An Assessment Of Heavy Metals Accumulation In Clarias gariepinus, (Burchell 1822) And Wastewater Reservoir Of Coca Cola Company In Maiduguri Borno State, Nigeria

Fabian Zira Lawrence* and Abubakar Abdulrahman Kotos**
Department of Zoology,Modibbo Adama University of Technology Yola, Adamawa State Nigeria
Zifabian2002@yahoo.com

Abstract: Routine laboratory analyses were carried out to determine the concentration of heavy metals in wastewater reservoir and fish tissue (Muscles and Gills). Wastewater has the highest concentration of heavy metals than in the fish tissue in the following order Cu>Fe>Cd>Mn>Zn, while Pb is below detection level. Among the tissue, gills were observed to have the highest concentration in the following order Fe>Cd>Cu>Mn>Zn, while Pb was also below detection limit. Mn concentration in fish has exceeded the international permissible limit by the World Health Organisatio, Food and Agricultural Organisation, and American Public Health Association. These therefore pose a threat to the consumer and the survival of the fish in the wastewater and necessitate the need for caution and stricter wastewater effluent discharge for a better water quality.

Keywords: Accumulation, Coca cola, Heavy metals, Wastewater reservoir,

INTRODUCTION

Water reservoirs are collectors of all materials spread by human, industrial and agricultural activities and these penetrates into the water reservoir via atmosphere, drainage, soil waters and soil erosion (Stainiskiene, et al., 2006). The reservoirs or river system and indeed all surface water may be excessively contaminated with heavy metals, and this contamination may have devastating effect on the ecological balance of the aquatic environment (Farombi, et al., 2007) and the diversity of organism become limited with extent of contamination.

Pollution of aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fishes (ECDG, 2002). Metal ions can be incorporated into food chain and concentrated in aquatic organisms. Of the effective pollutants are the heavy metals, which have drastic environmental impact on all organisms (Mason, 2002). The most anthropogenic source of metals is industrial, petroleum contamination and sewage disposal (Santos *et al.*, 2005). These metals include; Iron, Zinc, Cadmium, Manganese, Lead and Copper amongst others.

The general acceptance is that wastewater reuse in agriculture is justified on agronomic and economic grounds, although care must be taken to minimize adverse health and environmental impacts (Reitveld *et al.*, 2009; Sowers, 2009). Furthermore wastewater reuse is increasingly becoming important for supplementing drinking water needs in some countries around the world. The option of reuse of wastewater is becoming necessary as a result of increased climate change, thus leading to drought and water scarcity, and the fact that wastewater effluent discharge regulations have become stricter leading to a better water quality (Rietveld *et al.,* 2009).

This study will therefore determine the level of concentration of heavy metals in fish tissue (Muscles and Gills) and wastewater in the reservoir. The result will provide an early warning guide to fish consumer and regulatory agency for in the study area.

MATERIALS AND METHODS

Study area: Maiduguri is the capital of Borno State and is located between latitude 10^{o}09'and 13^{o}44' N and longitude 11^{o}36' and 14^{o}38' E. Most part of the state lies within the sahelian climate zone classified under the tropical continental climate with rainfall of 250 – 1000mm. the temperature regime of the state is relatively more constant than that of rainfall pattern. The hottest months of the year are March, April, and May with mean temperature of 29.5, 32.8, and 34.5^{o}C respectively (Aminu, and Omoyeni, 2001).The plant and reservoir constructed in 1978 are located at Pompomari industrial area and the reservoir covers an estimated area of about 43,200sq meter with an approximate depth of about 6 meters.

The study was conducted through routine analysis of detecting some heavy metals in the wastewater reservoir from July to December 2013. Wastewater samples were collected fortnight at 08:00Hrs local time at a depth of 30cm below the water surface. Wastewater samples were collected at three different points (Station 1, 2 and 3) which the reservoir is divided into for the purpose of this study. Station 1 is the area of discharge, station 2 is the area covered by macrophytes and station 3 is the open water were fishing activity takes place. Wastewater samples were collected in triplicate for the analysis.

Fish were sampled by the fishermen using various size of gill net; 2", 2.5" stretched mesh size, Malian traps which was set at 5.00pm and was retrieved early morning the following day. Fish were sorted out from the catch as described by Olurin and Aderibigbe (2006).

Fish tissue sample (muscles and gills) was digested as described by Oliafa *et al.,*(2004). Five grams (5g) each from Ten (10) fish samples was exercised from the tissues and was weighed with electronic balance (Model: SPU 411) placed in beaker of 10ml of freshly prepared trioxonitrate v acid ($HNO_{3)}$ and hydrogen peroxide (H_2O_2) (1:1) solution.

Collected water samples were digested with 1:1 nitric acid EPA (1996). The digested water samples were transferred to centrifuge tube before added with 2ml of 1% Sodium Sulfide and 30% of Aluminum Sulfate solutions. Water was left for five minutes before centrifugated at 1500rmp for 20minutes and the supernatant was discarded. The remaining was treated with 4ml M nitric acid at 50^{o}C before analysis using FAAS. Calibration curve: Aqueous stocks solutions was prepared for cadmium (Cd), Copper (Cu), Lead (Pb), Iron (Fe), Zinc (Zn) and Manganese (Mn) using the appropriate salts. Six working standard was prepared in duplicate for each metal by serial dilution of stock solutions for fish (120) while water samples was in triplicate for three sample area (54).

RESULTS

A comparative result of monthly variation of Iron concentration in water muscles and gills shows that Fe concentration was constantly high in water than in muscles and gills throughout the period understudy (July to December). The highest value of 1.62$\pm$0.09mg/l was recorded in water in the month of July and the lowest value of 1.14$\pm$0.25mg/l was recorded in September. Gills recorded the next higher concentration with its highest value of 0.72$\pm$0.16mg/kg recorded in the month of July and its lowest value of 0.26$\pm$0.07mg/kg recorded in November. Muscles recorded the lowest concentration of Fe among the three samples and have its highest value of 0.53$\pm$0.18mg/kg in the month of July and its lowest value of 0.29$\pm$0.08mg/kg recorded in the month of November (Table 1). The correlation between Iron in water, muscles and gills were all higher with the highest observed between the muscles and gills (r=0.9844). The monthly mean variation for the three samples ranged from1.3$\pm$0.15mg/l for Iron concentration in water and 0.38$\pm$0.09mg/kg for Iron concentration in muscles (Table 2). This variation was markedly significant statistically ($p<0.05$) and there was no significant difference between muscles and gills ($p>0.05$).

Similarly, the comparative results for monthly variation of Zn in water, muscles and gills revealed that zinc concentration was also high in water than in both the muscles and gills almost throughout the period of the study (July to December). The highest value of 0.47$\pm$0.04mg/l was recorded in water in the month of August and the lowest value of 0.30$\pm$0.09mg/l was recorded in December. Gills concentration was 0.33$\pm$0.05mg/kg in December and 0.22$\pm$0.25mg/kg in September Muscles recorded the lowest concentration of Zn among the three samples and have its highest value of 0.30$\pm$0.07mg/kg recorded in the month of December and its lowest value of 0.21$\pm$0.11mg/kg in the month of September (Table 1). The variables also revealed high correlation values with the highest observed between the muscles and gills. The monthly mean variation for the three samples ranged from 0.40$\pm$0.07mg/l for Zn concentration in water to 0.25$\pm$0.03mg/l for Zn concentration in muscles (Table 2). This variation was markedly significant statistically ($p<0.05$) and there is no significant difference between muscles and gills.

A comparative result of cadmium in water, muscles and gills again shows a constantly higher Cd concentration in water than both muscles and gills as observed for the period understudy (July to December). The highest value of 0.75$\pm$0.02mg/l was recorded in water in the month of July and its lowest value of 0.54$\pm$0.02mg/l in the month of October. Gills recorded its highest mean value of 0.68$\pm$0.12mg/kg in September and the lowest value of 0.37$\pm$0.12mg/kg in November. Muscles also recorded the lowest concentration of Cd among the three samples and have its highest value of 0.62$\pm$0.13mg/kg recorded in the month of August and its lowest value of 0.42$\pm$0.08mg/ recorded in the month of September (Table 1). Also the correlation was high within the variables and the highest was also observed between the muscles and gills. The monthly mean variation for the three samples ranged from o.6$\pm$0.075mg/l for Cd concentration in water to 0.55$\pm$0.07mg/kg for Cd concentration in muscles (Table 2). This variation was markedly not significant statistically ($p>0.05$).

Concentration variation of manganese is also revealed higher in water than the muscles and gills for the period understudy. The highest value of 1.30$\pm$0.07mg/l was recorded in water in the month of August and October and the lowest value of 0.79$\pm$0.08mg/kg was recorded in the month of July and December. Gills recorded its highest value of 0.54$\pm$0.11mg/kg in the month of August and its lowest value of 0.32$\pm$0.08mg/kg in November. Muscles also recorded the lowest concentration of Mn among the three samples and have its highest value of 0.42$\pm$0.11mg/kg recorded in the month of July and December and its lowest value of 0.30$\pm$0.07mg/kg recorded in the month of November (Table 1). High correlation values were observed between all the variables and the highest was between muscles and gills. The monthly mean variation for the three samples ranged from 1.08$\pm$0.23mg/l for Mn concentration in water to 0.39$\pm$0.04mg/l for Mn concentration in muscles (Table 2). This variation was markedly significant statistically ($p<0.05$) and there is no significant difference between muscles and gills ($p>0.05$).

The comparative monthly variation of copper in water, muscles and gills also revealed that the constantly higher concentration of copper in water than in both muscles and gills was observed throughout the period under study. The highest value of 2.19$\pm$0.15mg/l was recorded in September and its lowest value of 1.40$\pm$0.04mg/l was recorded in the month of July. Gills recorded its highest value of 0.74$\pm$0.18mg/kg in July and its lowest value of 0.29$\pm$0.13$mg/kg \pm$ in November. Also muscles recorded the lowest concentration of copper and its highest value of 0.74$\pm$0.18mg/kg in the month of July and its lowest value of

$0.35\pm0{,}07$mg/kg in the month of November and December (Table 1). Correlation was highest between muscles and gills. The monthly mean variation for the three samples ranged from 1.81 ± 0.31mg/l for copper concentration in water to 0.48 ± 0.14mg/kg for concentration of copper in both muscles and gills (Table 2). This variation was markedly significant statistically ($p<0.05$) with also no significant difference between muscles and gills ($p>0.05$).

DISCUSSION

Iron (Fe) is required for the nutrition of fish and is essential for its metabolism (NRC, 1993; Canli and Atli, 2003); however, the amount present in these fish is within the safety limits recommended by WHO for fish, i.e 43mg/kg (WHO, 2003; FAO,1985; APHA, 1990). The concentration of this metal observed in this study is higher in wastewater than in fish tissue (muscles and gills) which shows that water is the reservoir of pollutants. The highest concentration observed in water was $1.62\pm0.07 mg/l$ while in the fish tissue, gills recorded $0.72\pm0.16 mg/kg$ and the muscles recorded $0.53\pm0.18 m$g/kg all in the month of July. The presence of high concentration of Fe in the gills than muscles, agrees with Karuppasamy (2000) and Nussey (2001). Bogul (1997) reported the reduction in chemical parameters (Protein, Carbohydrate and Lipids) contents of the muscles and gills of fish when exposed to Iron for days. The correlation observed between the tissues might be as a result of the gills being the organs of respiration is always in contact with the metals in water and the unique nature of iron metabolism.

Zinc (Zn) is a nutritional requirement for aquatic animal growth and essential for metabolism (NRC, 1993; Canli and Atli, 2003). The concentration of Zinc in wastewater is also higher throughout the study than in the tissue (muscles and gills). The acceptable range of zinc in fish according to WHO (2003), FAO (1985) and APHA (1990) is within the range of 10-100mg/kg. The accumulation of zinc observed in this study is therefore not a hazard. The highest concentration observed in water was $0.47\pm0.04 mg/l$ in the month of September, while in fish tissue, the gills recorded the highest value of $0.33\pm0.18 mg/kg$ in December and the muscles recorded $0.30\pm0.07 mg/kg$ as its highest in December which also agrees with Karuppasmy (2001). Water being the reservoir of these metals has the highest concentration while the gills concentration may be due to its respiration function.

Although zinc is an essential element as it is carefully regulated by physiological mechanism in most organism. It is also regarded as potential hazards that can endanger animal health. Agency for Toxic Substances and Diseases Registry (ATSDR, 2005) states that ingesting high levels of zinc for several months may cause anemia, damage pancreas and decrease level of high density lipoprotein (HDL) cholesterol. Bogul, (1997), reported that biomonitoring of these metals is necessary for safe environment in the future.

Manganese is relatively non-toxic to aquatic biota and is seldom a problem in fish (USEPA, 1975). The mean value concentration is also observed to be higher in wastewater than in the fish tissue (muscles and gills) throughout the period of study. Roth (2006) reported that the exposure to high level of manganese causes toxicity and decreases the fitness of the organism. The acceptable limit of manganese in fish according to WHO (2003), FAO (1985) and APHA (1990) is 1.0mg/kg which the values obtained in this study are within the acceptable limit. The highest value recorded in water was $1.30\pm0.07 mg/l$ in August as well as in September, while the highest value in fish tissue was also observed in gills $0.54\pm0.11 mg/kg$ in August and the muscles recorded $0.42\pm0.11 mg/kg$ in July and December. Nussey (2001), reported that Manganese and Nickel behave similar to chromium when present in fish which affects the growth and reproduction even in sub-lethal level.

Cadmium have no Biological role and hence non-essential (Metcalf and Eddy, 2003; Hussein *et al.*, 2005). The mean monthly concentration of cadmium observed also revealed that wastewater have the highest concentration as compared to the fish tissue (muscles and gills) throughout the study period. Lethal concentration of cadmium in water may vary greatly,

ISSN 2229-5518

since the cadmium accumulation in fish depend on water quality (pH, DOC, etc), and sensitivity of the fish species (Hollis et al., 1999). Hollis *et al.*, (1999) also reported that cadmium is considered to be a respiratory toxicant. Sangalang and Freeman (1979) also reported that cadmium is a nephrotoxicant for fish. The acceptable limit of cadmium in fish according to WHO (2003), FAO (1985) and APHA (1990) is 0.05mg/kg and limit observed in this study exceed the permissible limit. The highest value recorded in water was $0.75 \pm 0.02 mg/l$ in July while the gills recorded the highest in fish tissue with a value of $0.68 \pm 0.12 mg/kg$ in the month of September and muscles recorded its highest value of $0.62 \pm 0.13 mg/kg$ in August. Vijaran (1998), reported that cadmium reduces the glycogen content in the muscles and liver of the fish, it inhibit the acid phosphate activity when present in the liver. Ruparallia *et al*., (1992), observed that cadmium behave in similar to Mercury when present in the tissue of fish by showing reduction in feeding, growth, oxygen consumption, and protein and SDH activity.

Copper (Cu) is also a nutritional requirement for aquatic animal growth and essential for metabolism (NRC, 1993; Canli and Atli, 2003). The concentration of Cu is higher in water than in fish tissue (muscles and gills) as also observed throughout the study period. The values in the fish tissue were well within the acceptable safe levels of 3.0mg/kg recommended by WHO (2003), FAO (1985) and APHA (1990). According to Beyer et al (2000), Cu at sub lethal concentration in fish decreases survival growth, productivity and mainly accumulates in gills and liver. The highest value observed in water was $2.19 \pm 0.15 mg/l$ while the muscles recorded the highest value in fish tissue with its highest monthly mean of $0.74 \pm 0.18 mg/kg$ in July and the gills also recording its highest value of $0.74 \pm 0.18 mg/kg$ also in the month of July. Gills are the organ of respiration and are always in contact with these metals. According to Wood (2001), the blood pH of fish was increased because of metabolic alkalosis in copper sub-lethal exposure, which may reflect in internal build up of ammonia in the plasma and tissue, a phenomenon that has indicated in reduced swimming performance. Furthermore, the decreased aerobic swimming performance of the fish may reflect changes in gills morphology, such as epithelial lifting, lamellar fusion, necrosis and apoptosis that is caused by cotisol (Bury *et al., 1998*). Patil and Dhande (2001) reported the effect of copper on reduction of cholesterol in the ovary of a fresh water fish, suggesting possibility that the heavy metal either block hormone action or the complex enzymes responsible for vitellogenesis. Also that copper induces hyperplasia in the kidney of a fish and reduces gonadosomatic index.

Lead which also have no biological role and hence non-essential (Metcalf and Eddy, 2003; Hussien *et al*., 2005), has been below detection limit in both water samples and fish tissue (muscles and gills) throughout the period of this study. The acceptable limit in fish tissue is 0.2mg/kg (WHO, 2003; FAO, 1985; APHA, 1990).

CONCLUSION AND RECOMMENDATION

This study found that the wastewater is a reservoir for heavy metals and the fish (*Clarias gariepinus*) was found to have accumulated heavy metals to various degrees in their tissue (muscles and gills) which is bioindicator for aquatic pollution. Therefore environmental quality and wastewater discharge regulations should be adhere to international standard for proper wastewater aquaculture and fisheries management in the region.

ISSN 2229-5518

Table 1: Monthly Variation in the Concentration of Heavy Metals in Coca Cola Wastewater Reservoir

	July	August	September	October	November	December	SEM
Iron	1.62	1.43	1.14	1.43	1.35	1.23	0.068928
Zinc	0.45	0.47	0.45	0.41	0.31	0.30	0.030596
Manganese	0.79[b]	1.30[a]	1.28[ac]	1.30[ac]	1.03[bc]	0.79[b]	0.10124****
Cadmium (ug/l)	0.75[a]	0.66[a]	0.68[a]	0.54[b]	0.56[b]	0.68[a]	0.032634**
Copper	1.40[b]	2.07[a]	2.19[a]	2.07[a]	1.53[b]	1.59[b]	0.138382****
Lead	ND	ND	ND	ND	ND	ND	

Table 2: Heavy metals concentrations in reservoir water, fish muscles and gills

Heavy metals	WATER	MUSCLES	GILLS
IRON	1.37[a]	0.38[b]	O.44[b]
ZINC	0.40[a]	0.25[b]	0.27[b]
CADMIUM	0.65	0.55	0.57
MANGANESE	1.08[a]	0.39[b]	0.45[b]
COPPER	1.81[a]	0.48[b]	0.48[b]

REFERENCES

[1] APHA (1990). (American Public Health Association) Standard methods for examination of water and west water, Clesceri L.S; AA Greenberg and R.R. Trussell 17th Edition pp 3144.

[2] ATSDR (2005). Agency for Toxic Substances and Disease Registry. Clifon road, Atlanta: division of toxicology.

[3] Aminu, R. and B. A. Omoyeni, (2001). Integrated Cultural, Economic and Environmental Requirements for Fish Production in Borno State.

[4] Beyer, W.N., Day, D., Melacon, M.J., and Sileo L. (2000). Toxicity of Anacostia River, Washington DC., USA, sedment fed to mute swam (Cygnus olor). Environmental Toxicology and Chemistry, 19. 731-735.

[5] Bogul, I. (1997). Water pollution by heavy metals and their impact on Fish and human health. Hrvatske vode. Pp 223-229.

[6] Bury, N. R., Codd, G. A., Wendelaar Bonga, S. E., and Flik G. (1998). Fatty acids from the cyanobacterium *Microcystiss aeruginosa* with potent inhibitors effects on fish gill Na^+/K^+-ATPase acitivty. *Journal of Experimental Biology* 2001: 81-89.

[7] Canli, M. and Atli, G. (2003). The relationship between heavy metals (Cd, Cu, Ni, and Pb) levels and the size of six Mediterranean fish species. Environmental pollution chemistry. 121, 129-136.

[8] ECDG (2002). European Commission DG ENV. E3 Project ENV. E3/ETU/0058. Heavy Metals in waste. Final report.

[9] EPA, (1996). U. S. Environmental Protection Agency, American Society of Civil Engineers, and American Water Works Association. Technology Transfer Handbook: Management of Water Treatment Plan Residuals. EPA/625/R-95/008. Washington DC.

[10] Farombi, E.O; Adelowo, O.A. and Ajimoko, Y.R. (2007). Biomarkers of Oxidative Stress and Heavy Metal Level As Indicators of Enviromental pollution in African Catfish, *Clarias gariepinus* from Nigeria, Ogun River, *International Journal of Environmental Research and Public Health Vol.* 4 (2) pp 150-155.

[11] Food and Agriculture Organization (1985). Codex Alimentarius Commission: Contaminants: Joint FAO/WHO Food Standard Programme. Codex Allimentarius, Vol. XVII, Ed, Roma, pp 1-13.

[12] Hollis, L., McGeer, J. C., McDonald D. G. and Wood, C.M. (1999). Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long term sub lethal Cd exposure in rainbow trout. Aquatic Toxicology 46: 101-119.

[13] Hussein H, Farag S, Kandil K, and Moawad, H. (2005). Tolerance and uptake of heavy metals by Pseudomonas. Process Biochem. 40:955-961.

[14] Karuppasamy, R. (2000). Tissue histopathology of *Channa Punctatus* (Bloch) in relation to short and long exposure to Phenyl mercuric cetate. *Journal Plat. Jubilee A.U.* 40: 179pp.

[15] Mason, C. F. (2002). Water Pollution Biology. In: Causes, Effects and Control. Ed: R. M. Harrison. 82-112.

[16] Metcalf and Eddy (2003). Wastewater Engineering: Treatment and Reuse. In: Wastewater Engineering, Treatment, Disposal and Reuse. Techobanoglous G, Burton FL, Stensel HD (eds), Tata McGraw-Hill Publishing Company Limited, 4th edition. New Delhi, India.

[17] National Research Council (NRC), (1993). Nutritional requirement of fish. National Acad.

[18] Nussey, G; Van Vuren, J.H.J and Du Preez, H.H. (2001). Bioaccumulation of Cr, Mn, Ni and Pb in the tissue of Moggel, Lobeo Umbratus (cyprinidae) from witbank Dam, Mpumalanya, South Africa. Water S.A. 26 (2): 269-284.

[19] Olaifa, F. F; OlaifaA.A; Adelaji and Owolabi, A.G. (2004). Heavy Metals Contamination of *Clarias gariepinus* from a Lake and Fish farm from Ibadan.*Nigerian Journal of Biomedical research* Vol. (7)pp 145-148.

ISSN 2229-5518

[20] Olurin, K. B. and Aderibigbe, O. A. (2006). Length weight relationship and condition factor of pond reared juvenile *Oreochromis niliticus*. *World Journal of Zoology* 1(2): 82-85. Idosi Publication.

[21] Phatil, M. and Dhande, I. (2001). Accumulation and Distribution of Copper in Both Water and Some Vital Tissues of Two Fish Specie of lake quran Fayoum Province, Egypt, *Pakistan Journal of Aquatic Biology and fisheries.*

[22] Reitveld, L.C, Meijer L, Smeets P.W.M.H, and van der hoek J.P (2009). Assessment of *Cryptosporidium* in wastewater reuse for drinking water purposes: a case study for the city of Amsterdam, The Netherlands. Water SA, 35(2): 211-215.

[23] Roth, J.A. (2006). Homeostasis and toxic mechanisms regulating manganese uptake, retention, and elimination. Biological research, Vol.39 No.1 (April 2005), pp. 45-57,ISSN 0716-9760.

[24] Rupallaria, C; Canl, M. and Atti, G. (1992). The relationship between (Cd, Cr, Fe, Pb, and Zn) levels and the size of six Meditranean fish species. Environmental pollution.

[25] Sangalang, G. B. and Freeman, H. C. (1979). Tissue uptake of cadmium in brook trout during chronic sub lethal exposure. Archives of Environmental Contamination and Toxicology 8: 77-84.

[26] Santos, I. R., E. V. Silva-Filho, C. E. Schaefer, M. R. Albuquerque- Filho and L. S. Campos. (2005). Heavy metals contamination in coastal sediments and soils near the Brazilian Antarctic Station, King George Island. Mar. Poll. Bull., 50: 85-194.

[27] Sower, A. D., (2009). The effect of wastewater effluent on fish and amphibians species. A Doctoral Dissertation presented to the Graduate School of Clemson University

[28] Stainiskiene, B; Matusvicus, P. Budrecklene, R. Skibniewska (2006). Distribution of Heavy Metals in a Fresh Water Water Fish in Lithuania. *Journal of environmental studies Vol. 15*(4) pp 585-591.

[29] US Environmental Protection Agency (USEPA) (1975). Volunteer Lake Monitoring a methods manual, EAPA 4404-91-002 Office of Water Washington D.C.

[30] Vijaran, S. (1989). Monitoring of Environmental Heavy Metals in Fish From Nasser Lake. Environmental International.

[31] Wood, C. M. (2001). Toxic responses of the gill. In: target Organ Toxicity in Marine and Freshwater Teleosts, Volume 1 – organs, Ed: D. W. Schlenkand W. H. Benson pp: 1-89.

[32] World Health Organization (2003). Heavy Metals Regulations: legal notice no. 66 www.discoverlife.2008