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Bacteriological and metal analyses of water samples from Awotunde fish pond and river

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A R T I C L E   I N F O

A B S T R A C T

The microbiological and metal analyses of water samples obtained from Awotunde fish pond and river in Ila-Orangun, Osun State, Nigeria were determined. The total bacterial and coliform counts were done using pour plating technique. Mineral was analyzed using Atomic Absorption Spectrophotometry techniques. The physicochemical parameters were done using standard methods. The total bacterial and coliform count of the water samples ranged 6.4×10^4 CFU/ml - 7.4×10^4 CFU/ml and 1.1×10^3 CFU/ml - 4.0×10^3 CFU/ml respectively. The values obtained for the physicochemical parameters ranged as follows: temperature (24 - 26) ^{o}C, HCO_3 (2.0 - 4.6), pH (6.0 - 7.2), alkalinity (40 - 100) mg/l, hardness (60 - 120) mg/l, conductivity (200 - 400)µS/cm, dissolved oxygen (1.1 - 2.0) mg/l, BOD (0.06 - 0.22) mg/l, chloride (28.4 - 106.4) mg/l, nitrite (4.0 - 188.0) mg/l, sulphate (14.0 - 640.0) mg/l, nitrate (0.0 - 90.0) mg/l and phosphate (1.6 - 83.2) mg/l. The mean values of the mineral contents of the water samples are as follows; potassium (94.4 mg/l), sodium (94.7 mg/l), magnesium (89.3 mg/l) and calcium (94.9 mg/l). The mean value obtained for the heavy metal concentrations are as follow; copper (0.1 mg/l), manganese (0.13 mg/l), nickel (2.09 mg/l), lead (0.03 mg/l) and iron (0.26 mg/l**).** This shows that heavy metal concentrations were below critical limit according to the WHO standard. However, the microbial counts obtained portend the fact that the fish harvested from the pond might be a source of food poisoning if such fishes are not adequately cooked. Since the river serve as a source of water for domestic usage, an outbreak of water

borne diseases could be imminent. Hence there must be a need for the Nigeria fish farmers to improve on their fish farming management which includes fish food preparation, environmental sanitation and monitoring the quality fish yield.

1. Introduction

In recent years, a tremendous amount of attention has been directed toward pollution of soil and water supplies and the subsequent effects on the life of many animals and human. Meanwhile, pollution of the environment simple entails making the environment unclean and unhealthy by aiding unwholesome states or condition. Water, a ubiquitous chemical substance basically composed of hydrogen and oxygen with the molecular formula of H_2O (Parker *et al.*, 2001); is the cheapest indispensable and most universal substance to man as it comes first among all the environmental factors that affect the survival of known forms of life including plants, animals and microorganisms (Prescott *et al.*, 1999; EPA, 1996).

Heavy metals are thus commonly defined as those having a specific density of more than $5g/cm^3$. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic (Lenntech, 2008). Trace quantity of many metals such as Nickel (Ni), Manganese (Mg), Lead (Pb), Chromium (Cr), Cadmium (Cd), Zinc (Zn), Copper (Cu) and Iron (Fe) are though important constituents of most waters as they are necessary for growth of biological life in water, but numerous metals have received attention as they serves as environmental contaminants and toxicological hazards to human. For instance, the occurrence of arsenic, cadmium and lead in drinking water is considered an important pathway of potential exposure of man's various deadly diseases such as cancer, kidney diseases and impaired cognitive function (Jarup *et al.*, 2000). Furthermore, the usability/portability of water is being affected most especially when some metals (such as Zinc, Copper, Iron, Cadmium e.t.c.) are found in concentrations higher than the desirable amount (Pelczar, 1993) as they tend to biomagnifies and pose high risk to human health especially due to the recalcitrant nature of these contaminations (Fonseca, 2000; Lapygina *et al.*, 2002).

A sustainable aquaculture system which depends upon eco-friendly, economically and socially viable system entails the recycling of organic wastes for fish culture thereby serving the dual purpose of cleaning the environment (by avoiding the problem of waste disposal) and providing economic benefits. The recycling of animal dung/waste in fish ponds for natural fish production serves to reduce the expenditures on costly feeds and fertilizers which form more than 50% of the total input cost for the system (Schroeder, 1980). However, the indiscriminate use of their manures in fish ponds, instead of improving the pond productivity, may also lead to pollution and vast contamination which pose several risk on the intended plankton production, growth and subsequently on human health (Hojoh and Sonng, 2003). Leaching into ground water is another major part of the concern, especially due to the recalcitrant nature of some contaminants from the pond and nearby farm lands as the population grows and urbanization increases, more water is required and greater demand is made on ground and surface water which is of great risk due to less availability of potable water. Therefore, it is necessary to know the standard doses of these wastes which would keep the physicochemical parameters of the pond water in a favorable range required for the survival and growth of fish with minimal or zero percent (0%) toxigenic consequence on human and their immediate environment.

Awotunde fish pond is located in the ancestral town of Ila-Orangun, Osun-State, a western part of Nigeria, with an estimated population of about 100,000. The pond is located along the course of a flowing river (Awotunde River) by the use of dam. Poultry is constructed on the pond which supplies poultry waste (organic matter) for the feeding of the fishes. Also, the pond's location is such that, run-off water from adjacent and nearby farmlands flows into the river and subsequently the pond. This river passes through the pond and drains many other farmlands. Therefore, the pesticides, herbicides and residues of fertilizers applied in adjacent farms could serves as contamination to the pond water and subsequently the river hence serves as sources of toxic heavy metals, minerals and nutrients which could get to human through "Biomagnifications"; by their incorporation into fishes which are subsequently eaten by the populace (Eja *et al.*, 2003). The toxic metal and other nutrients can find their way into the underground water table through percolation and leaching and thus proves hazardous to the

population (Jarup *et al*., 2000). Also, crops grown in areas which heavy metals contaminated water drains the soil may absorb the metals through cation exchange and later find their ways into human diet. Furthermore, the rivers at some point in its course serves as a source of water supply for domestic and drinking purposes for some people and thus endanger the health of such people.

This paper is aimed to ascertain the microbiological and chemical quality of the water available in Awotunde pond and river located in Ila-Orangun; use for the natural fish production which serves the major source of fish consumed by the town bearer and also serves as source of water for domestic and drinking purposes.

2. Materials and methods

2.1. Collection of water sample

Water samples were collected aseptically with the aid of a pre-sterilized screwed-capped glass bottles (sampling bottles); having a capacity of about 200ml and transported in ice to the Microbiology laboratory for analysis within 4-6 hrs after collection. Samples were collected at seven different sampling points are as follow:

A- Upstream along the river 15m before the fish ponds.
B- Point along the river just before the fish ponds.
C- Fish pond on the east of the river.
D- Fish pond on the west of the river.
E- Point along the river between the two ponds on both sides.
F- Point along the river just after the fish ponds.
G- Downstream along the river 15m after the fish ponds.

2.2. Enumeration of total bacterial counts (TBC)

Determination of bacterial load in the water samples were done in triplicates. Bacterial plate counts were carried out using the pour plate method with nutrient agar. This method was based on the serial dilution of water sample, which were then pipetted into each sterile Petri-dish. About 20ml of sterilized molten Nutrient agar was cooled to 45°C and poured into each Petri-dish containing 1ml of the water sample. Plates were allowed to cool and set after which were incubated in an inverted position at 37°C. After 24hrs of incubation, the plates were counted using colony counter (Barrow and Feltham, 1993; Olutiola *et al*., 1991).

2.3. Estimation of total coliform count [TCC]

Using the membrane filtration technique as described by (Olutiola *et al*., 1991). The water sample was filtered using a membrane filter thereby retaining the bacteria cells on the upper surface of the membrane filter. A 20ml of sterile molten MacConkey agar (without salt) plate was inoculated by placing the membrane filter on it, and inoculated at 37°C for 24hours in an inverted position. After incubation, membrane filter was transferred to an absorbent pad saturated with methylene blue solution (0.01%) and allowed to stain for 1minute. Subsequently, membrane filter was saturated with sterile distilled water so as to remove excess stain. Colonies are counted on membrane filter and used to calculate the number of coliforms per ml of the original sample recorded in CFU/ml unit.

2.4. Physicochemical analysis

About 5cm^3 of concentrated Hydrochloric acid was added to 250cm^3 (v/v) of water sample and evaporated to 25cm^3. The concentrate was transferred to 50cm^3 standard flask and diluted to the mark with distilled de-ionized water (AOAC, 2005). The pH was measured with a KENT EIL 7020 (Kent industrial measurement Limited, Surrey, England) pH-meter, temperature was measured with a simple thermometer calibrated in centigrade, conductivity was measured with CDM83 conductivity meter (By Radiometer A/S Copenhagen, Denmark) after standardization with KCl solution, various standard methods were used for the determination of other parameters. Total alkalinity was determined by acidometry using bromocresol green-methyl red mixed indicator; total hardness and calcium hardness by EDTA titration using Erichrome Black-T-indicator (AOAC, 2005).

2.5. Mineral analysis

Zinc, iron, copper, lead, nickel and manganese were analyzed in the three matrices using a Perkin Elmer model 306 Atomic Absorption Spectrophotometer. While potassium, magnesium, sodium and calcium were analyzed from solution obtained by means of Atomic Absorption Spectrophotometer (PYE Unicam Sp 9, Cambridge, UK) (AOAC, 2005) all were recorded in milligram per liter.

3. Results

The total bacterial and coliform counts of water samples from different points of Awotunde fish pond and the inflow river are depicted in Fig. 1. The total bacterial and coliform counts of the samples from the fish pond and river ranged from 6.4×10^4 CFU/ml to 7.4×10^4 CFU/ml and 1.1×10^3 CFU/ml to $x\ 4.0 \times 10^3$ CFU/ml respectively (Fig. 1).
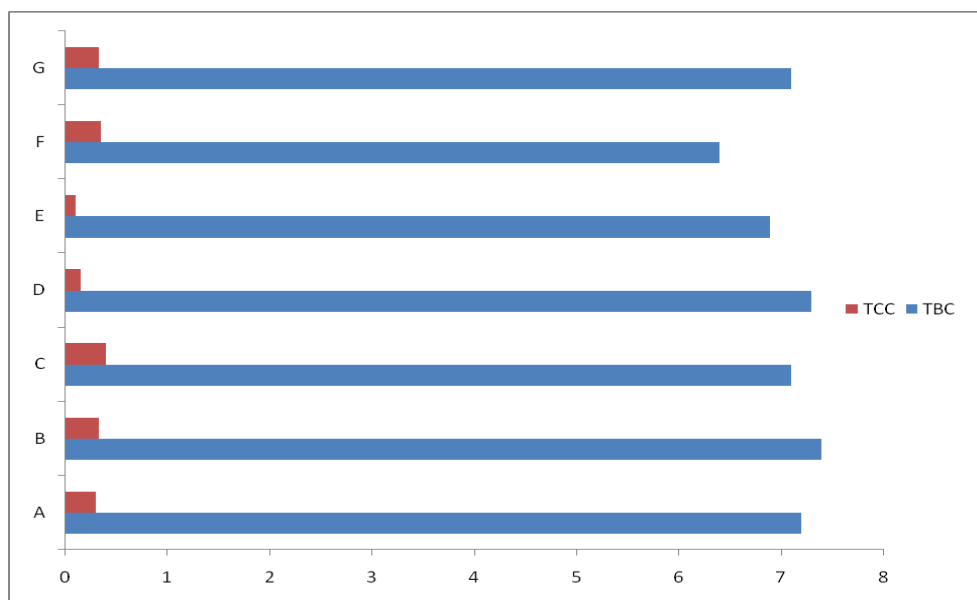

Fig. 1. Mean total bacterial and coliform counts (CFU/ml) of water samples from Awotunde fish pond and river.

Table 1
Physicochemical values of water samples from Awotunde fish pond and river.

Parameters	A	B	C	D	E	F	G	Mean values of the parameters in the water samples
Temperature (^{o}C)	26.0	25.0	25.0	26.0	24.0	25.0	24.0	25.0
HCO_3^{-2}	4.60	4.60	2.60	2.00	2.10	2.20	2.50	2.90
pH	7.20	6.90	6.60	6.50	6.50	6.20	6.00	6.60
Alkalinity (mg/l)	100	80	50	60	60	50	40	62.9
Hardness (mg/l)	120	128	60	66	84	84	82	89.1
Conductivity(μS/cm)	400	390	200	200	200	200	200	255.7
Dissolved O_2 (mg/l)	1.30	1.10	2.00	2.00	1.50	2.00	1.80	1.70
BOD (mg/l)	0.22	0.06	0.2	0.18	0.06	0.16	0.16	0.14
Chloride (mg/l)	106.4	67.4	28.4	42.5	35.5	35.5	42.5	51.2
Nitrite (mg/l)	188	35	4.00	12.8	8.00	8.80	6.00	37.5
Sulphate (mg/l)	640	86	32	36	32	14	14	122
Nitrate (mg/l)	52	90.0	14.0	4.0	24.0	-	7.20	31.9
Phosphate (mg/l)	83.2	16.0	4.00	4.80	2.40	2.60	1.60	16.4

KEY: A, B, C, D, E, F and G as depicted in materials and methods.

The values of the physicochemical parameters ranged as follow: temperature (24 – 26) ^{o}C with a mean value of 25°C, HCO$_3$ (2.0 - 4.6) with mean value of 2.94, pH (6.0 - 7.2) with mean value of 6.55, alkalinity (40 – 100) mg/l with mean value of 62.9 mg/l, hardness (60 – 120) mg/l with mean value of 89.14 mg/l, conductivity (200 – 400) μS/cm with mean value of 255.7 μS/cm, dissolved oxygen (1.1 - 2.0) mg/l with mean value of 1.67 mg/l, BOD (0.06 - 0.22) mg/l with mean value of 0.14 mg/l, chloride (28.4 - 106.4) mg/l with mean value of 51.2 mg/l, nitrite (4.0 - 188.0) mg/l with mean value of 37.5 mg/l, sulphate (14.0 - 640.0) mg/l with mean value of 122 mg/l, nitrate (0.0 - 90.0) mg/l with mean value of 31.9 mg/l and phosphate (1.6 - 83.2) mg/l with mean value of 16.4 mg/l (Table 1).

The values of mineral contents ranged as follows: potassium (80.3 - 110.5) mg/l having mean value of 94.4 mg/l, sodium (70.9 - 112.5) mg/l having mean value of 94.7 mg/l, magnesium (76.9 - 102.7) mg/l having mean value of 89.3 mg/l and calcium (77.5 - 110.7) mg/l having mean value of 94.9 mg/l (Table 2).

Table 2
Mineral values (mg/l) of water samples from Awotunde fish pond and river.

Mineral	A	B	C	D	E	F	G	Mean of concentration of mineral in water sample
K	80.5	80.3	89.9	98.9	105.3	119.5	95.5	94.41
Na	112.5	96.7	70.9	95.9	100.7	105.3	80.9	94.69
Mg	102.7	101.3	80.9	80.6	97.5	84.9	76.9	89.25
Ca	101.4	89.7	77.5	105.1	91.2	110.7	88.9	94.92

Legend: A, B, C, D, E, F and G as depicted in materials and methods.

The value of heavy metal contents ranged as follow: copper (0.0 - 0.1) mg/l having mean value of 0.1mg/l, manganese (0.0 - 0.2) mg/l having mean value of 0.13 mg/l, nickel (1.5 - 2.45) mg/l having mean value of 2.09 mg/l, lead (0.0 - 0.2) mg/l having mean value of 0.03 mg/l and iron (0.1 - 0.6) mg/l having mean value of 0.26 mg/l (Table 3).

Table 3
Heavy metal values (mg/l) of water samples from Awotunde fish pond and river.

Heavy Metal	A	B	C	D	E	F	G	Mean of concentration of heavy metals in water samples
Cu	-	-	-	-	0.1	-	0.1	0.10
Mn	-	0.1	0.1	0.1	0.2	0.2	0.1	0.13
Ni	1.5	2.0	2.7	2.5	2.5	1.9	1.6	2.09
Pb	2.0	1.0	1.0	1.0	1.0	1.0	-	1.17
Zn	0.5	0.2	0.2	0.2	0.3	0.4	0.5	0.33
Fe	0.2	0.1	0.1	0.2	0.4	0.6	0.2	0.26

Legend: A, B, C, D, E, F and G as depicted in materials and methods.

4. Discussion

The microbial values indicated very high bacteriological contamination due to influence of anthropogenic activities. This is in accordance with the study of (Fafioye, 2011) who stated that bacterial population was in a high yield in Kajola fish pond in Ibadan, Nigeria. The high bacterial and coliform loads could be attributed to the poor sanitary and unhygienic practices around the pond and management's workers, activities of human in and around the inflow river/spring. As observed in the study location, the micro-flora of the soil around the river could also find their way into the river and consequently contaminate the river and ponds. However, high coliform loads observed in this study is an indication of faecal contamination from the animals in the poultry that is constructed on the pond which supplies poultry waste as feeds (Pelczar, 1993; Odeyemi *et al.*, 2011).

The mean values of the physicochemical parameters gotten from this study are within the range the Aqua culture (Tucker and Robinson, 1990). For instance, the recorded pH 6.55 falls within recommended value of 5.9 and 6.8 according to Randall (1991), the mean dissolved oxygen of 1.67 mg/l is slightly below but temperature of 25°C is above the recommended value according to Neil and Bryan (1991). The mean of total hardness of 89.1 mg/l falls within 75-150 mg/l according to Boyd (1990) while the recorded mean alkalinity of 62.9 mg/l will support nitrification process as documented by Neil and Bryan (1991).

According to the results gotten from the river and pond water samples, the concentrations of the heavy metals were slightly different in variation. Water samples from point E (Point along the river in between the two ponds on both sides) and F (Point along the river just after the fish ponds) have the highest concentration of the heavy metals. Furthermore, concentration of Ni and Pb showed the highest amounts in all the sampling points/samples. However, the heavy metal concentrations were still below critical limit as suggested by Ward (1995) and in the tolerable average heavy metal concentration range for the world rivers (WHO, 1995; Lokhande and Kelkar, 1999).

The presence of several compounds in the pond as observed in this study can best be discussed considering the fact that run-off water from adjacent and nearby farmlands flows into the river that feed the pond, thereby having in possession several inorganic toxic compound from residues of fertilizers. With this, coupled with the microbial counts obtained portends the fish harvested from the pond be a vehicle for food poisoning and since the river serve as a source of water for domestic usage for some people upstream, an outbreak of water borne diseases could be imminent.

5. Conclusion

The bacteriological and physicochemical condition of the Awotunde fish pond and river therefore justify the conclusion that the pond was becoming increasingly polluted and thus, becoming dangerous to fish and human, as it inhibits proper growth of fish and ultimately decrease fish production and consequently affects the entire human and their assets. However, since apparent fish related disease outbreak has not been reported at the time of this investigation, it should be put on awareness that consumption of the infected fish by humans might lead to outbreak of infection. Furthermore, the presence of bacteria suggests the need for following strict hygiene during the management of fish pond to prevent the transfer of potentially pathogenic bacteria to human. Similarly, there is need for the Nigeria fish farms and industry to improve on their fish farming management, such as, fish food preparation, environmental sanitation and monitoring for good quality fish yield.

References

AOAC, 2005. 18th Ed. Association of official Analytical Chemists.

Barrow, G.I., Feltham, R.K.A., 1993. 3rd edn. Cambridge: Cambridge University Press.

Boyd, C.E., 1990. Water quality in pond for Aquaculture Auburn: AL: Auburn University/Alabama Agricultural Experiment Station.

Eja, M.E., Ogiri, O.R.,- Arikpo, O.E., 2003. Bioconcentration of Heavy Metals in surface sediments from thgreat Kwa River Estuary, Calabar, Southeastern Nigeria. J. Nig. Environ. Soc. 1, 247-256.

ENVIRONMENTAL PROTECTION AGENCY, 1996. Quality Criteria for water U.S. EPA, 440/9-76-023. Environmental Protection agency Washington D. C.

Fafioye, O.O., 2011. Preliminary studies on water characteristics and bacterial population in high yield Kajola fish ponds. J. Agric. Exten. Rur. Develop. 3(3), 68-71.

Fonseca, L.F., 2000. Concentration of hardness, alkalinity and nitrate in water used for cleaning milk equipment in Brazilian diary farms. Proc. X[th] ISAH Maastricht, the Netherlands. 100-103.

Forstener, 2001. Metal pollution in the Aquatic Environment. Springer-Verkigi Berlin, 220-238.

Hojoh, B.O., Sonng, A.W., 2003. Sources of water and Microorganisms Associated with water. J. Environ. Microb. 22, 10-12.

Jarup, L., Hellstrom, L., Alfven, T., Carlsson, M.D., Grubb, A., Persson, B., 2000. Low exposure to cadmium and early kidney damage:the OSCAR study. Occup. Environ. Med. 57, 668-72

Lapygina, E.V., Lysak, L.V., Zvyaginstsev, D.G., 2002. Tolerance of soil bacterial complexes to salt shock. Microbiol. 71, 143-147.

Lenntech, 2008. Water treatment & air purification Holding B.V.Rotterdamseweg, 402M 2629 HH Delft, The Netherlands,

Lokhande, R.S., Kelkar, N., 1999. Studies on heavy metals in water of Vasai Creek , Maharashtra. Indian J. Environ. Protect. 19, 664-668.

Neill, W.H., Bryan, J.D., 1991. Responses of Fish to temperature and oxygen and response integration through metabolic scope. Aquaculture and Water quality, Brune D. E and J. R. Tomasso, eds Baton Rouge. World Aquacult. Soc. 62-115.

Odeyemi, A.T., Dada, A.C., Akinjogunla, O.J., Agunbiade, O.R., 2011. Bacteriological, physicochemical and mineral analysis of water used in abattoirs in Ado-Ekiti, Southwest Nigeria. J. Microbiol. Biotech. Res. 1(2), 14-20.

Olutiola, P.O., Famurewa, O., Sonntag, H.G., 1991. An Introduction to Microbiology. A Practical Approach. Printed in the federal Republic of Germany.

Parker, W.I., Thompson, I.R., Melcer, R.M., 2001. Fate of volatile organic compound in municipal activated sludge. J. wat. Environ. Res. 65, 58-90.

Pelczar, M.J., 1993. Microbiology of natural waters, drinking water and waste water. Microbiol. Concepts Appl. 29, 821-823.

Prescott, L.M., Harley, P.T., Klein, N.R., 1999. Microbiology.5th ed. Pg. 510-527.

Randall, D., 1991. The Impact of Variation in water pH on fish. Aquaculture and Water Quality, Brune, D.E and J. R. Tomasso, eds Baton Kouge, LA: World Aquaculture Soci. Pp, 79-103.

Schroeder, G.L., 1980. Fish farming in manure loaded ponds, p 73-86. In ICLARM-SEARCA Conf. Agri. Aquac. Farm System, Manila, Philippines.

Tucker, C.S., Robinson, E.H., 1990. Channel Catfish Farming Handbook New York: Van Nostrand Reinhold, pp. 67-85.

Ward, N.I., 1995. Environmental Analytical Chemistry. In Trace Elements (Eds Fifield, F. W. and Haines, P. J.), Blackie Academic and Professional, UK, pp. 320-328.

World Health Organisation, 1995. Lead. Environmental Health Criteria, vol. 165. Geneva: World Health Organisation.