EFFECTS OF COPPER AND LEAD APPLIED SINGLY AND IN MIXTURE WITH CHITOSAN ON SOME SERA PARAMETERS OF *Clarias gariepinus*

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ABSTRACT

Effects of copper (Cu) and lead (Pb) applied singly and in mixture with chitosan (CT) on sera aspartate aminotransferase (AST), alanin aminotransferase (ALT) activities, glucose, total protein and cholesterol levels of *Clarias gariepinus* were studied after exposing the animals to 5.0 ppm Cu and 1.0 ppm Pb singly and in mixture with 75 ppm Chitosan over 1, 7 and 15 days. Sera parameters were measured using an auto analyzer and statistical evaluation of the experimental data was carried out by Variance Analysis and Student Newman Keul's Procedure (SNK).

Abundant biosorbants such as chitin and chitosan are known to form stable complexes with many metal ions. Chitin is widely distributed in nature, especially in the exoskeletons of marine invertebrates such as prawn, crab and lobster whereas its derivative chitosan has reactive amino groups which forms complexes between metal ions and the polymer chain.

No mortality was observed during the experiments. Exposure to Cu-CT mixture decreased sera glucose level at all exposure periods, sera cholesterol level on day 15, sera ALT activity on day 1 and sera total protein level on day 15 compare to Cu alone. Exposure to Pb-CT mixture decreased sera glucose, cholesterol levels and AST and ALT activities on day 15 compared with Pb alone. This might be due to chitosan has reactive amino groups which forms complexes between metal ions and the polymer chain.

KEY WORDS:
Copper, lead, chitosan, sera parameters, *Clarias gariepinus.*

1. INTRODUCTION

Heavy metals are natural components of aquatic environments and enter to these environments by natural phenomenon such as volcanic eruptions and erosion. The levels of these metals, however, increased significantly mainly by anthropogenic activities [1].

Copper is necessary in trace amounts for the functioning of various biological mechanisms [2], while lead have no biological function and is toxic even at very low concentrations [3]. Discharge of various metal mixtures containing urban, agricultural and industrial waste waters to freshwater environments result in a number of physiological and biochemical disturbances to organisms living in these environments [4-5].

Various biosorbents such as chitin and chitosan (CT) are known to form stable complexes with many metal ions. Chitin is widely distributed in nature, especially in the exoskeletons of marine invertebrates such as prawn, crab and lobster whereas its derivative CT has reactive amino groups which forms complexes between metal ions and the polymer chain.

Clarias gariepinus is a widely distributed species in inland waters and drainage channels of Mediterranean region and is consumed commonly as a protein source. Due to its wide tolerance against pollutants and its habitat being under direct influence of agricultural and industrial activities, the species was chosen as an experimental fish.

Effects of heavy metals result in significant variations in blood parameters. CT is well known as an excellent biosorbent for metal cation removal in near-neutral solutions because of its large number of NH_2 groups. Hence sera AST and ALT activities, glucose, cholesterol and total protein levels were determined after exposing the animals to 5.0 ppm Cu and 1.0 ppm Pb singly and in mixture with 75 ppm CT over 1, 7 and 15 days.

2. MATERIAL AND METHODS

C. gariepinus was obtained from a private fish farm in Silifke-Mersin. The mean length and weight of the animals were 21.9 ± 1.5 cm and 73 ± 3.11g respectively. Fish were adapted to laboratory conditions for one month in glass

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aquaria 40x120x40 cm in height. Experiments were run in triplicate being 3 fish in each replicate, hence 9 fish were placed in each aquarium. The same sized five aquaria were used in the experiments. The first four aquaria were filled with 120 L of 5.0 ppm Cu, 1.0 ppm Pb, 5.0 ppm Cu + 75 ppm CT and 1.0 ppm Pb + 75 ppm CT, respectively while the fifth one was filled with the same amount of copper and lead free tap water and used as control. Copper sulphate ($CuSO_4{:}5H_2O$) and lead nitrate ($Pb(NO_3)_2$) were used in the preparation of experimental solutions and trisodium citrate ($C_6H_5Na_3O_7.5H_2O$) was used to prevent precipitation and adsorption of the metals. Experimental solutions were replaced daily, by serial dilutions of freshly prepared 1000 ppm stock solution of the metals. 1 % acetic acid was used to prepare CT stock solution. Some physical chemical properties of experimental water are given in Table 1.

TABLE 1 - Physical and chemical properties of the experimental water.

Illumination regime	12h/12h light/dark
Temperature	22 ± 1^{0}C
Total alkalinity	305 ± 0.5 mg $CaCO_3$/L
Dissolved oxygen	7.1 ± 0.5mg/L
pH	8.2 ± 0.5

Fish were fed once a day with readymade fish feed (Pınar, Pellet No: 2) at amounts of 2% of total biomass. Three fish were removed from each aquaria at the end of each exposure period. Fish were anesthetized with MS222 to prevent changes in the studied parameters. They were then washed with tap water and dried with Whatman filter papers.

Blood samples to be used in determining sera parameters were obtained by cutting caudal peduncle vertically. They were transferred to anticoagulant free centrifuge tubes and centrifuged at 4000 rpm for 10 minutes. Obtained sera samples were then transferred to sera tubes and analyzed using an auto-analyzer.

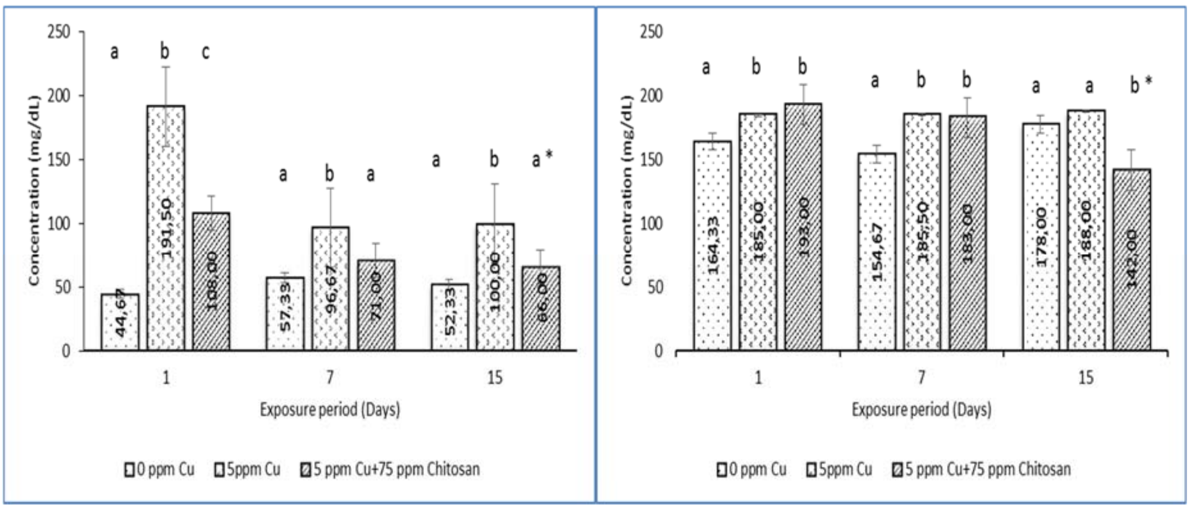
Statistical analysis of the data were carried out by Analysis of Variance and Student Newman's Procedure (SNK) using SPSS-16 statistical package program [6].

3. RESULTS

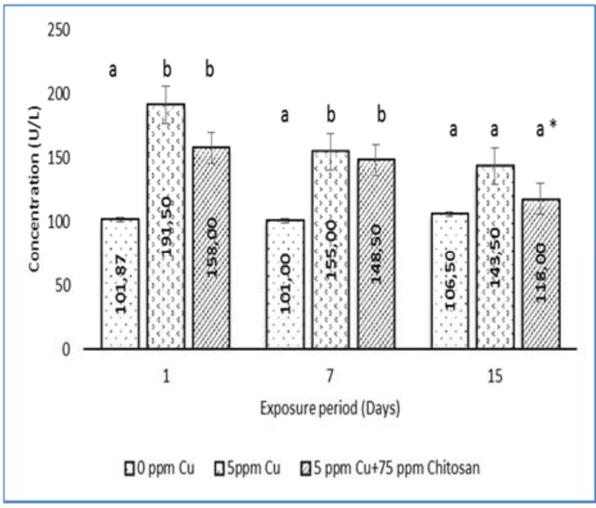
No fish mortality was observed during the experimental period. Sera glucose levels increased significantly compared to control when exposed to Cu alone, whereas it decreased significantly in Cu-CT mixture compared to Cu alone at the exposure periods tested (Fig. 1a; $P<0.05$). There was a significant increase in sera cholesterol levels on days 1 and 7 under the effect of copper compared with control (Fig. 1b; $P<0.05$). No significant change in sera cholesterol level, however, was observed under the effect of Cu-CT mixture during these periods (Fig. 1b; $P>0.05$). Sera cholesterol was significantly lower in fish exposed to Cu-CT mixture than in those exposed to copper alone at the end of 15 days (Fig. 1b; $P<0.05$).

There was a significant increase in sera AST and ALT activities of *C. gariepinus* when exposed to Cu for 1 and 7 days compared to control (Fig. 1c-1d; $P<0.05$). AST and ALT activities remained unchanged when exposed to Cu-CT mixture compared with Cu alone at the exposure periods tested. Sera total protein increased significantly compared with control when exposed to Cu alone at all exposure periods (Fig. 1e; $P<0.05$). Exposure to Cu-CT mixture decreased sera total protein levels at the end of 15 day compared to Cu alone (Fig. 1e; $P<0.05$).

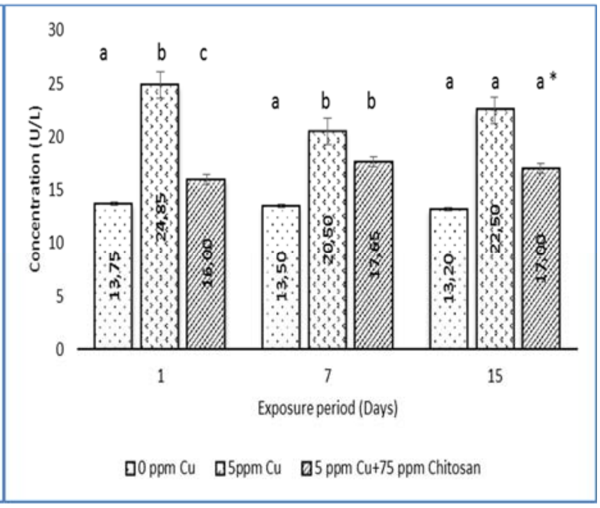
Sera glucose and cholesterol levels were not affected by exposure to Pb alone and Pb-CT mixture except for day 15. Their levels were significantly lower in fish exposed to Pb-CT mixture compared to Pb alone after 15 days (Fig. 2a-2b; $P<0.05$).



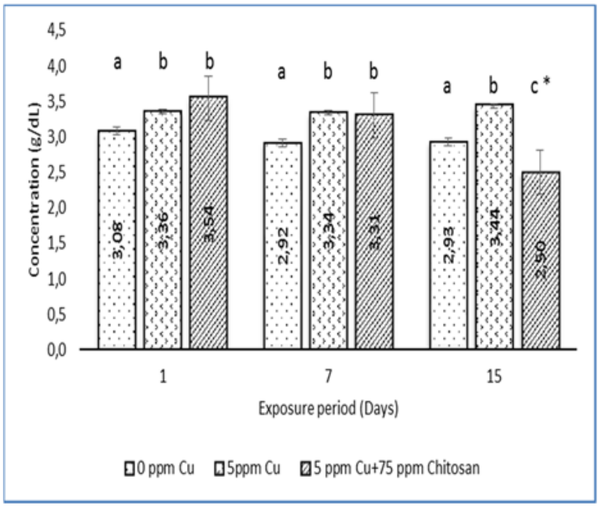
a. Glucose (mg/dL) b. Cholesterol (mg/dL)



c. Aspartate aminotransferase (AST) (U/L)

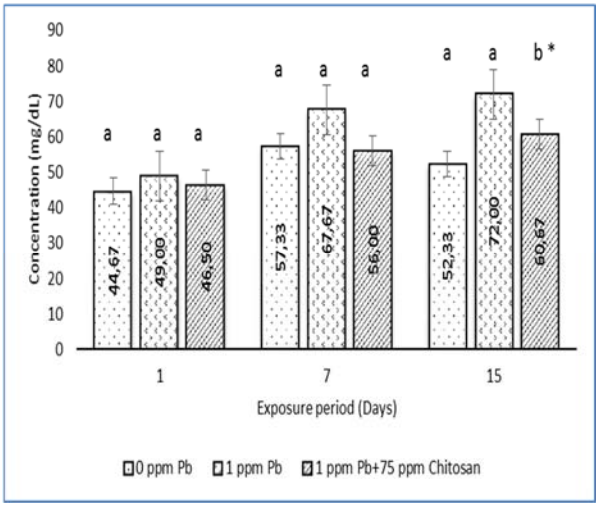


d. Alanine aminotransferase (ALT) (U/L)

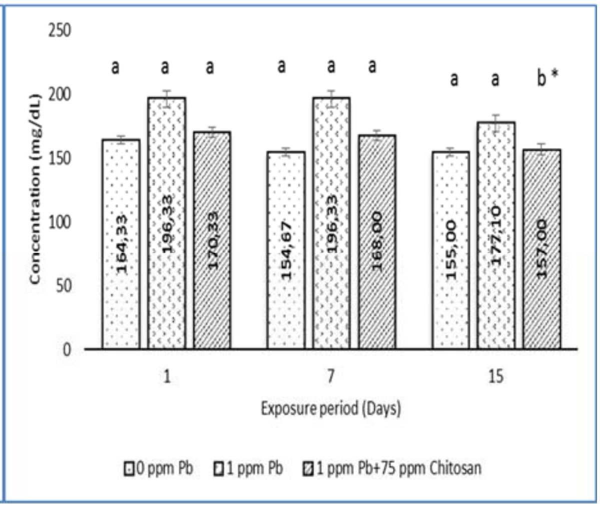


e. Total protein (g/dL)

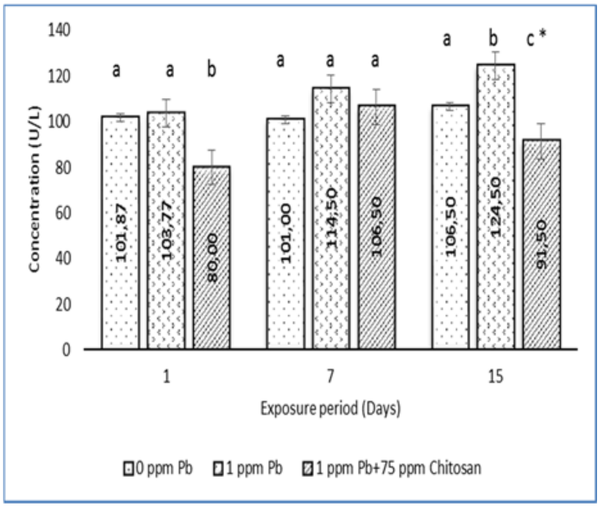
FIGURE 1 - Effects of Cu and with Cu-CT mixture on some sera parameters of *C. gariepinus*. *=SNK; Letters a, b, c show differences among concentrations. Data shown with different letters are significant at the P<0.05 level.



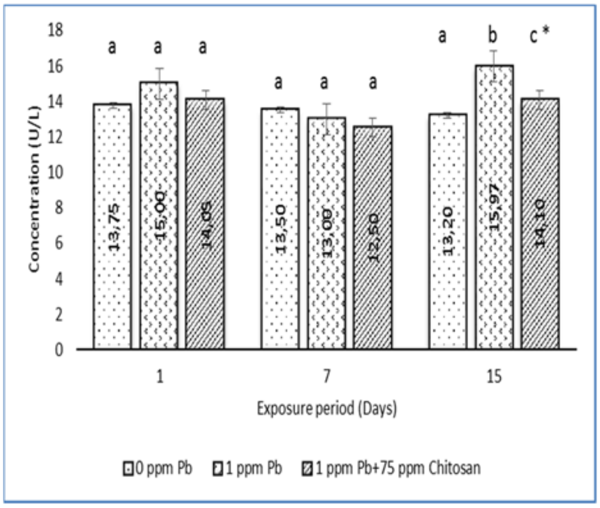
a. Glucose (mg/dL)



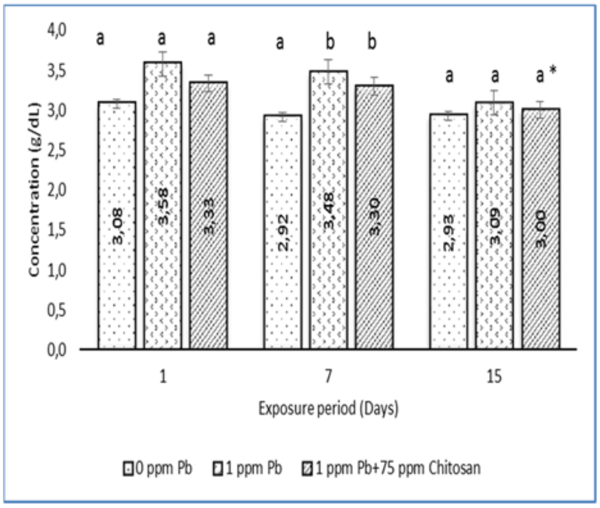
b. Cholesterol (mg/dL)



c. Aspartate aminotransferase (AST) (U/L)



d. Alanine aminotransferase (ALT) (U/L)



e. Total protein (g/dL)

FIGURE 2 - Effects of Pb and Pb-CT mixture on some sera parameters of *C. gariepinus*. *=SNK; Letters a, b, c show differences among concentrations. Data shown with different letters are significant at the P<0.05 level.

Exposure to Pb alone and Pb-CT mixture did not affect sera AST and ALT activities on days 1 and 7 (Fig. 2c-2d; P>0.05). Sera AST and ALT activities increased significantly compared with control when exposed to Pb alone for 15 days, while Pb-CT mixture decreased their levels significantly compared to Pb alone at a given period (Fig. 2c-2d; P<0.05). Sera total protein increased significantly when exposed to Pb alone compared with control after 7 days (Fig. 2e; P<0.05).

4. DISCUSSION

There was no mortality *C. gariepinus* exposed to 5.0 ppm Cu and 1.0 ppm Pb over 15 day which was probably due to short exposure periods and low metal concentrations.

Heavy metals above given concentrations activate stress conditions in fish as other stress factors, such as temperature and anaerobic conditions, which in turn increases the need for energy. The increase in energy demand stimulates the release of glucocorticoids such as cortisol, epinephrine, and catecholamine causing mobilization of liver and muscle glycogen by glycogenolysis and result in hyperglycemia.

Copper increases sera glucose and cortisol levels in *Salmo trutta* depending on exposure concentration and period [7]. McLeay [8] reported that sera glucose levels increased in *Oncorhynchus mykiss* exposed to zinc for 7 days. Exposure to lead for 4 days increased sera glucose level in *O. niloticus* [9]. Sera glucose level increased significantly compared with control when exposed to Cu alone at all exposure periods. This might result from stimulation of glycogenolysis in liver and muscle depending upon the energy demand under the effect of metal stress.

Cholesterol is the main structural component of lipoproteins, bile acids and steroid hormones. Effects of heavy metals on cholesterol levels in fish varies between fish species and metals tested. Sera cholesterol levels increased in *O. niloticus* exposed to Ag, Zn, Cr, Cu and Cd singly [10] while long term effect of lead decreased sera cholesterol levels in *Prohilodus lineatus* [11]. Short term exposure to Cu and Pb increased sera cholesterol levels of *C. gariepinus* whereas its level decreased with increasing periods. This might be due to tissue damage caused by the metal, breakdown in cholesterol synthesis and/or the use of cholesterol in the synthesis of steroid hormones.

Heavy metals not only induces stress conditions in fish but also cause tissue damages. Stress conditions in fish increase energy requirements which are derived primarily from carbohydrates, such as glucose and from non-carbohydrate sources, such as proteins and lipids, through gluconeogenic enzymes, namely AST and ALT. The levels of gluconeogenic enzymes are low under normal conditions. Zinc increased sera AST and ALT activities in *O. niloticus* at both short and long exposure periods [12]. Exposure to Cu and Pb for four and twenty one days increased sera AST and ALT activities in *O. niloticus* [9]. Sera AST and ALT activities also decreased in *C. gariepinus* when exposed to Cu for 1 and 7 days and to Pb for 15 days.

Trace elements are transported between various tissues by binding to proteins such as albumin, globulin and ceruloplasmin. Sera total protein levels of *C. carpio* increased under the effect of Cu and Zn [13]. Four days of exposure to lead did not affect sera total protein level in *O. niloticus* [9]. Sera total protein levels of *Oncorhynchus. mykiss* increased with increasing exposure periods when the fish were fed with 489.8± 1.9 mg Cu/kg food [14]. Copper increased sera total protein levels of *C. gariepinus* at all exposure periods and increased on the 7 day of exposure to Pb alone. The increase in synthesis of metal binding proteins and time depended increase in protein catabolism might explain these variations.

CT is produced by alkaline *N*-deacetylation of chitin, which is widely found in the exoskeleton of shellfish and crustaceans. It is widely known that the excellent adsorption behaviors of CT for heavy metal removal is attributed to: high hydrophilicity of CT due to large number of hydroxyl groups, large number of primary amino groups with high activity and flexible structure of polymer chain of CT making suitable configuration for adsorption of metal ions [15]. McKay et al. [16] reported that the maximum adsorption capacities of CT for Hg, Cu, Ni, and Zn. CT had an inhibitory effect on Cd intake in *O. mykiss* fed with Cd and Cd-CT mixed food [17]. Copper and CT complex decreased oxidative stress in *C. carpio* [18].

Exposure to Cu-CT mixture decreased sera glucose level at all exposure periods, sera cholesterol level on day 15, sera ALT activity on day 1 and sera total protein level on day 15 compared to Cu alone . Exposure to Pb-CT mixture decreased sera glucose, cholesterol levels, and AST, ALT activities on day 15 compared to Pb alone. This might be resulted from reactive amino groups of CT form complexes with metal ions through its polymer chains.

It was concluded that the studied metals concentrations caused significant alterations in the carbohydrate and protein metabolism by effecting sera parameters. Chitosan, which is a natural adsorbent, seemed to decrease the harmful effects of Cu and Pb on sera parameters especially on long term exposures.

The authors have declared no conflict of interest.

REFERENCES

[1] Naito, W., Kamo, M,. Tsushima, K. and Iwasaki, Y. (2010) Exposure and Risk Assessment of Zinc in Japanese Surface Waters. Sci. Total Environ. 408(20), 4271–4284.

[2] Nussey, G., Van Vuren, J.H.J. and Du Preez, H.H. (1995) Effect of Copper on the Hematology and Osmoregulation of the Mozambique Tilapia, *Oreochromis mossambicus* (Cichlidae). Comp. Biochem. Physiol., 111C (3), 369–380.

[3] Cicik, B. (2003) Bakır-Çinko Etkileşiminin Sazan (*Cyprinus carpio* L.)'nın Karaciğer, Solungaç ve Kas Dokularındaki Metal Birikimi Üzerine Etkileri. Ekoloji, 12(48), 32-36.

[4] Gobeil, C., Rondeau, B. and Beudin, L. (2005) Contribution of Municipal Effluents to Metal Fluxes in the St. Lawrence River. Environ. Sci. Technol. 39, 456–464.

[5] Shaw, J.R., Dempsey, T.D., Chen, C.Y., Hamilton, J.W. and Folt, C.L. (2006) Comparative Toxicity of Cadmium, Zinc and Mixtures of Cadmium and Zinc to Daphnids. Environ. Toxicol. Chem. 25, 182–189.

[6] Sokal, R.R. and Rohlf, F.J. (1995) Biometry: The Principles and Practice of Statistics in Biological Research. 3rd edition. W.H. Freeman and Co., New York.

[7] Nemcsok, J. and Hughes, G. M. (1988) The Effect of Copper Sulphate on Some Biochemical Parameters of Rainbow Trout. Environ. Pollut., 49, 77–85.

[8] Mcleay, D.J. (1977) Development of a Blood Sugar Bioassay for Rapidly Measuring Stressful Levels of Pulmill Effluents in Salmonid Fish. J. Fish. Res. Bd. Can. 34, 477-485.

[9] Fırat, Ö., Çogun, H.Y., Yüzereroğlu, T.A., Gök, G., Fırat, Ö., Kargın, F. and Kötemen, Y. (2011) A Comparative Study on the Effects of a Pesticide (cypermethrin) and Two Metals (copper, lead) to Serum Biochemistry of Nile Tilapia, *Oreochromis niloticus*. Fish Physiol. Biochem., 37, 657–666

[10] Öner, M., Atlı, G. and Canlı, M. (2007). Changes in Serum Biochemical Parameters of Freshwater Fish *Oreochromis nilotıcus* Following Prolonged Metal (Ag, Cd, Cr, Cu, Zn) Exposures. Environ. Toxicol. Chem., 27(2), 360–366.

[11] Martinez, C.B.R., Nagae, M.Y., Zaia, C.T.B.V. and Zaia, D.A.M. (2004) Acute Morphological and Physiological Effects of Lead in the Neotropical Fish *Prochilodus lineatus*, Braz. J. Biol., 64 (4), 797-807

[12] Younis, E.M., Abdel-Warith, A.A. and Al-Asgah, N.A. (2012) Hematological and Enzymatic Responses of Nile Tilapia *Oreochromis niloticus* during Short and Long Term Sublethal Exposure to Zinc. Afr. J. Biotechnol., 11(19), 4442-4446.

[13] De Smet H. and Blust R. (2001) Stress Responses and Changes in Protein Metabolism in Carp *Cyprinus carpio* during Cadmium Exposure. Ecotoxicol. Environ. Safe., 48(3), 255-262.

[14] Handy, R.D., Sıms, D.W., Gıles, A., Campbell, H.A. and Musonda, M.M. (1999) Metabolic Trade-off between Locomotion and Detoxification for Maintenance of Blood Chemistry and Growth Parameters by Rainbow Trout (*Oncorhnchus mykiss*) during Chronic Dietary Exposure to Copper. Aquat. Toxicol., 47, 23-41.

[15] Babel, S. and Kurniawan, T.A., 2003. Low-cost Adsorbents for Heavy Metals Uptake from Contaminated Water. J. Hazard. Material. 97(B), 219–243.

[16] McKay, G., Blair, H.S. and Findon, A. (1989) Equilibrium Studies for the Sorption of Metal Ions onto Chitosan. Ind. J. Chem. 28(A), 356–360.

[17] Ren H., Jia H., Kim S., Maita M., Sato S., Yasui M., Endo H. and Hayashi T (2006) Effect of Chinese Parsley *Coriandrum sativum* and Chitosan on Inhibiting the Accumulation of Cadmium in Cultured Rainbow Trout *Oncorhynchus mykiss.* Fisheries Sci,, 72, 263-269.

[18] Dautremepuits, C., Betoulle, S., Paris-Palacios, S. and Vernet, G. (2004) Humoral Immune Factors Modulated by Copper and Chitosan in Healthy or Parasitized Carp (*Cyprinus carpio* L.) *Ptychobothrium sp*. (Cestoda). Aquat. Toxicol., 65, 325-338.

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