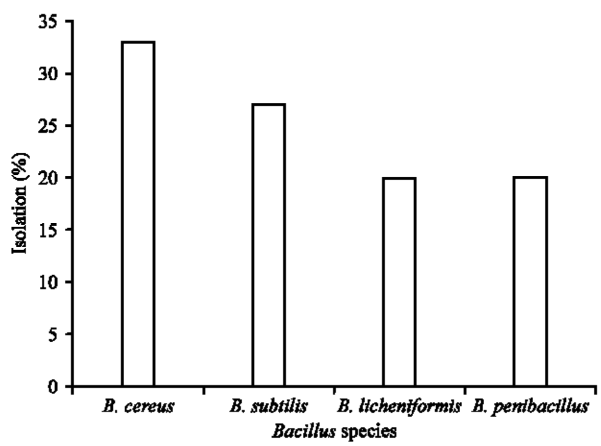

Fig.4: Frequency isolates of *Bacillus* species of Nile water

isolate. It was recorded (82%) of the isolates. Then followed by *Micrococcus, Staphylococcus* and *Pseudomonas*, they found in 62, 41 and 38% of isolates, respectively. The genera *Streptococcus* and *Aeromonas* were recorded the lowest proportion of the isolates. They are found in 24 and 13% of the isolates, respectively. The genus of *Bacillus* of three Nile water was identified to species level. Figure 4 shows that, *B. cereus* was the most common species (33%), followed by *B. subtilis* (27%), *B. licheniformis* (20%) and *B. penibacillus* (20%). The most frequent coliforms isolated from Nile water were *Enterobacter aerogenes, Enterobacter cloacae, Enterobacter agglomerans, Klebsiella oxytoca, Klebsiella planticola, Serratia* spp. and *Citrobacter* spp. (Table 2). Then the faecal coliform isolates were identified as two groups; *Echerichia coli* type I and type II using IMViC tests (Table 3).

Table 3: Results of morphological and biochemical tests of *Echerichia coli* isolates

						IMViC tests				
Shape	Gram stain	Oxidase test	Catalase test	Motility	Growth in air	Indole	Methyl red	Citrate	V/p	Organism
Rod	-	-	+	+	-	+	+	-	-	*E. coli* type I
Rod	-	-	+	+	-	-	+	-	-	*E. coli* type II

DISCUSSION

The results of this study indicate that, the most dominant three bacteria isolated from Nile water were *Bacillus* a typical soil inhabits organism, which represents the greater number of isolates and many species of this genus demonstrate a wide range of physiologic ability that allows them to live in many natural environments (Ahn *et al.*, 2001). They are omnipresent in terrestrial and fresh water domain and are widely distributed in seawater (Ruger, 1989). Followed by *Aerococcus,* a typical airborne organism and have been isolated from different sources from air, vegetation, dust, hospital, water, human and animals (Ruoff, 2007; Vela *et al.*, 2007; Volterra *et al.*, 1986) and then *Micrococcus* a free organism of environmental existence (Brenner, 1984). Similar results were obtained by Ahmed (2005) and El-Tom (1997); they reported that *Bacillus* and *Aerococcus* were the two most common bacterial genera found in water. However, Elrofaei (2000) noticed that *Bacillus* and *Micrococcus* were the two most common genera found in drinking water. These results were in agreement with Oyeleke and Istifanus (2008) who reported that Gram-positive genera, especially the genus *Bacillus* were the most abundant bacteria isolated from Kaduna River in Nigeria. These results also agree with the findings of Hassan *et al.* (1995) who was found that *Bacillus* spp. in abundance in water. It thus reflects the predominance of spore formers in sands and soils. A among the other identified genera *Pseudomonas* is widespread in soil and water (Lightfoot, 2003). *Streptococcus, Staphylococcus* and *Aeromonas* were found in considerable numbers in water and they are non-faecal indicators of water quality. However, the existence of *Aeromonas* genus is the possible representatives of different sewage originating microbes in Nile water. Szczuka and Kaznowski (2004) were pointed that these bacteria are found in a wide range in the environment, particularly in surface water and sewage. Kueh *et al.* (1995) revealed that there is a significant relationship between *Aeromonas* levels and gastrointestinal illness.

The most abundant one genera (*Bacillus*) was identified up to the species-level Fig. 4 (*B. cereus, B. subtilis, B. licheniformis* and *B. penibacillus*) that deals with human food poisoning. It is reported that *B. cereus* was causes serious and potentially fatal non-gastrointestinal-tract infections and its pathogenicity, whether intestinal or non-intestinal, is closely associated to the production of tissue-destructive exoenzymes (Bottone, 2010). Other *Bacillus* spp. has more rarely been identified as agents of food borne diseases characterized by diarrhea and/or vomiting (Anonymous, 2005). However, *B. subtilis* has been involved in many cases of food poisoning (Gilbert *et al.*, 1981; Kramer *et al.*, 1982) as cited by Logan (1988) and therefore, the main symptom of food poisoning is acute vomiting, although diarrhea quite commonly follows. Similar species were identified by Bal *et al.* (2009) who reported that *B. cereus, B. subtilis* and *B. licheniformis* were the species identified from *Bacillus* genus isolated from seawater of Orissa coast in India. Contamination of water resources by microorganisms of faecal origin is a current world-wide public health concern. Coliform bacteria and faecal coliform bacteria were used as indicator bacteria of water contaminated with the faecal origin. In this study, the Nile water was faecal contaminated (Data not shown).

Thus, the common genera coliform found were *Enterobacter*, *Klebsiella*, *Citrobacter*, *Serratia* and *Echerichia*. This finding agreement with LeChevallier *et al.* (1983) who stated that the common members of coliform bacteria are the genera of *Escherichia*, *Enterobacter*, *Klebsiella* and *Citrobacter*. However, the presence of *Echerichia coli* (type I and II) Table 3 indicated that the faeces of human and animal were taking place into the Nile water. Comparatively high numbers of coliform bacteria are discharged (2×109 coliforms/day/capita) in human and animal faeces (Bitton, 2005). *Enterobacter* species are important causing agents of hospital sand other infections (Iversen and Forsythe, 2003; Deal *et al.*, 2007). *Enterobacter* infections are of concern because they are becoming increasingly common. Moreover, their antibiotic resistance is especially alarming (Anonymous, 2010). Faecal coliforms and fecal streptococci (including the sub-group enterococci) are traditionally used as the indicator organisms to guarantee microbiological safety of drinking water, natural water resources (Sidhu and Toze, 2009). Primary routes of coliform pollution of natural water sources comprises vegetation, flood, run-offs and untreated or partly treated waters, including domestic, agricultural and industrial wastes. Microbial contamination of the soil and natural water occurs from land application of sludge, which results from sewage and wastewater treatments (Sidhu and Toze, 2009). Although many coliform bacteria and other indicator organisms may not be pathogenic, their presence in water indicates the presence of potentially pathogenic agents, including viruses from the intestinal tract of warm-blooded animals (Haller *et al.*, 2009; Noble *et al.*, 2003). Thus, coliform bacteria, particularly faecal indicator bacteria, are used to evaluate the microbiological safety of water resources.

Bacteria levels in surface waters are frequently correlated with the levels of pathogenic organism in human, thus, those who interested with public health were concerned with the bacterial levels in surface waters. (USEPA, 1986). However, the Nile water was faecal contaminated and consumption of this water poses a risk to human health. So, further research is needed in Nile water for the presence of food borne pathogens.

CONCLUSION

From the results, it can be concluded that the Nile water (Blue, White and Main River Nile) were microbially contaminated. The presence of coliforms bacteria such as *Enterobacter* spp., *Klebsiella* spp., *Serratia* spp. and *Citrobacter* spp. indicates the existence of potentially pathogenic bacteria in water, especially *Escherichia coli* as the faecal indicator, could be risked for public health.

REFERENCES

APHA, 1995. Standard Methods for the Examination of Water and Wastewater. 19th Edn., American Public Health Association, Washington, DC., USA.

Abdel Magid, H.M., I.S. Ibrahim and H.A. Dirar, 1984. Chemical and microbiological examination of well and nile water. Environ. Int., 10: 259-263.

Ahmed, I.F., 2005. Microbiological quality of water in some food factories storage cisterns in Khartoum North Industrial area. Ph.D. Thesis, Faculty of Science, University of Khartoum, Khartoum, Sudan.

Ahn, T.S., S.H. Hong, O.S. Kim, J.J. Yoon, S.O. Jeon and S.I. Choi, 2001. The changes of *Bacillus* spp. in municipal waste-water treatment plant with B3 process. Kor. J. Microbiol., 37: 209-213.

Anonymous, 2005. Opinion of the scientific panel on biological hazards on bacillus cereus and other *Bacillus* spp. in foodstuffs. EFSA J., 175: 1-48.

Anonymous, 2010. *Enterobacter* for the health care provider. http://www.wechealthunit.org/diseases-conditions/fact-sheets/HCP_Enterobacter.pdf

Atiribom, R.Y., S.I. Ovie and O. Ajayi, 2007. Bacteriological quality of water and fish samples from kainji lake and the effects of animal and human activities. Proceedings of the Conference on Fisheries Society of Nigeria, November 12-16, 2007, Kebbi State, Nigeria, pp: 209-218.

Bal, S., R.R. Mishra, B. Rath, H.K. Sahu and H.N. Thatoi, 2009. Characterization and extracellular enzyme activity of predominant marine *Bacillus* spp. isolated from sea water of Orissa Coast, India. Mala. J. Microbiol., 5: 87-93.

Barrow, G.I. and R.K.A. Feltham, 1993. Cowan and Steel's Manual for Identification of Medical Bacteria. 3rd Edn., Cambridge University Press, Cambridge, UK., ISBN-13: 978-0521326117, Pages: 351.

Bitton, G., 2005. Microbial indicators of faecal contamination: Application tomicrobial source tracking. Report submitted to the Florida Storm Water Association, pp: 1-71.

Bottone, E.J., 2010. *Bacillus cereus*, a volatile human pathogen. Clin. Microbiol. Rev., 23: 382-398.

Brenner, J.D., 1984. Facultatively Anaerobic Gram-negative Rods. In: Bergey's Manual of Systematic Bacteriology, Krieg, N.R. and J.G. Holt (Eds.). 1st Edn., Williams and Wilkins, Baltimore, USA., pp: 409-598.

Deal, E.N., S.T. Micek, D.J. Ritchie, R.M. Reichley, W.M. Dunne and M.H. Kollef, 2007. Predictors of in-hospital mortality for bloodstream infections caused by *Enterobacter* species or *Citrobacter freundii*. Pharmacotherapy, 27: 191-199.

Dirar, H.A., 1986. Coliform bacteria counts in the Nile water at Khartoum. Environ. Int., 12: 571-576.

El-Tom, A.M., 1997. Microbiology of Port-Sudan water supply. Ph.D. Thesis, University of Khartoum, Khartoum, Sudan.

Elrofaei, N.M., 2000. Microbiological examination of drinking water for the displaced people living around Khartoum State. Ph.D. Thesis, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.

Gilbert, R.J., P.C.B. Turnbull, J.M. Parry and J.M. Kramer, 1981. *Bacillus cereus* and other *Bacillus* species: Their Part in Food Poisoning and other Clinical Infections. In: The Aerobic Endospore-Forming Bacteria: Classification and Identification, Berkeley, R.C.W. and M. Goodfellow (Eds.). Academic Press, London, pp: 297-314.

Haller, L., J. Pote, J.L. Loizeau and W. Wildi, 2009. Distribution and survival of faecal indicator bacteria in the sediments of the Bay of Vidy, Lake Geneva, Switzerland. Ecol. Indic., 9: 540-547.

Harrigan, W.F., 1998. Laboratory Methods in Food Microbiology. 3rd Edn., Academic Press, London.

Hassan, E.S., I.M. Banat and A.H. Abu-Hilal, 1995. Post-Gulf-War nutrients and microbial assessments for coastal waters of Dubai, Sharjah and Ajman Emirates (UAE). Environ. Int., 21: 23-32.

Iversen, C. and S. Forsythe, 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. Trends Food Sci. Technol., 14: 443-454.

Kramer, J.M., P.C.B. Turnbull, G. Munshi and R.J. Gilbert, 1982. Identification and Characterization of *Bacillus cereus* and other *Bacillus* species Associated with Foods and Food Poisoning. In: Isolation and Identification Methods for Food Poisoning Organisms, Corry, J.E.L. (Ed.). Academic Press, London, pp: 261-286.

Kueh, C.S.W., T.Y. Tam, T. Lee, S.L. Wong and O.L. Lloyd *et al*., 1995. Epidemiological study of swimming-associated illnesses relating to bathing-beach water quality. Water Sci. Technol., 31: 1-4.

LeChevallier, M.W., S.C. Cameron and G.A. McFeters, 1983. New medium for improved recovery of coliform bacteria from drinking water. Applied Environ. Microbiol., 45: 484-492.

Lightfoot, N.F., 2003. Bacteria of Potential Health Concern. In: Heterotrophic Plate Counts and Drinking-Water Safety, Bartram, J. J. Cotruvo and M. Exner (Eds.). IWA Publishing, London, UK., ISBN-13: 9781843390251, pp: 61-79.

Logan, N.A., 1988. *Bacillus* species of medical and veterinary importance. J. Med. Microbiol., 25: 157-165.

Mahgoub, D.M. and H.A. Dirar, 1986. Microbial pollution of the blue and white niles at khartoum. Environ. Int., 12: 603-609.

Mahgoub, D.M., 1984. Coliform bacteria in the Nile at Khartoum. M.Sc. Thesis, University of Khartoum, Khartoum, Sudan.

Noble, R.T., D.F. Moore, M.K. Leecaster, C.D. McGee and S.B. Weisberg, 2003. Comparison of total coliform, fecal coliform, and enterococcus bacterial indicator response for ocean recreational water quality testing. Water Res., 37: 1637-1643.

Obasohan, E.E., D.E. Agbonlahor and E.E. Obano, 2012. Water pollution: A review of microbial quality and health concerns of water, sediment and fish in the aquatic ecosystem. Afr. J. Biotechnol., 9: 423-427.

Oyeleke, S.B. and N. Istifanus, 2008. The effects of Nigeria State water treatment plant effluent on its receiving river (Kaduna). Afr. J. Biotechnol., 7: 1530-1535.

Ruger, H.J., 1989. Benthic studies of the northwest African upwelling region: Psychrophilic and psychrotrophic bacterial communities from areas with different upwelling intensities. Mar. Ecol. Prog. Ser., 57: 45-52.

Ruoff, K.L., 2007. *Aerococcus, Abiotrophia* and Other Aerobic Catalase-Negative, Gram-Positive Cocci. In: Manual of Clinical Microbiology, Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (Eds.). 9th Edn., ASM Press, Washington, DC., USA., pp: 443-454.

Sidhu, J.P.S. and S.G. Toze, 2009. Human pathogens and their indicators in biosolids: A literature review. Environ. Int., 35: 187-201.

Szczuka, E. and A.Kaznowski, 2004. Typing of clinical and environmental *Aeromonas* sp. strains by random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR and enterobacterial repetitive intergenic consensus sequence PCR. J. Clin. Microbiol., 42: 220-228.

USEPA, 1986. Ambient water quality criteria for bacteria. Report, EPA440/5-84-002.

Vela, A.I., N. Garca, M.V. Latre, A. Casamayor and C. Sanchez-Porro *et al*., 2007. Aerococcus suis sp. nov., isolated from clinical specimens from swine. Int. J. Syst. Evol. Microbiol., 57: 1291-1294.

Volterra, L., L. Bonadonna and F.A. Aulicino, 1986. Fecal streptococci recoveries in different marine areas. Water Air Soil Pollut., 29: 403-413.