

Bacterial and Heavy Metals Analyses in Fish at Shawaka Area of Tigris River

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Abstract
This study was conducted from October to December 2012. 35 fish were collected from the Shawaka area of the Tigris river for quality analyses using bacteriological and chemical parameters. Results of bacteriological analysis showed that the total viable bacteria count in the fish gut, skin and gill were ranged from 35.35×10^3 - 6×10^3 cfu/g, 34.9×10^3 -21×10^3 cfu/g and 30.1×10^3 - 1.9×10^3 cfu/g respectiveluy. 151 bacterial isolates were obtained and identified into 14 genera (13 Gram negative bacteria + one Gram positive bacteria). The predominant bacteria from different organs were *Staphylococcus spp*., *E. coli*, *Proteus spp*., *Citrobacter spp*., *Enterobacter spp*. respectively. The results of study indicated that most fish samples were polluted with high levels of heavy metal Pb, Cd and Hg. Concentration of these heavy metals exceed limits for the fish recommended by the World Health Organization (WHO) and the Centre for Environment, Fisheries and Aquaculture Science (CEFAS).
Key word: Tigris river, Fish, Bacteria, Heavy Metals

1. Introduction

River Tigris is considered the main source of water in Baghdad and the change in water quality result to inadequate sanitation and sewerage. The Shawaka area water polluted through municipal wastewater, industrial wastewater and fisher men residues. There are clear interaction between fish species and the level of heavy metals (1) and fishes may carry bacteria before and after death (2). Bacteriological analysis of *Clarias gariepinus* fish in Asia river (Nigeria), including total heterotrophic count, total coliform and thermotolerant coliform counts revealed a high level of faecal pollution of the river (3). Recent study showed that *Vibrio spp*. are basically used as test organism for contamination of sea food (4). *Salmonella spp*. may reach aquatic environments through faecal contamination and it has been isolated from freshwater fish culture ponds in many countries (5). Untreated water and the improper way of sewage disposal system is one of the main sources for microbial water contamination which results in the accumulation of pathogenic bacteria species in the commercial edible fish (6). The mean of total aerobic bacteria load in the skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus*, samples were high and range from 10^{12}- 10^{13} cfu/g (7). The hygienic limits of heavy metals in fish muscle for human food are defined for copper, nickel, chromium, lead and cadmium (8). Absorbed zinc and other heavy metals can be distributed quickly to the other tissues and organs (e.g. bone, gills, kidneys, muscle) rather than accumulating in the liver and in the skin (9, 10).

This is the first study in Iraq was undertaken to investigate the bacterial and the heavy metals analyses in fish species of the Shawaka area of Tigris river.

2. Materials and Methods

2.1 Sampling site: Sampling was conducted from the Shawaka area on the Tigris river in Baghdad (Figure 1). The Shawaka area consider the central area for fishing, transport to another side of the river, swim area, inhabited and many pipes of sewerage.

2.2 Fish Sampling: Sampling activities were carried out from October to December 2012. Fresh caught live healthy fish were collected using conventional fish net traps in the early hours of the day. All captured fish were placed immediately into container containing river water and transported to the laboratory for fish identification, bacterial and heavy metals analyses within one hour of collection.

2.3 pH of Water: Water pH of each sample was measured by using a digital pH – meter (HANNA lnsturments).

2.4 Bacterial Enumeration and Identification: skin, gill, and gut samples of each fish were removed aseptically and collected separately in sterile containers containing sterile normal saline. Serial dilution was prepared in normal saline (0.85% NaCl) and plated onto Nutrient Agar (for total bacteria count) and different differential and selective media (Mannitol Salt Agar, MacConkey Agar, and Eosin Methylene Blue Agar). Tetrathionate broth and S. S. Agar for *Salmonella spp.* isolation. Alkaline peptone , TCBS and red ring test for *Vibrio spp* isolation. All the plates were incubated at 37^0 C for 24-48 hours. Various tests were used to identify the bacterial isolates and these include: indol, methyl red, Vp, citrate, TSI, urease, Gelatin, glucose, manitol, sorbitol, rhamnose, sucrose, melbiom, amygdalin and arabinose (11).

2.5 Determination of Heavy Metals (Pb, Zn, Cd, Cu and Mg): Muscle, bone and skin organs were dried using electric oven at 150^0 C for over night. One gram of each dry tissue mixed with 10 ml concentrated HNO_3 for one hour. Heating and evaporate until red vapor appears then cooling and added 5 ml of 30% H_2O_2 and reheating until end digest. Cooling the sample and added deionized distilled water to volume 25ml. The heavy metals concentration (Pb, Zn, Cd, Cu) were determined using atomic absorption flame (12). For mercury determination, mixed 1ml from the digested sample with 1 ml of separated solution (dithozone+ CCL_4) in separated funnel. Repeat the separation three times. The organic layers washed by weak alkaline NH_4OH three times until change the organic layer of green to orange and the last wash by acetic acid (2M). The mercury concentrations were determined using UV-visible spectrophotometer (485λ) (13).

2.6 Statistical Analysis; Results are presented as mean ±S. D.

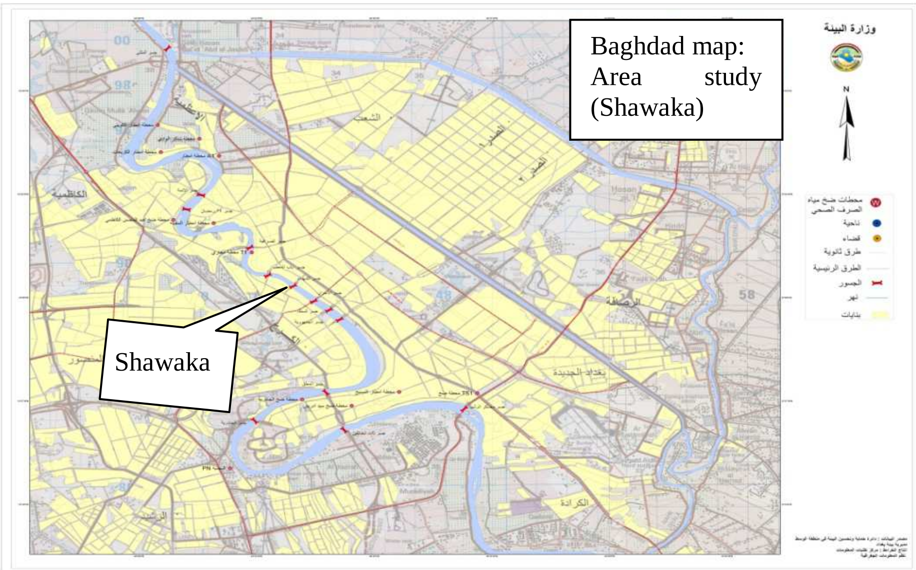

Figure 1: Map of sampling location, Tigris river in Baghdad, Iraq

3. Result

The present study showed that the 35 fish under study were classified into eleven genera (11 species) (table 1).

Table 1: Fish species checklist from Tigris river (Shawaka area)

No.	Fish species	Common name	Number of samples
1	*Liza abu*	khishni	3
2	*Cyprinus carpio*	Common Karp	2
3	*Cyprinion macrostomus*	Bunni Kaper	5
4	*Varicorhinus damascinus*	Tela Demascus	5
5	*Chondrostoma regium*	Baloot Muluki	4
6	**Mystus pelusius*	Abu Zummair	5
7	*Barbus luteus*	Himri	3
8	*Gara rufa*	Karkoor Ahmar	3
9	*Carassius auratus*	Carp Thahabi	3
10	*Mastacembelus Mastacembelus*	Marmaritc	1
11	**Heteropneustes fossilis*	Ab- Alhaka	1
	Total		35

*Exotic

Table 2 revealed the highest mean density of total bacteria was 35.35×10^3 cfu/g in guts of *Cyprinus carpio* but the lowest value was 6×10^3 cfu/g in *Mastacembelus mastacembelus.* It was found that the highest density of total bacteria in gill was 30.1×10^3 cfu/g in *Mystus pelusius* but the lowest value was 1.9×10^3 cfu/g in *Mastacembelus mastacembelus*. Thereafter, the highest density of total bacteria in skin was 34.9×10^3 cfu/g in *Mystus pelusius* and lowest value was 2.1×10^3 cfu/g in *Carassius auratus*.

Table 2: Mean (×10^{3}cfu/g) of total bacteria counts obtained from skin, gill and gut of fish samples

No.	Fish species	skin	Gill	Gut
1	*Liza abu*	3.866	3.166	11.2
2	*Cyprinus carpio*	18.7	3.7	35.35
3	*Cyprinion macrostomus*	9.675	13.4	31.72
4	*Varicorhinus damascinus*	8	16.86	18.92
5	*Chondrostoma regium*	10.6	14.55	12.4
6	*Mystus pelusius*	34.9	30.1	26.86
7	*Barbus luteus*	13.36	4.63	30
8	*Gara rufa*	6.45	20.11	25
9	*Carassius auratus*	2.1	16.3	25
10	*Mastacembelus Mastacembelus*	9.5	1.9	6
11	*Heteropneustes fossilis*	3	2	18

In this study, 151 bacterial isolates were obtained and identified into 14 genera (13 Gram negative bacteria + one Gram positive bacteria). Figure 2 showed high number of bacteria species in the guts and *Escherichia coli* was found the most prevalent organism (16/83), thereafter, *Proteus vulgaris*, *Staphylococcus spp*., *Citrobacter freundii*, *Enterobacter agglomerus* and *Citrobacter diversus* respectively. The highest number of bacterial isolates in gill was *Staphylococcus spp.* (8/22), then *E. coli*, *C. freundii* and *Klebsiella oxytoca* respectively. But only one bacterial isolate recorded for *Salmonella* sp., *Yersinia enterocolitis*, *Providencia rettgeri* and *Morganella morganii* from gut, and *Serratia liquefacient* from skin (figure 2).

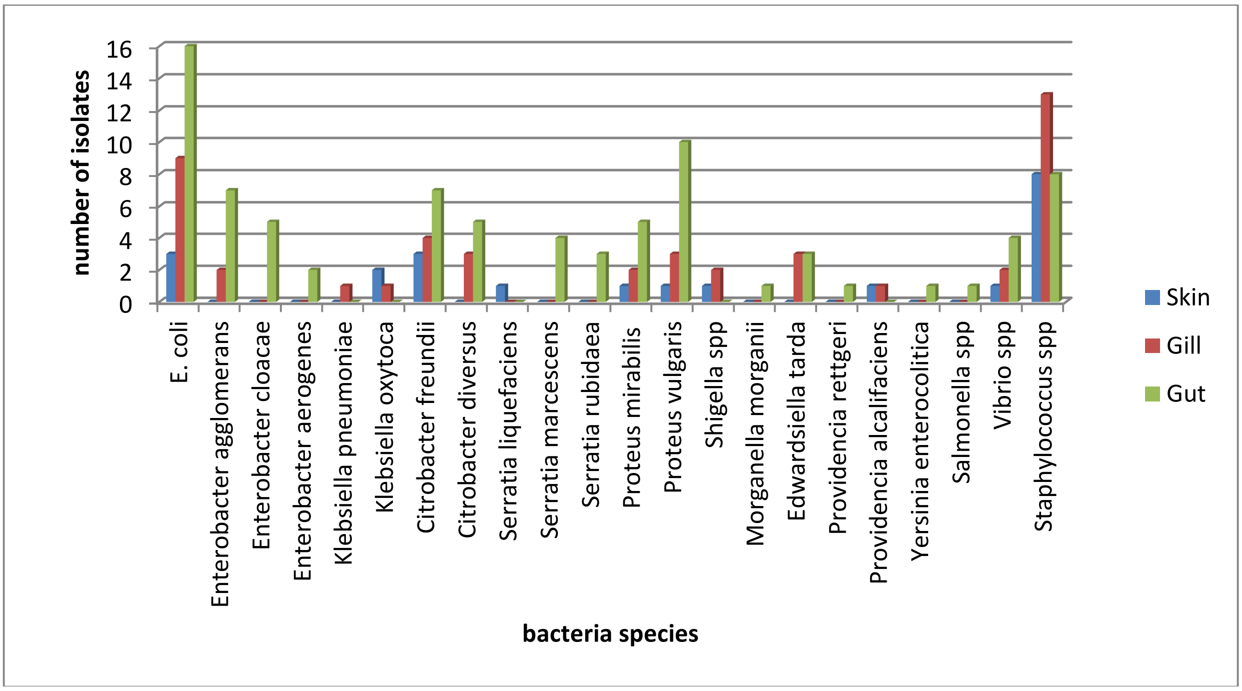

Figure 2: Type and Number of Bacterial Isolates

Table 3 showed the mean concentration of Pb in the tissue of fish species studied varied from 0.6 mg/kg in *C. carpio* to 5.6 mg/kg in *B. lutus*. But Zn was ranged from 10.5mg/kg in *C. carpio* to 576 mg/kg in *B. luteus*.

Highest mean concentration of Cu was 4.5mg/kg in *B. luteus* and lowest value was 0.5mg/kg in *C. carpio*. Cd concentration was ranged from 0.5 to 2.2 mg/kg in *M. mastacembelus* and in *H. fossilis* respectively. Finally Hg concentration recorded maximum value in *H. fossilis* (3.4 mg/kg) and minimum value in *M. mastacembelus* (0.16 mg/kg).

Table 3: Mean concentration (mg/kg) of Pb, Zn, Cu, Cd and Hg in the tissues of the fish species (mean± standard deviation).

No.	Fish species	Pb	Zn	Cu	Cd	Hg
1	*Liza abu*	3.625 ±1.187	26.825 ±3.946	1.4 ±0.294	0.9575 ±0.507	1.365 ±0.935
2	*Cyprinus carpio*	0.6 ±0.141	10.5 ±0.707	0.5 ±0	0.35 ±0.070	0.48 ±0.169
3	*Cyprinion macrostomus*	3.44 ±0.661	21.74 ±12.95	1.28 ±0.676	0.84 ±0.357	1.284 ±0.733
4	*Varicorhinus damascinus*	3.18 ±1.314	21.94 ±7.537	1.26 ±0.450	0.84 ±0.427	1.612 ±1.100
5	*Chondrostoma regium*	4.4 ±1.507	37.475 ±32.60	2.275 ±1.059	1.488 ±1.157	2.38 ±1.619
6	*Mystus pelusius*	3 ±1.867	21.25 ±10.64	1.425 ±0.699	0.838 ±0.534	1.32 ±1.248
7	*Barbus luteus*	5.667 ±0.503	576.267 ±233.809 2	4.533 ±0.450	1.3 ±0.278	1.567 ±0.404
8	*Gara rufa*	2.933 ±1.504	13.167 ±5.107	1.1 ±0.173	0.8 ±0.7	1.553 ±1.207
9	*Carassius auratus*	3.3 ±0.964	29.6 ±15.245	2 ±0.7	0.4 ±0.360	0.913 ±1.124
10	**Mastacembelus Mastacembelus*	1.0	17.5	0.6	0.15	0.16
11	**Heteropneustes fossilis*	5.5	66.0	2.9	2.2	3.4

*Data without replication

The present results showed that the pH of water samples during the research period was 6.

4. Discussion

The river fish are economically important. Shawaka area is characterized as an important center for fishing and sale of large and small fish. Poor families depend on consuming small fish for low price. Therefore, the study tended to determine the bacterial content and heavy metals concentration of economically important fish and exotic fish in the same area. It found that nine fish species important economically and two fish species exotic (*M. pelusius* and *H. fossilis*) (table 1).

Water river are harbour to bacterial contamination and cause health risks to human. The Shawaka area is highly polluted by domestic discharges wastewater (14). The present study revealed variable total viable bacteria count for different fish organs (gill, gut and skin). Results showed that the total viable counts in the guts were ranged from 35.35×10^3 to 6×10^3cfu/g. Such results have been noticed, Gotas *et al* reported that the range from 14×10^3 to 7×10^3cfu/g (15). Many authorities report that the highest counts of bacterial flora is the digestive tract reach to 10^{13} cfu/g (7,16). The total viable bacteria counts in the skin and gills were ranged from 34.9×10^3 to 21×10^3cfu/g and 30.1×10^3 to 1.9×10^3cfu/g respectively. Bacteria recovered from the skin and gills may be transient rather than residing on the fish surfaces (17).

Different species of bacteria were isolated and identified. It was recorded 83/151 bacterial isolates from guts , 46/151 from gills and 22/151 from skin. The predominant bacterial isolates were *Staphylococcus spp*., *E. coli*, *Proteus spp*., *Citrobacter spp*., *Enterobacter spp*., *Serratia spp*., *Vibrioo spp*., *Edwardsiella sp*., *Klebsiella spp*., *Shigella spp*., *Providencia spp*., *Morganella sp*., *Yersinia spp.* and *Salmonella spp.* respectively. *Proteus* and

Staphylococcs were more predominant with frequency of 100% and 70% respectively(18). Previous research revealed that the *Staphylococcus spp*. were not found in water samples from fishpond cultivation, but isolated from tegument, gut and fresh fillets (19). This untreated and the improper way of sewage disposal system is one of the main sources for microbial water contamination which results in the accumulation of these pathogenic bacteria species in the commercial edible fish (6).

Results showed that the high levels of heavy metals Pb, Cd and Hg found in the muscle of different fish species according to WHO and FDA. Pb concentration in fish muscle species was ranged from 0.6 to 5.6 mg/kg and exceed limits for fish recommended by WHO is 0.3 mg/kg and FAO is 0.5mg/kg (20, 21).High level of Cd concentration found in 90% of fish muscle samples and exceed limits recommended by WHO is 0.2 mg/kg for fish. Hg showed high concentration in 81% of fish muscle samples and exceed level permitted in CEFAS is 0.1-0.3mg/kg (20, 22). Both of Zn and Cu showed only high concentration in 9% of fish muscle samples when compared with level permitted Zn 75 mg/l and Cu 3 mg/l (23).

The concentration of heavy metals limits in Iraqi water sources according to Environmental Protection and Improvement Directorate in Centric Region, 2012 are Pb 0.05 mg/l, Zn 0.5 mg/l, Cu 0.05 mg/l, Cd o.oo5 mg/l and Hg o.oo1 mg/l (24). There are more than ten fold for heavy metals concentration in fish muscle. The heavy metal accumulation in small and large fish is important in the food chain (25).

The present study revealed that the pH of water samples was 6. The average pH of water ranged between 6.8±0.1 to 6.9±0.3 showing the slightly acidic condition (16), The pH of the aquatic system is an important indicator of the water quality and the pollution in watershed areas (26).

Conclusion

The results of this study conclude that the total viable count, bacteria species and heavy metal concentration reflect the validity of fish consumption from such area. Thus, it is necessary that biological monitoring of the water and fish to ensure the continuance safety.

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References

1. El-Shehawi A.M., Ali F.K. and Seehy M.A. Estimation of water pollution by genetic biomarkers in tilapia and catfish species shows species-site interaction. African Journal of Biotechnology. 2007, 6(7): 840-846.
2. Dave S.M. Isolation and identification of bacteria originating from a larvivorous fish, *Aphanius dispar*. Life Sciences Leaflets. 2011, 19:823-829.
3. Kolawole O.M., Ajayi K.T., Olayemi A.B. and Okoh A.I. Assessment of water quality in Asia river (Nigeria) and its indigenous *Clarias gariepinus* fish. Int. Environ. Res. Public Health. 2011,8: 4332-4352.
4. Adebayo-Tayo B.C., Okonko I.O., John M.O., Odu N.N., Nwanze J.C. and Ezediokpu. Occurrence of potentially pathogenic *Vibrio* species in sea foods obtained from Oron Creek. Advances in Biological Research. 2011, 5(6): 356- 365.
5. Lotfy N.M., Hassanein M.A., Abdel-Gawad F.Kh., El-Taweel G.E. and Bassem. Detection of *Salmonella spp*. in aquatic insects, fish and water by MPN-PCR. World Journal of Fish and Marine Sciences. 2011,3(1): 58-66.
6. Sujatha K., Senthilkumaar P., Sangeetha S. and Gopalakrishnan. Isolation of human pathogenic bacteria in two edible fishes, *Priacanthus hamrur* and *Megalapis cordyla* at Royapuram waters of Chennai, India. Indian Journal of Science and Technology. 2011, 4(5):539-541.
7. Emikpe B.O., Adebisi T. and Adedeji O.B. Bacteria load on the skin and stomach of *Clarias gariepinus* and *Oreochromis niloticus* from Ibadan, South West Nigeria: public health implications. Journal of Microbiology and Biotechnology Research. 2011, 1(1): 52-59.
8. Kacaniova M., Andreji J., Stranai I., Hascik P., Cubon J. and Felsociova. Microbiological quality of fish meat and the effect on the heavy metals contents. Slovak J. Anim. Sci. 2007, 40(4):185-188.
9. Al-Weher S.M. Levels of heavy metal Cd, Cu and Zn in three fish species collected from the Northen Jordan Valley, Jordan. Jordan Journal of Biological Sciences. 2008, 1(1): 41-46.
10. Yilmaz A.B. Comparison of heavy metal levels of grey muller (*Mugil cephalus L.*) and sea bream (*Sparus aurata L.*) caught in Lskendrum Bay (Turkey). Turk. J. Vet. Anim. Sci. 2005, 29: 257-262.
11. Forbes B.A., Sahm D.F. and Weissfeld A.S. Diagnostic Microbiology.11th edition,Bailey & Scotts. Mosby, Missouri. 2002.
12. Novozamsky I., Houba V.J.G., V-Eck R. and Vark W. A novel digestion technique for multi-element plant analysis. Communications in soil science and plant analysis. 1983, 14: 239-248.

13. Stecher P.G.The Merck index: An encyclopedia of chemicals and drugs. 8th edition, published by Merck. Co., Inc. USA, 1968.
14. Ibrahim I.AJ., Ismail M.I. and Ibrahim Y.AJ. Estimation of validity Tigris river water for swimming in Baghdad city. Advances in Physics Theories and Applications. 2013, 18:14-21.
15. Golas I., Lewandowska D., Zymslowska I. and Teodorowicz. Sanitary and bacteriological studies of water and European catfish (*Silurus glanis L*.) during wintering.Archives of Polish Fisheries. 2002, 10(2):177-186.
16. Roy R.P. and Barat S. Influence of water quality on the bacterial contamination of resident loach, lepidocephalichthys guntea (*Hamilton buchanan*) and on a Terai river Lotchka of Derjeeling District, West Bengal, India. Archives of Environmental Science. 2011,5: 116-123.
17. Cahill M.M. Bacterial flora of fishes: A review. Microbial Ecology. 1990, 19:21-41.
18. Atuanya E.L., Edefetah M.A. and Nwogu N.A. Microbiological qualities and some heavy metals (Mercury and Cadmium) levels of fresh and dry fish species solid in Benin city, Edo State, Nigeria. Bulletin of Environment, Pharmacology and Life Sciences. 2011, 1(1): 10-14.
19. Boari C.A., Pereira G.I., Valeriano C., Silva B.C., Morais V.M., Figueiredo H.C.P. and Piccoli R.H. Bacterial ecology of tilapia fresh fillets and some factors that can influence their microbial quality. Ciència e Tecnologia de Alimentos. 2008, 28(4): 863- 867.
20. WHO. Review of potentially harmful substances-cadmium, lead and tin. WHO,Geneva.(reportandstudiesNo.22.MO/FAO/UJESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Aspects of Marine Pollution)1985. (Cited by: Sary A.A. and Mohammadi M. Human health risk assessment of heavy metals in fish from freshwater. Research Journal of Fisheries and Hydrobiology. 2011, 6(4): 404-411
21. FAO. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fisheries Circular No. 764. Food and Agriculture Organization of the United Nations,Rome, October, 1983.
22. CEFAS (The Central for Environment, Fisheries & Aquaculture Science). Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1994. Science series, Aquatic Environment Monitoring Report No. 47, 1997.
23. Nwani C.D., Nwachi D.A., Okogwu O.I., Ude E.F. and Odoh G.E. Heavy metals in fish species from lotic freshwater ecosystem at Afikpo, Nigeria. Journal of Environmental Biology. 2010, 31(5): 595-601.
24. - Environmental Protection and Improvement Directorate in Centric Region.
Ministry Environment/ Republic of Iraq 2012 (Arabic Text).
25. Demanou H. and Brummett R.E. Heavy metal and faecal bacterial contamination of urban lakes in Yaounde, Cameroon. Food Africa. 2003, 5-9 May.
26. Yisa, J. and Jimoh, T. Analytical Studies on Water Quality Index of river Landzu. American Journal of Applied Science, 2010,7 (4): 453-458.