# AN ASSESSMENT OF THE UPTAKE OF LEAD ACETATE ON *Oreochromis niloticus (*AFRICAN TILAPIA)

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**ABSTRACT**
*An assessment of the concentrations of lead acetate in the gills of Tilapia (Oreochromis niloticus) samples collected from a fishpond in Michael Okpara University of Agriculture, Umudike, Abia State was carried out in this study. The levels of heavy metal concentration on the gills were relatively higher compared to other body parts which may be attributed to the fact that the gills are the route of uptake of water (which invariably serves as the medium of entry). Photomicrography from gills of Oreochromis niloticus exposed to lead acetate showed prominent secondary lamella with moderate epithelial necrosis and marked congestion of venous sinuses, scant pillar cells, and mucous cells. Correlations between metal concentrations and fish size were not significant (P<0.05). Although, the level of bioaccumulation of Lead (Pb) generally did not exceed the safe level for human consumption. However, the constant presence of heavy metal in concentrations closer to limits (0.2 mg/kg of fresh weight) should be a reason for concern. The study further advised the populace who constantly consume fish from polluted rivers to avoid eaten the gills and reduce consumption.*

**Keywords: lead acetate, fish gills, toxicity screening, Tilapia, pollution**

## INTRODUCTION

The rapid development of industry and agriculture has resulted in increased heavy metals pollution which is a significant environmental hazard for invertebrates, fishes, and humans (Uluturhan and Kukuksezgin, 2007). Amongst the contaminants, heavy metals are of importance due to the implications of their bioaccumulation in ecosystems (Nadmituo *et al*., 2014). The heavy metal, Lead (Pb), is the most common toxicant that can be found in the marine environment. Lead (Pb) enters the aquatic environment from both natural pathways and a variety of anthropogenic sources (Young-Joo, 2003) with negative impacts on aquatic ecosystems, the food chain, and human health. This trace element is regarded as a serious environmental pollutant because of its toxicity, lack of degradability, and persistence (Dodaradaran *et al*., 2010; Jinadasa *et al*., 2013). The toxicity of this metal has long been of concern, considering that, they are not removed from aquatic ecosystems by self-purification (Xie *et al*., 2014) and accumulate in suspended particles and sediments ( De Jonge *et al*., 2012), thereby, potentially threatening human health and ecosystems via the food web (Eslami *et al*., 2011). Consequently, evaluating ecological risks has become an area of keen interest (Xu *et al*., 2012). Accumulation of Lead (Pb) in fish is a clear indication of pollution (Arena *et al*., 2013; Ikenakaa *et al*., 2013). Significant quantities of Lead (Pb) are discharged into rivers where they can are bioaccumulated and biomagnified in resulting in sub-lethal effects or death in the local fish population (Xu *et al*., 2014). The concentration of ~~(~~Lead~~)~~ in biological compartments such as fish muscle is a complex combination of biological and ecological variables (He *et al.,* 2014). In fish, Lead (Pb) can cause disturbances in growth and reproduction as well as, histopathological alterations in the skin, gills, liver, spleen, and kidney (Vitek *et al*., 2007). *Oreochromis niloticus* is a common table food usually consumed for protein nourishment (Prasad *et al*., 2007). Other aquatic animals contain essential amino acids, protein, vitamins, and minerals; and serve as an easily digestible and cheaper source of these nutrients. A study of Tilapia for Lead (Pb) toxicity would be of benefit due to its high acceptability and consumption. This study investigated the uptake of Lead (Pb) by Tilapia (*Oreochromis niloticus*) when exposed to environmental concentrations of Lead Acetate.

## MATERIALS AND METHODS

This study was carried out in the laboratory of the College of Natural Resources and Environmental Management (CNREM), Department of Environmental Management and Toxicology in Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. A total of 120numbers of Oreochromis *niloticus* fingerlings were used for the experiment. The acclimation period was fourteen (14) days, and the fishes were fed once a day on 3% of their body weight in a large aquarium (80L capacity) with well-aerated dechlorinated tap water. After the acclimation period, a static bioassay was employed for this investigation during which the set-up was continuously aerated. The fish were randomly distributed into the test containers for toxicity test after conducting a range-finding test. Before the

commencement of the toxicity test, the fish were not fed. Each container/tank contained 10L of water and 10 experimental fish each. Tilapia was exposed to lead acetate, to determine the toxic effects in a short-term continuous static bioassay. The first ten fish (ten in each tank) of mean weight 20.5g and mean length of 10cm respectively were added in the test solution containing the lead acetate. The test containers were covered with a net to prevent the fish from jumping out of the aquaria. All the fish were then monitored for mortality within a 96hours experimental period. Concentrations of lead (Pb) was determined, and lead acetate was introduced into each of the aquaria at 30mg, 40mg, 50mg, 60mg, 70mg, and control (0mg). The percentage survival in each aquarium was determined after 24hours until 96hours. Fish showing no movements were considered dead. Tilapia were collected from fishponds in Michael University of Agriculture, Umudike. Lead acetate was gotten from one of the chemical industries in Nigeria. After 96hours, gills were excised from the fish samples and stored with formalin. The gills were taken to the laboratory for histopathological analysis, slides were prepared and interpreted. The physicochemical parameters of the tanks were carried out using a Horiba U7 series water quality checker designed to monitor five parameters namely, temperature, pH, dissolved oxygen, conductivity, and turbidity. All results obtained were subjected to statistical analysis. Standard deviation was obtained for all mean values calculated. All data were subjected to one-way analysis of variance (ANOVA), and their mean values were compared using Duncan's multiple range test to determine significant differences ($P < 0.05$).

For histopathological examination of the gills from the control and experimental fishes, samples were gently rinsed in physiological saline solution (0.9% NaCl) to remove the blood and adhering tissues and immersed in fixative composed of glacial acetic acid, formaldehyde, and ethanol (1:3:7). The fixed tissue was passed through ascending grades of alcohols: 70%, 95%, 95%, and 100%, dehydration, and finally cleared in xylene. Tissues were then infiltrated with molten paraffin wax in a hot air-dry oven at 58°C and finally embedded in paraffin wax. After embedding, the blocks were trimmed, sectioned at 5microns on a rotary microtome (Leitz Rotary). The sections were stained with haematoxylene and eosin (H&E). The stained slides were covered with DPX mountant slips. The slides were observed under a microscope attached with a digital camera (Olympus E420 camera) and photographed.

## RESULTS AND DISCUSSION

The results revealed 60% to 80% mortality among the different concentrations while no mortality was recorded among the control fish which did not show any observed change in the gills. The lack of mortality among the control fish could be a result of the absence of the test solution, which implies that the 60% to 80% mortality recorded could reveal that the test solution was responsible. The results of the physicochemical parameters of experimental water (Table 1) revealed a difference in characteristics: Temperature values for the different concentrations were within the range of 26.4 - 27.2 °C with the mean of 26.65°C. The dissolved oxygen content was within the range 7.0 - 7.6mg/L with a mean of 7.38mg/L. The pH of the water sample was within the range of 6.5 - 6.8 with a mean of 6.6. The electrical conductivity of water measured is 82, 79, 80, 78, 84, and 80µScm respectively with the mean of 80.5µScm. Results of histological examination on the organ of the target (gill) are presented in Plates.

**Table 1: Results of Physico-Chemical Parameters of Water in Experimental Tanks.**

| Parameters | A<br>0(Control) | B<br>30mg | C<br>40mg | D<br>50mg | E<br>60mg | F<br>70mg |
|---|---|---|---|---|---|---|
| Temperature ($^{O}$C) | 26.4± 0.60 | 26.0± 0.63 | 27.0± 0.59 | 26.4± 0.60 | 27.2± 0.60 | 26.9± 0.61 |
| Dissolved Oxygen (mg/L) | 7.6± 0.40 | 7.4± 0.44 | 7.2± 0.50 | 7.6± 0.40 | 7.5± 0.40 | 7.0± 0.50 |
| pH | 6.5± 0.30 | 6.6± 0.33 | 6.5± 0.30 | 6.5± 0.32 | 6.7± 0.34 | 6.8± 0.35 |
| Conductivity (µScm) | 82± 0.15 | 79± 0.10 | 80± 0.10 | 78± 0.13 | 84± 0.20 | 80± 0.10 |

**Keywords:** A, B, C, D, E, F= Experimental Treatments

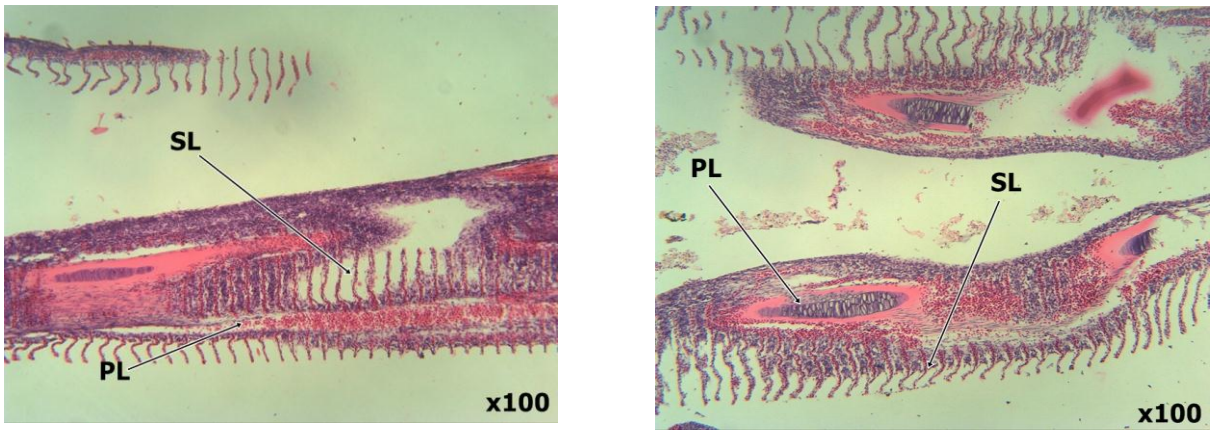



Plate 1(A) (Control): Photomicrographs from gills of *Oreochromis niloticus* exposed to lead acetate
**Key: PL- Primary Lamella; SL-Secondary Lamella**

Photomicrographs show fish gills, show primary lamella with prominent cartilaginous support, afferent and efferent arterioles as well as intact epithelial covering. The secondary lamella is well developed and consists of epithelial cells, mucous cells, and pillar cells with surrounding lacunae. The venous sinuses are filled with red blood cells.

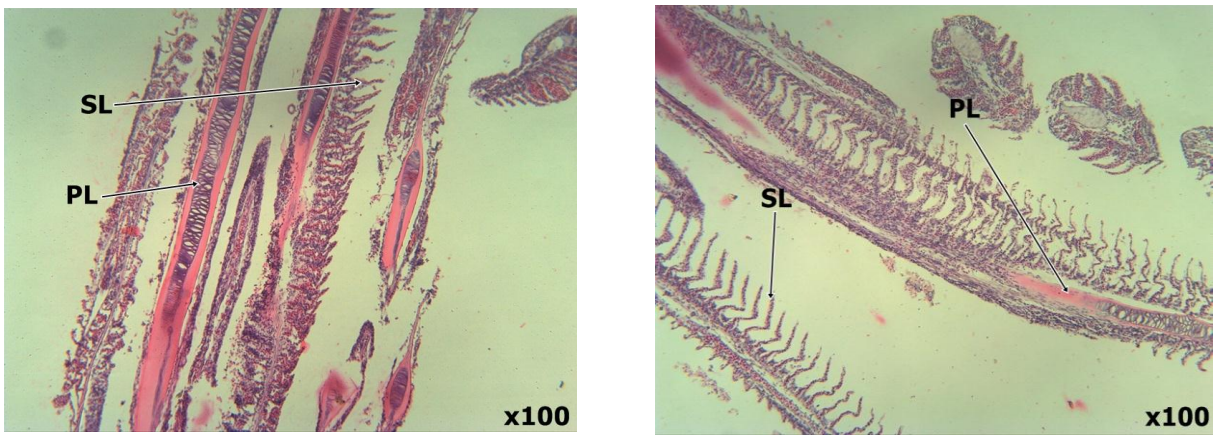



Plate 2(B) 30mg: Photomicrography from gills of *Oreochromis niloticus* exposed to lead acetate.
Compared to 'A' (control) there is prominent secondary lamella with moderate epithelial necrosis and marked congestion of venous sinuses, scant pillar cells, and mucous cells.

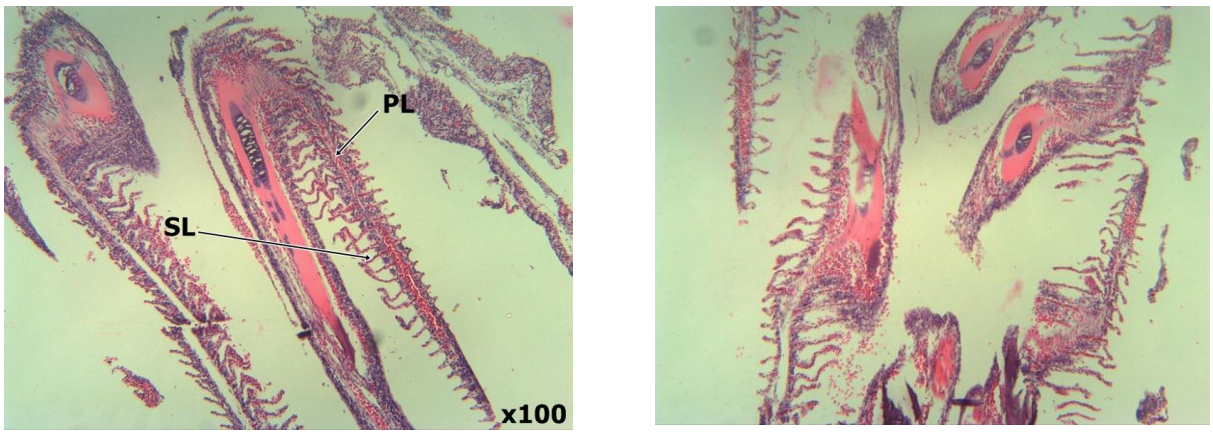
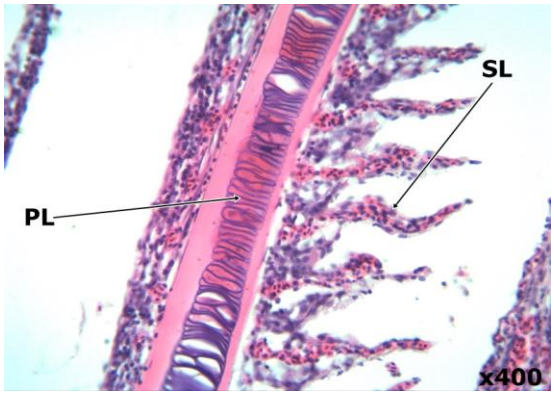



Plate3(C)40mg: Photomicrographs from gills of *Oreochromis niloticus* exposed to lead acetate
Compared to 'A' (control) there is prominent secondary lamella with moderate epithelial necrosis and marked congestion of venous sinuses, scant pillar cells, and occasional mucous cells.



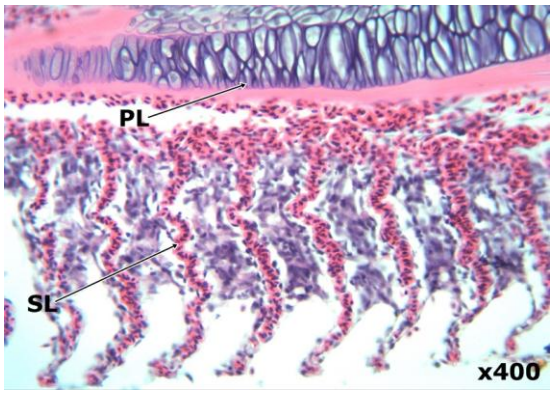
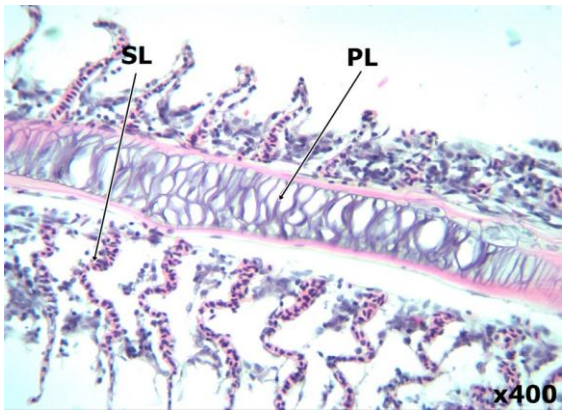




Plate 4(D) 50mg: Photomicrographs from gills of *Oreochromis niloticus* exposed to lead acetate
Compared to 'A' (control) there is prominent secondary lamella with moderate epithelial necrosis and marked congestion of venous sinuses, scant pillar cells, and mucous cells.



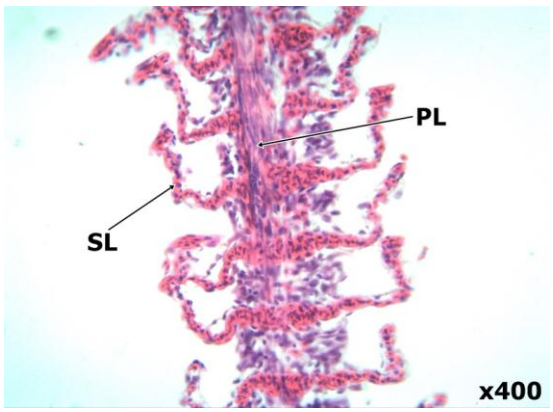
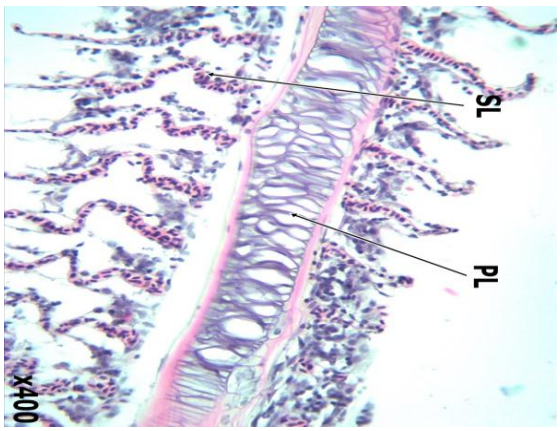




Plate 5(E)60mg: Photomicrographs from gills of *Oreochromis niloticus* exposed to lead acetate
Compared to 'A' (control) there is prominent secondary lamella with severe epithelial necrosis and marked congestion of venous sinuses, occasional pillar cells, and mucous cells. The epithelial covering of the primary lamella is moderately affected.



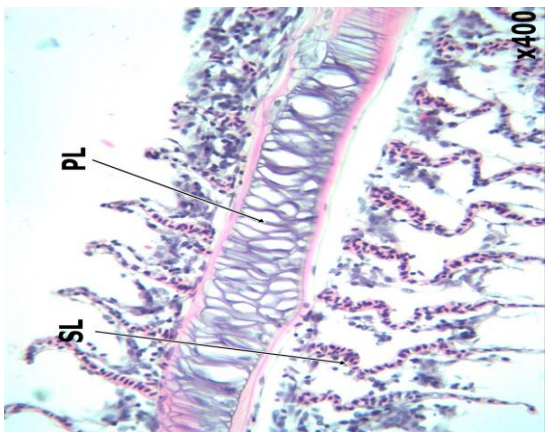




Plate 6(F)70mg: Photomicrographs from gills of *Oreochromis niloticus* exposed to lead acetate
Compared to 'A' (control) there is prominent secondary lamella with severe (total) epithelial necrosis and marked congestion of venous sinuses, absent pillar cells, and mucous cells.
The epithelial covering of the primary lamella is severely affected.

The behavioral pattern observed during the exposure period includes: the fish stood upright with the snout above the water surface gasping for air, change of the skin color, the initial increase in opercula movement, loss of balance, erratic swimming, stillness, the white colouration of the gills and finally death. Mortality was used as the endpoint of the toxicity test in agreement with the findings of (Odiete, 1999). Mortality increased with increased concentration of lead acetate. This observation is in agreement with the earlier reports of Omoregie and Ufodike (1991), Avoaja and Oti (1997). The mortality indicated that lead acetate was toxic to *Oreochromis niloticus* (Neff *et al*., 2002). The progressive uptake of lead in this study was in line with the findings by Abdel- Baki *et al*. (2011), Lead is carcinogenic, and its accumulation and eventual consumption in contaminated fish could pose health hazards especially to pregnant women and children. From the analysis of the fish samples, the effects of lead acetate on the histology of the gills are presented in Plates 1- 6. Plate 1A showed a photomicrograph with primary lamella with prominent cartilaginous support, afferent and efferent arterioles as well as, intact epithelial covering. The secondary lamella was well developed and consists of epithelial cells and pillar cells with surrounding lacunae. The venous sinuses were filled with red blood cells. However, treatment B, C, D, E, F when compared with the control, had prominent secondary lamella with moderate epithelial necrosis and marked congestion of venous sinuses, scant pillar cells, and mucous cells which ranged to severe (total) epithelial necrosis and absent pillar cells and mucous cells. Results obtained indicated significant changes in fish gill exposed to concentrations as low as 30mg. These effects can lead to changes in biodiversity if exposure is prolonged in the ecosystem. The effects of lead acetate in this study led to mortality and accumulation which poses a threat to life when consumed via the food chain.

## CONCLUSION

From this analysis, it is recommended that heavy metals should not be discharged into our aquatic bodies especially wastewater from paint industries that use lead during production. Also, during disposal, the wastes should be thoroughly treated before discharge to avoid causing harm to aquatic life within that environment. It is also advisable that consumption of fish gills should be reduced or eliminated during fish processing to avoid the consumption of such organs that may have been contaminated, and over a long period may pose danger to human health. Thus, from this study, the gills should not be considered for human consumption.

## REFERENCES

Abdel -Baki, A. S., AlQuarishy, S. and Dkhil, M. A. (2011). Bioaccumulation of some heavy metals in Tilapia fish relevant to their concentration in water and sediment of Wadihanifan, Saudi Arabia. *Africa Journal of Biotechnology, 10: 2541-2547.*

Arena, G., Copat, C., Fallico, R., Ferrante, M., Fiore, M., Ledda, C., and Sciacca, S. (2013). Heavy metals concentration in fish and shellfish from Eastern Mediterranean Sea: Consumption advisories. Food and Chemical Toxicology: Elsevier, 53: 33-37.

Avoaja, D. A., and Oti, E.E. (1997). Effect of sub-lethal concentrations of some Africa catfish 'Heteroclarias' (Hybrid). *Nigeria Journal of Biotechnology 8(1): 40-45*.

De Jonge, M., Bel-Paire, C., Geeraerts, C., De-Cooman, W., Blut, R., and Bervoets, L. (2012). Ecological impacts assessment of sediment remediation in a metal-contaminated low land river using translocated zebra mussels and resident macroinvertebrate. *Journal of Environmental Pollution: 171: 99-108.*

Dobaradaran, S., Ghaedi, H., Nadaffi, K., and Nazmara, S. (2010). Heavy metal (Lead) (Pb) content in two fish species of Persian gulf in bushen port, Iran. *African Journal of Biotechnology, 37: 6191-6193.*

Eslami, S., H., Moghaddam, A., Jafari, N., Nabavi, S. F., Nabavi, S. M., and Ebrahimzadeh, M. A. (2011). Trace element in different tissue of *Rutilus frisii* collected from Tajan River, Iran. Biological Trace Element Research. 143: 965-973.

He, M., Wang, Z., and Tang, H. (1998). The chemical, toxicological and ecological studies in assessing the heavy metal pollution in Le and river, China. Water Resources. 32:510-518.

Ikenakaa, Y., Ishizukaa, M., Nakayamaa, S.M. M., Saengtienchaia, K.W.A., and Yohannesa, Y.B. (2013). Organochlorine pesticides and heavy metals in fish from lake Awassa, Ethiopia. Insights from stable isotopes analysis, chemosphere, Elsevier, 91: 857-863.

Jinadasa, B. K. K. K., Edirisinghe, E. M. R. K. B. and Ahmad, S. B. N. (2013). A study of trace metals level in selected fisheries harbours in south and West coast of Sri. Iranka. *Journal of the National Aquatic Resources and Development Agency, 42: 47- 58.*

Nadmituo, B., Hong, S., In-Kang, S., Chu, J. M., Gomboev, B., Janchivdvorji, L., Lee, C. H., and Khim, J. S. (2015). Large scale monitoring and assessment of metal

contamination in surface water of the Selenga River Basin (2007-2009*). Environment Science Pollution Research International 22(4):2856-2867*.

Neff, J. M., Hillman, R.E., and Waugh, J.J. (2002). Bioaccumulation of trace metals from drilling mud barite by benthic marine animals. Pages 461-480. In: F.R. Engelhardt, J.P. Ray, and A.H. Gillam, eds. Drilling waste. Elsevier Applied Sciences Publishers, London.

Odiete, W.O. (1999). Environmental Physiology of Animals and Pollution. Diversified Resources Limited, Lagos. 12p.

Omoregie, E., and Ufodike, E.B.C. (1991). Histopathology of *Oreochromis niloticus* exposed to acetelli 25, Ec. *Journal of Aquatic Sciences 6:13-17.*

Prasad, Y., Kumar, P., and Nandi, D. (2007). Healthful perspectives of fish. Veterinary World 6(1):30-32.

Uluturhan, E., and Kucuksezin, F. (2007). Heavy metal contaminants in Red Pandora (*Pagellus erythrinus*) tissue from the Eastern Aegean Sea Turkey. Water Resources. 41:1185-1192.

Vitek, T., Spurny, P., Mares, J., and Zikova, A. (2007). Heavy metal contamination of the Loucka River water ecosystem. Acta Veterinaria. Brno 76: 149- 154.

Xie, Z., Sun, Z., Zhang, H. and Zhan, I. J. (2014). Contamination assessment of heavy metals in a typical abandoned estuary wetland, a case study of the Yellow River Delta, Natural Reserve. Environmental Monitoring. Assessment. 186(11):7211-7232.

Xu, T., Huang, Y., and Chen, J. (2014). Metal distribution in the tissue of two benthic fish from Pandy fields in the middle reach of the Yamgtze river. Bulletin of Environmental Contamination and Toxicology 92(4):446-450.

Xu, Y. J., Liu, X. Z., and Ma, A. J. (2004). Current Research on Toxicity Effect and Molecular Mechanism of Heavy Metals on Fish. Marine Science: 28: 67-70.

Young-Joo. A. (2003). Bioaccumulation in freshwater fish with emphasis on the dietary influence. Water Resources: 34: 4234-4242.