Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 1019 - 1032

Research Article **Allied Science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPARATIVE STUDY OF ARSENIC AND CADMIUM COMBINED METAL TOXICITY IN FRESH WATER FISH WITH YAMUNA WATER FISH

ARTI SRIVASTAVA, SOHINI SINGH* AND TANUALLEN

Amity Institute of Biotechnology, Amity University, Noida, India

ABSTRACT

Yamuna River is one of the most important natural water resources essential for survival and growth. Over exploitation of this resource has made it one of the most polluted rivers in the world. Heavy metals such as Arsenic and Cadmium enter the aquatic environment due to rapid industrialization and other anthropogenic activities. High cadmium level definitely harms the fish health, since the arsenic present also tends to bioaccumulate in the fish organs, there are possibilities of alteration in toxicity due to the presence of both these metals together. Hence the objective of present study was designed to compare the toxicity due to two metals present together in Yamuna water on the fish C*hanna punctatus*. Accumulation of metals was significantly higher ($p < 0.05$) in metals in combination as compared to Yamuna water fish with control while lipid peroxidation was significantly higher in Yamuna water fish. Level of catalase, SOD, GSH and GST were significantly lower in both Yamuna water fish and metal treated fish compared to control.

KEYWORDS: **Arsenic, Cadmium, Yamuna river, Toxicity**

SOHINI SINGH
Amity Institute of Biotechnology, Amity University, Noida, India

INTRODUCTION

Rapid industrialization, growth, increased demand for resources and materials have led to the exploitation of natural resources[1, 2] Yamuna one of the most important water body for survival and growth originating from Yamunotri, the main tributary of the river Ganga is turning into one of the most polluted rivers in the world, especially the Delhi stretch where city dumps about ~58% of its waste into it[3].Elevated level of various heavy metals was reported in the river and land around Delhi regions[4, 5]. Heavy metals such as arsenic (As) and cadmium (Cd) enters the aquatic environment by mining, burning of fossil fuels, pesticide application, industrial activities like electroplating, fabrics, plastics, ceramic and glass, vehicle and electronics[6,7,8] which can be toxic to the aquatic organism in even a very small amount. These metals when present in water bodies were usually get bioconcentrated, bioamplified and bioaccumulate in the tissue of aquatic organisms through which they become the part of various food chains and prove to be toxic to both humans and other organisms[9,10]**.**Oxidative stress is the major consequence of water pollution in aquatic organisms. Fish are endowed with an antioxidant defense system to protect them from this stress[11]. However when there is imbalance in antioxidant defences, leading to the generation of reactive oxygen species (ROS), which can be detoxified by metallothioneins (MT), superoxide dismutase (SOD), catalase (CAT) and glutathione related enzymes**.** Antioxidant enzymes level can be used as a biomarker of oxidative stress subsequent to pollutants exposure in aquatic animals[12, 13]**.**Arsenic and cadmium result in induction of antioxidant defense mechanisms[14,15]. Being a major source of food and a part of the very high healthy diet for human; fish have great demand in market. They were, however, going to be in danger mainly because of water pollutants[16]**.** Fish can be used as bioindicator of any aquatic health system as they occupy the top of an aquatic food chain and constitute an excellent model to understand mechanistic aspects of metal toxicity[17]**.**According to ATSDR (Agency for Toxic Substances and Disease Registry)**,** of the U.S. Department of Health and Human Service**s** arsenic and cadmium are listed as the first and seventh most commonly found toxic substance in the United States. Due to their known or suspected toxicity these metals are established to pose the most significant potential threat to human health. Central Water Commission (CWC)[18] have reported the status of toxic metal content of Indian Rivers and found that arsenic concentration was within the acceptable limit while cadmium content was more than the acceptable limits of Bureau of Indian Standard (BIS) in river Yamuna at Delhi Rly Bridge. Cadmium was found above the acceptable level of Bureau of Indian Standard in Yamuna water obtained from Delhi-Nizammudin Bridge[19]. High cadmium level definitely harms the fish health, since arsenic is also there to bioaccumulate in the fish organs, there are possibilities of alteration in toxicity due to the presence of both these metals together. Hence the present study was designed to compare the toxicity due to two metals in combination with Yamuna water.

MATERIALS AND METHODS

Healthy specimens of fresh water Murrel, *Channa punctatus* (Bloch) were procured from fish farm. They were acclimatized individually in all glass aquaria in dechlorinated water at laboratory conditions (25 ± 2°C) for 1 month. During acclimatization fish were fed with goat liver. For Yamuna water analysis surface water samples were collected from Okhla barrage and taken to the laboratory for the estimation of pH, temperature, and dissolved oxygen (DO) and biochemical oxygen demand (BOD). Reagent bottles of 250ml were used for collection of water samples. For dissolved oxygen (DO), we used 1 ml of each Winkler's solutions A (Manganous sulphate) and B (Potassium hydroxide in Potassium iodide) to fix the oxygen content of the water. For BOD, water samples were immediately wrapped in black polyethylene bags and transported to the laboratory. Fish were caught with the help of local fisherman, preserved in ice and transported to the laboratory. In laboratory liver

tissues and scales were collected and washed with deionized water then preserved at -80°C for further analysis. Average wet weight (g) and length (cm) of both Yamuna water fish and laboratory maintained fish were 19.5 ± 2.25 gm and 11.5± 0.2 cm respectively.

Test chemicals

For the present study all reagents and chemicals of highest purity were used. Analytical-grade arsenic tri-oxide (As_2O_3) (99.5%) and cadmium chloride ($CdCl_2$. H_2O) (99%), phenazine methosulphate and nitro-blue tetrazolium manufactured by Central Drug House (P) Ltd. have been used. Thiobarbituric acid was purchased from Loba Chemie (Mumbai). 5-5-Dithiobis-2-nitrobenzoic acid, 1-Chloro-2, 4-dinitrobenzene, glutathione, bovine serum albumin and tricaine methanesulfonate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide was purchased from Fisher Scientific. Tetra- Sodium pyrophosphate was purchased from Merck Specialities (P) Ltd Mumbai.

Experimental Procedure

The physicochemical properties of test water, viz. temperature, pH, dissolved oxygen, chloride, were analyzed using standard methods. Before starting the experiment the median lethal concentration of heavy metals for 96 h (96 h LC50 value) was calculated by pursuing the 24 h renewal bioassay system and Trimmed Spearman–Karber method[20]**.**The acclimatized fish were divided into 4 groups. Group A fish were introduced to an aquarium containing Arsenic tri oxide (1 mg/ml, 10% of 96 h LC50). Group B fish were introduced with Cadmium chloride (0.8 mg/L,10% of 96 h LC50) and group C fish were introduced to a combination of Arsenic tri-oxide + Cadmium chloride (1 mg/L + 0.8 mg/L) and group D fish were treated as a control (without any metal). Concentration of metals in the water of experimental aquaria was kept constant. A record of their mortality was also maintained. All groups contained five fishes with 20 L of water in each aquarium. Experiment was performed in triplicates.

Sample preparation

After exposure of 15 days, the fish from each group were euthanized; liver tissues and scales were collected. Samples were preserved at -80°C for further analysis.

1. ***Scales***

   Scales were removed from Yamuna water fish (mentioned above), heavy metal treated (individual and combination groups) and control fish. They were stained with Borax Carmine and chromatophore patterns were observed under the light microscope[21]**.**

2. ***Lipid Peroxidation assay***

   The lipid peroxidation in the liver tissue was assayed by determining microsomal malondialdehyde[22].

3. ***Protien concentration***

   Protein was estimated in liver tissue by method of Lowery[23]using bovine serum albumin as the standard.

4. ***Estimation of Metals***

   Toanalyze the metal concentration in liver tissue, tissue was digested in concentrated nitric acid and then diluted with double distilled water. 50 mg of liver tissue was digested in 1 ml of concentrated nitric acid at 80 °C for 1 h and it was evaluated through Atomic Absorption Spectrophotometer (Perkin Elmer AA800).

5. ***SOD activity***

   SOD activity was analyzed using the method described by[24]. Reaction mixture contained 2 ml sodium pyrophosphate buffer (pH8.3), 0.1 ml phenazine methosulphate, 0.2 ml nitro blue tetrazolium and 0.2ml tissue supernatant. Reaction was initiated by adding 0.2 ml NADH at 30^{o}C and stopped by addition of 2 ml glacial acetic acid. Color intensity of the solution was measured at 560 nm. The SOD activity was expressed as % of inhibition.

6. ***Catalase activity***

   Catalase activity was quantified with the procedure described by[25]. The reaction

mixture contained 1.96 ml of 50 mM sodium phosphate buffer (pH 7.0) and 1 ml of 40 mM H_2O_2. The reaction was initiated by adding 40 µl of tissue supernatant. The CAT activity was determined by measuring the rate of disappearance of H_2O_2 at 340 nm for 1 min. Enzyme activity was expressed as I M H_2O_2 decomposed/min/mg protein.

7. ***Reduced Glutathione (GSH)***
Amount of reduced glutathione in the liver was estimated by using Ellmans' reagent[26]. Reaction of GSH and DTNB (5-5-dithiobis-2-nitrobenzoic acid) was resulted in the formation of a yellow colored product 2-nitro-5-thiobenzoic acid. GSH concentration was determined by measuring the absorbance at 412 nm. The unit of glutathione was expressed as nM GSH /mg protein.

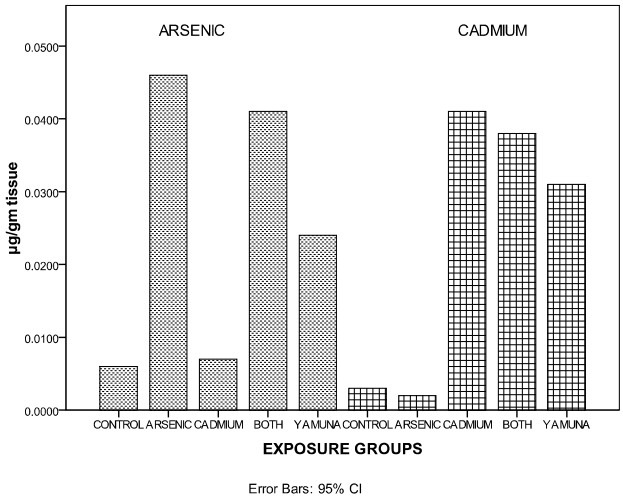
8. ***GST activity***
GST activity was calculatedspectrophotometrically at 30°C by following the formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (cDNB) at 340 nm using an extinction coefficient of 9.6 mM^{-1} cm^{-1} [27]. The reaction mixture contained 2 ml volume of 0.1 M sodium phosphate buffer (pH 6.5), 50 mM GSH, 50 mM cDNB in ethanol and the enzyme solution. GST activity was expressed as nM cDNB conjugates/min/mg of protein

Statistical analysis
Statistical analysis was done using SPSS.

RESULTS

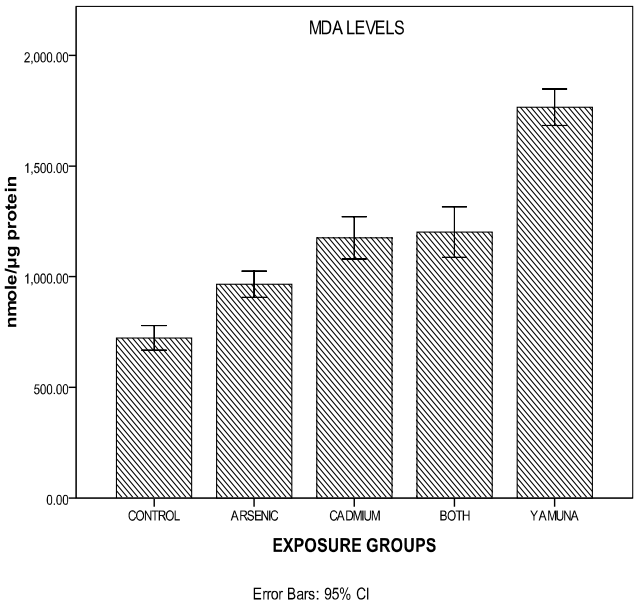
The physiochemical properties of the experimental water and Yamuna water were measured in which Dissolved Oxygen was 7.34 mg/l for experimental water and 1.61 mg/l for Yamuna water. The temperature of experimental water and Yamuna water was same 18.0 - 20.0 °C. The pH values for experimental water and Yamuna water were 7.0 – 7.1 and 7.8 – 8.0 respectively. As shown in Fig 1 accumulation of metals (arsenic and cadmium) in liver of Yamuna water fish was significantly higher ($p<0.05$) than the liver tissue of control fish whereas it was lower when compared to the individual metal (arsenic and cadmium) treated group. Accumulation of arsenic was found to be more when given individually than compared to combination. Same observations were also found in group of cadmium treated fish.

Figure 1
Metal concentration in liver tissue



Exposure of arsenic and cadmium individually for 15 days significantly ($p<0.05$) increased the level of lipid peroxidation in the liver as compared to the control group. Same observation was also found with Yamuna water fish (Fig 2). Lipid peroxidation was significantly higher in Yamuna water group when compared with individual and combined metals group and control group.

Figure 2
MDA levels after exposure. n = 5 in each exposure; values are represented as mean ± SE, p < 0.05



The antioxidant enzymes like SOD and CAT decreased significantly (*p<0.05*) in metals treated group and Yamuna water group. Moreover, decrease in SOD and CAT level in Yamuna water fish is more significant than metal treated group.

Figure 3
SOD activity after exposure. n = 5 in each exposure; values are represented as mean ± SE, p < 0.05

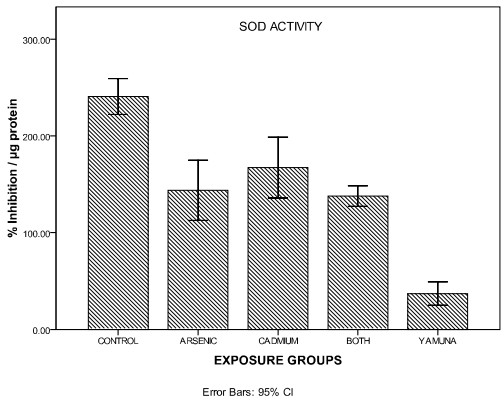
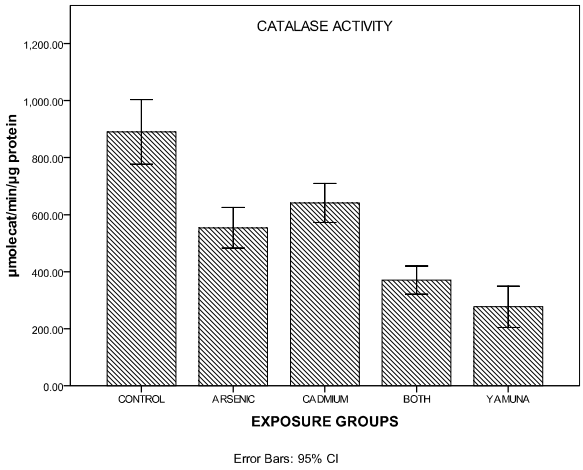
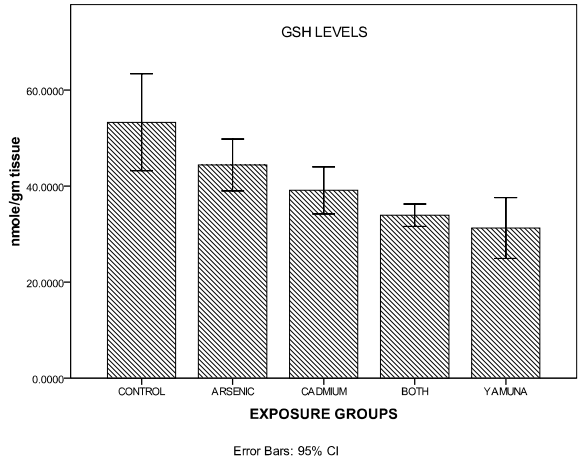


Figure 4
Catalase activity after exposure. n = 5 in each exposure; values are represented as mean ± SE, p < 0.05



The result shown in the Figure 5 shows significantly reduced level of GSH (*p<0.05*) in fish exposed to metal arsenic, cadmium individually, in combination and in Yamuna water fish as compared to control. However, decline in reduced glutathione is more significant in Yamuna water fish than metal treated group individually.

Figure 5
GSH levels after exposure. n = 5 in each exposure; values are represented as mean ± SE, p < 0.05



Significant (*p<0.05*) reduced level of GST enzyme were observed in fresh water fish *Channa punctatus* exposed to As, Cd individually, in combination and in Yamuna water fish when compared with control.

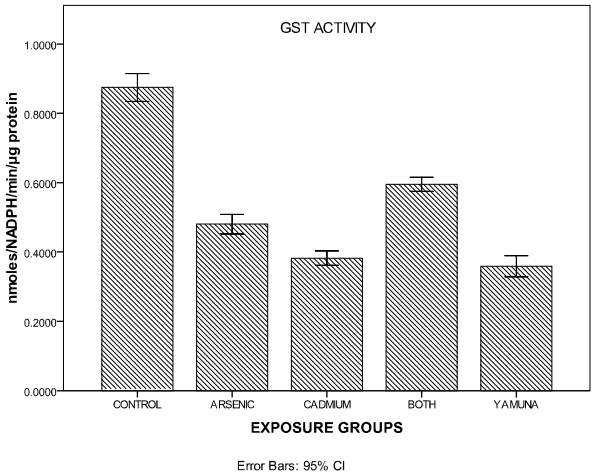


Figure 4
GST activity after exposure. n = 5 in each exposure; values are represented as mean ± SE, p < 0.05

Accumulation of metals in the scales produces the variation in the chromatophores position, shape and density which was shown in the respective figures. In the control group the chromatophores were punctuated and densely present. However, in arsenic treated group number of chromatophores was reduced, but reticulated and a specific linear pattern was visualized. In cadmium treated groups, the chromatophores were very less in number and dispersed throughout the scale. In combined metals group, the number of chromatophores reduced. In Yamuna water fish, chromatophores had maximum variation in shape, size and distribution.

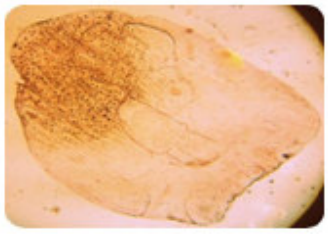


Figure 8
Scale of control fish

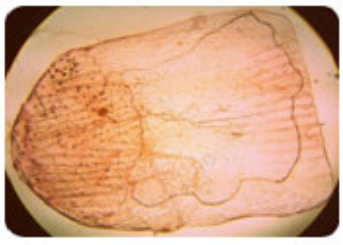


Figure 9
Scale of cadmium treated fish

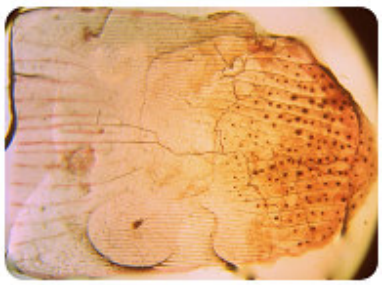


Figure 10
Scale of Arsenic treated group

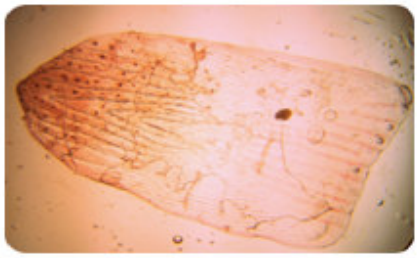


Figure 11
Scales treated with both Arsenic and Cadmium

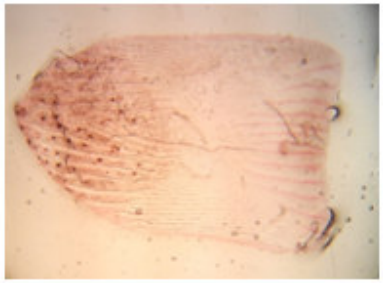


Figure 12
Scales treated with Yamuna water

DISCUSSION

The present work was intended to study the level of heavy metals and certain biomarkers of oxidative stress in fish caused by exposure of metals in laboratory condition, and in Yamuna water fish *Channa punctatus* . Our results clearly indicated the accumulation of metals in *Channa punctatus* which caused an elevation of malondialdehyde and alternation in the other antioxidant enzymes in fish. Exposures to certain metals are effective inducer of oxidative stress, furthermore, they have toxic and carcinogenic effects[28, 29.] The liver is the major target organ of inorganic arsenic, which is explained by the affinity of As (III) toward vicinal dithiol in hepatic cytosolic proteins. The binding of arsenic in target organs was postulated as a first step of arsenic detoxification, this binding is also responsible for the major intoxication symptoms: hepatic and renal failure and cardiovascular and neurological effects[30]. Birben[31]enlightened oxidative stress, one of the major consequences of elevated levels of metal toxicity, is a well-known mechanism for the production of reactive oxygen species (ROS).It occurs when there is an imbalance between productions of ROS and the cellular antioxidant

defense system which results in formation of superoxide anion radicals, hydrogen peroxide, hydroxyl radical, alkoxyl radical, peroxyl radical. Oxidation of proteins and lipids, modification in gene expression, and changes in cell redox status is aftermath of metal exposure through ROS mediated reactions[32,17]. Malonyldialdehyde is a complex secondary end-product of lipid degradation induced by metals, perpetuate the disruption of cell integrity hence, its presence in tissue showed a reliable biomarker of oxidative stress[33, 34]. Therefore we included the evaluation of MDA content in the liver of metal treated and Yamuna water fish. The results of present study clearly revealed that an exposure of arsenic and cadmium individually for 15 days, significantly ($p<0.05$) increased the level of lipid peroxidation in the liver as compared to the control group. Same observation was also found with Yamuna water fish. Elevation in the lipid peroxidation was significantly higher in Yamuna water group than the metal treated group and control. Accumulation of metals (arsenic and cadmium) in Yamuna water fish was although significantly higher than the control, it was lower when compared to the arsenic and cadmium treated group. The increase in lipid peroxidation in Yamuna water fish may be attributed to the presence of other pollutants in the Yamuna water. Our result was supported by a previous study performed on different fish tissue in Yamuna water[35, 36]. Increased level of LPO was observed by many investigators in heavily polluted sites. Ferreira and Bacanskas[37, 38] found elevated level of lipid peroxidation from polluted site of river Douro Estuary, Portugal in two resident species, mullet *(Mugil cephalus)* and flounder *(Platichthys flesus)* and in killifish *(Fundulus heteroclitus)* inhabiting a polluted inlet of the Elizabeth River respectively. Induction of TBARS content in three-spined stickleback *(Gasterosteus aculeatus L.)* have been observed, impacted by various sources of contaminations in comparison to fish from uncontaminated sites[39].Superoxide-dismutase (SOD) is antioxidant metalloenzyme which reduces superoxide radicals to water and molecular oxygen[40] and consequently catalase (CAT) reduces hydrogen peroxide to molecular oxygen and water[41.] Since both enzymes are linked functionally therefore, similar parallel trend in the activities of both enzymes should be there. In support to this, we found that at an exposure of 15 days, SOD and CAT activities decreased significantly (*$p<0.05$*) in metals treated group and Yamuna water group as compared to control, although, decrease in SOD and CAT level in Yamuna water fish is more significant than metal treated group indicating more damage to the antioxidant enzymatic defence in Yamuna water fish. Similar findings were also observed by some other investigators[42, 43]. They found decreased SOD and CAT activity impacted with toxicity of other metals. Farombi[44] also observed a decrease in CAT activity in *Clarias gariepinus* from Nigeria Ogun River. In contrast to our result[45,46] have reported hepatic SOD activity decreased with increased level of CAT which might be because decreased SOD level demonstrated damage in antioxidant mechanism due to metal exposure. In contrary to this, increase in CAT showed its participation to cope up with elevated level of oxidative stress as shown by[47]. Reduced glutathione has been considered as nonenzymatic antioxidant, pertaining important cellular defence against oxidative stress and products formed due to oxidative damage[48]. Glutathione sulfotransferase (GST), an imperative glutathione associated enzyme catalyzes the conjugation of GSH with various electrophilic substances to detoxify various reactive intermediates and keep equilibrium between ROS production and elimination[45, 49]. There were disparity in the results observed by various workers in the content of GSH and GST in fish tissue exposed to metals where it was found due to their organ specific response[50, 51, 52, 53]. Results from present study showed significant reduced level of GSH and GST after 15 days, in fresh water fish *Channa punctatus* exposed to As, Cd individually, in combination and in Yamuna water fish as compared to control. Reduction in the GSH level can be explained by their conjugation with metals, hence declining the total GSH pool available[54]. Results obtained indicated that GSH might

have participated in protection against oxidative stress due to heavy metal toxicity and consequently decreased sharply in its level. Atli and Canli, Lima[50, 55] observed a significant increase in level of GSH at exposure of Cd, Cu and contaminated effluents respectively in liver of *O.niloticus*, whereas Cao[56] investigated decreased level of reduced glutathione and GST in Japanese flounder juveniles due to cadmium exposure in liver, while Jia[57] found increased level of GSH in liver of Oujiang colored common carp *Cyprinus carpio* Var. color. Present study clearly gives a view of oxidative stress in Yamuna water fish when compared with other treatment groups. The metals hampered the antioxidant defense hence reduced antioxidants activity was observed in fish of metal treated individually and combined group. Compared to metal treated groups, Yamuna water fish had higher level of lipid peroxidation indicating more oxidative stress to the fish. While, their antioxidant defense is not reduced in the same manner. Thus definitely showing a better antioxidative defense in Yamuna water fish. Chromatophores are specialized cells which can produce pigments and are originated in neural crest during embryonic development. Depending on the species, they have various types of hormone receptors and neurotransmeters which participate in aggregation and dispersion of chromatophrores[58, 59]. Considering this fact fish chromatophores were also observed in this study to perceive change in pigmentation due to altered surrounding conditions**.** In the present study, there was marked changes in fish chromatophores noticed in both Yamuna water and metal treated group. Accumulation of metals in the scales produced the variation in the chromatophores position, shape and density. Our study reports that in arsenic treated group, numbers of chromatophores were reduced but they become reticulated and there was also a specific linear pattern visualized. In cadmium treated group, the chromatophores were very less in number and dispersed throughout. In combined metals group, the chromatophores greatly reduced and almost disappeared showing the degeneration of chromatophores. Yamuna water group has shown maximum variation in shape, size and density**.** This variation is again attributed to variety of stress Yamuna water fish have to face. Similarly Bajpaiand Tripathi, Kaur and Dua[60,61]also found dispersion of chromatophores in fresh water fish *Channa punctatus and*an alteration in the shape, density and dimension after fluoride exposure in stinging catfish *Heteropneustes fossils* (Bloch) respectively from water channel Tung Dhab drain.Due to metal toxicity chromatophores number reduced greatly. Thus it can be predicted in order to survive in the toxic environment, the fish might have maintained its antioxidants defense and hence chromatophores are not entirely damaged or reduced speculating their protection against toxicity to survive in pollutants loaded environment.

REFERENCES

1. Li X.B., Hou X.L., Mao Q., Zhao Y.L., Cheng Y.X., and Wang Q., Toxic Effects of Copper on Antioxidative and Metabolic Enzymes of the Marine Gastropod, *Onchidium struma.* Arch Environ Contam Toxicol*,* 56: 776–784, (2009)
2. Ameur W.B., Lapuente J.de., Megdichea Y.El., Barhoumi B., Trabelsi S., Camps L., Serret J., López D.R., Linares J.G., Driss M.R. and Borràs M., Oxidative stress, genotoxicity and histopathology biomarker responses in mullet *(Mugil cephalus)* and sea bass *(Dicentrarchus labrax)* liver from Bizerte Lagoon (Tunisia), Marine Pollution Bulletin*,* 64: 241–251, (2012)
3. Paul M.S., Varun M., D'Souza R., Favas P.J.C., Pratas J. Metal Contamination of Soils and Prospects of Phytoremediation in and Around River Yamuna: A Case Study from North-Central India, In:C.Maria and Hernandez-Soriano

Environmental Risk Assessment of Soil Contamination, InTech, 2014, DOI: 10.5772/57239. Available from: http://www.intechopen.com/books/environmental-risk-assessment-of-soil-contamination/metal-contamination-of-soils-and-prospects-of-phytoremediation-in-and-around-river-yamuna-a-case-study

4. Kaur S., Assessment of Heavy Metals in Summer & Winter Seasons in River Yamuna Segment Flowing through Delhi, India. Journal of Environment and Ecology, 3(1): 149-165, (2012)
5. Sehgal M., Garg A., Suresh R. and Dagar P., Heavy metal contamination in the Delhi segment of Yamuna basin. Environ Monit Assess, 184 (2): 1181-1191, (2012)
6. An M.I., An K.W. and Choi C.Y., Changes in Antioxidant Enzyme Activity and Physiological Responses to admium and Tributyltin Exposure in the Ark Shell, *Scapharca Broughtonii.* Mol. Cell. Toxicol*,* 5(4): 273-282, (2009)
7. Bissen M., Frimmel F.H., Arsenic- a review. Part I: occurrence, toxicity, speciation, and mobility. Acta Hydrochim Hydrobiol, 31: 9–18 (2003a)
8. Bissen M. andFrimmel F. H., Arsenic—a review. Part II: oxidation of arsenic and its removal in water treatment. Acta Hydrochim Hydrobiol, 31: 97–107, (2003b)
9. Alex E., Lawrence I.E. and Frances A., Heavy metal concentrations in surface water and bioaccumulation in fish *(Clarias gariepinus)* of river Owan , endostate , Niegeria. European International Journal of Science and Technology. 2(7): 31-39 (2013)
10. Peixoto F.P., Carrola J., Coimbra A.M., Fernandes C., Teixeira P., Coelho L., Conceicao I., Oliveira M.M. and Fontainhas-Fernandes A., Oxidative stress responses and histological hepatic alternations in Barbel *Barbus bocagei*, from Vizela river. Portugal. Rev. Int. Contam. Ambie, 29(1) 29-38, (2013)
11. Yildirim N.C., Benzer F. and Danabas B., Evaluation of environmental pollution at Munjur River of Tunceli applying oxidative stress biomarkers in *Capoeta trutta* (Heckel, 1843). The Journal of Animal & Plant Sciences 21(1): 66-71 (2011)
12. Jia X., Zhang H. and Liu X., Low levels of cadmium exposure induce DNA damageand oxidative stress in the liver of Oujiang colored common carp *Cyprinus carpio* var. color. Fish Physiol Biochem, 37: 97–103 (2011)
13. Valone M., Valbona A., Fahri G., Qenan M., Dhurat K. and Fatmir C., Evaluating Environmental pollution by applying Oxidative stress biomarker as bioindicator of water pollution in fish. Pol. J. Eviron Stud, 22(5): 1519-1523 (2013)
14. Almeida J.A., Barreto R.E., Novelli E.L.B., Castro F.J. and Moron S.E., Oxidative stress biomarkers and aggressive behavior in fish exposed to aquatic cadmium contamination. Neotrop Ichthyol, 7: 103–108 (2009)
15. Bagnyukova T.V., Luzhna L.I., Pogribny I.P. and Lushchak V., Oxidative stress and antioxidant defenses in goldfish liver in response to short-term exposure to arsenite. Environ Mol Mutagen, 48(8) 658-65 (2007)
16. Sethuraman V., Haq B., Chezhian A., Shanker S. and Selvan D.S., Changes due to the effect of the Heavy Metals ($HgCl_2$ and $ZnSO_4$) concentration on the marine fish, *Tilapia Mossambica* (Peters, 1852). Archives of Applied Science Research, 3(6): 333-341 (2011).
17. Sevcikova M., Modra H., Slaninova A. and Svobodova Z., Metals as a cause of oxidative stress in fish: a review. Veterinarni Medicina*,* 56(11): 537–546 (2011)
18. Annual report 2013-14 Central Water Commission, River Data Directorate. Planning & Development Organisation, New Delhi Accessed on "May 30,2014" http://www.indiaenvironmentportal.org.in/files/file/Status%20of%20Trace%20and%20Toxic%20Metals%20in%20Indian%20Rivers.pdf
19. Singh S., Malik D., Thakur J., Kaur A., Singh R.K. and Nijhawan S., A

Comparative Analysis of the Physico-chemical Properties and Heavy Metal Pollution in Three Major Rivers across India. International Journal of Science and Research, 3(8): 1936-1941 (2014)

20. Hamilton M.A., Russo R.C. and Thurston R.V., Trimmed Spearman Karber method for estimating median lethal concentrations in toxicity bio-assays. Environ Sci Technol, 11: 714–719 (1977)
21. Hogben L. and Slome D., The pigmentary effector system: IV. The dual character of endocrine co-ordination in amphibian color change. Proceedings of Royal Society of London, 108B: 10–53 (1931)
22. Jordan R.A. and Schankman J.B., Relationship between malon-dialdehyde production and arachidonate consumption during NADPH supported microsomal lipid peroxidation. Biochem. Pharmacol, 31: 1393-4000 (1982)
23. Lowry O.H., Rosenbrough N.J., Farr A.L. and Randall R.J., Protein measurement with folin phenol reagent, J Biol Chem193: 265-275 (1951)
24. Kakkar P., Das B. and Viswanathan P.N., A modified spectroscopic assay of superoxide dismutase. Indian J Biochem Biophys, 21: 130–132 (1984)
25. Aebi H., Catalase in vitro In: Packer L (ed) Methods in enzymology, Academic Press, New York, Vol 105: pp. 121–126 (1984)
26. Ellman G. L., Tissue sulfhydryl groups Arch. Biochem, 82: 70-77 (1959)
27. Habig W.H., Pabst M.J. and Jakoby W.B., Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. J Biol. Chem, 249: 7130–7139 (1974)
28. Valko M., Morris H. and Cronin M.T., Metals, toxicity and oxidative stress. Curr Med Chem, 12(10): 1161-208 (2005)
29. Flora S.J.S., Pandey M., Kannan G.M. and Mehta A., Lead induced oxidative stress and its recovery following co-administration of melatonin or N-acetylcysteine during chelation with succimer in male rats. Cell Mol Biol,50: OL543-OL551 (2004)
30. Devaraju, T., Sujatha, K., Madhava Rao, S., and K. Jayantha Rao. Impact of sodium arsenate on selected enzymes and histopathological studies in albino mice. International Journal of Pharma and Bio sciences, 1(3): 1-7 (2010).
31. Birben E., Sahiner U.M., Sackesen C., Erzurum S. and Kalayci O., Oxidative stress and antioxidant defence. World Allergy Organ J, 5(1): 9-19 (2010).
32. Saglam D., Atli G., Dogan Z., Baysoy E., Gurler C., Eroglu A. and Canli M., Response of the Antioxidant System of Freshwater Fish *(Oreochromis niloticus)* Exposed to Metals (Cd, Cu) in Differing Hardness. Turkish Journal of Fisheries and Aquatic Sciences, 14: 43-52 (2014)
33. Barrera G., Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncology 2012: 1-22 (2012)
34. Viarengo A., Bettella E., Fabbri R., Burlando B. and Lafaurie M., Heavy metal inhibition of EROD activity in liver microsomes from the bass *Dicentrarchus labrax* exposed to organic xenobiotics: Role of GSH in the reduction of heavy metal effects. Marine Environmental Research, 44(1): 1-11 (1997)
35. Elahi A., Ahmad J., Nauman M., Bashir H., Khan F. and Qureshi M.I., Biomarkers Analysis of Oxidative Stress as Indicator of Environmental Pollution in Liver of Walking Cat Fish (*Clarias Batrachus*) from Yamuna River, Delhi. Journal of Natural Science, Biology and Medicine, 2(2): 153-154 (2011)
36. Panday S., Parwez S., Sayeed I., Haque R., Bin Hafeez B., and Raisuddin S., Biomarkers of oxidative stress: a comparative study of river Yamuna fish *Wallago attu* (Bl. And Schn.). Sci Total Envion 309 (1-3): 105-15 (2003)
37. Ferreira M., Moradas-Ferreira P. and Reis-Henriques M.A., Oxidative stress biomarkers in two resident species, mullet *(Mugil cephalus)* and flounder *(Platichthys flesus)*, from a polluted site in River Douro Estuary, Portugal. Aquat Toxicol, 71(1): 39-48 (2005)

38. Bacanskas L.R., Whitaker J. and Di Giulio R.T., Oxidative stress in two populations of killifish *(Fundulus heteroclitus)* with differing contaminant exposure histories. Mar Environ Res*,* 58(2-5) 597-601 (2004)
39. Sanchez W., Ait-Aissa S., Palluel O., Ditche J.M., and Porcher J.M., Preliminary investigation of multi-biomarker responses in three-spined stickleback *(Gasterosteus aculeatus L.*) sampled in contaminated streams. Ecotoxicilogy, 16(2): 279-87 (2007)
40. McCord J.M., Keele B.B., and Fridovich I., An enzyme- based theory of obligate anaerobis: The physiological functions of superoxide dismutase. Proc Nati Acad Sci USA, 68: 1024-31 (1976)
41. Gutteridge J.M., Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 41: 1819–28 (1995)
42. Ruas C.B., Carvalho Cdos S., de Araujo H.S., Epindola E.L., and Fernandes M.N., Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. Ecotoxicol Environ Saf. 71(1): 86-93 (2008)
43. Saliu J.K. and Bawa-Allah K.A., Toxicological Effects of Lead and Zinc on the Antioxidant Enzyme Activities of Post Juvenile *Clarias gariepinus.* Resources and Environment, 2(1): 21-26 (2012)
44. Farombi E.O., Adelowo O.A. and Ajimoko Y.R., Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish *(Clarias gariepinus)* from Nigeria Ogun River. Int J Environ Res Public Health, 4(2): 158-65 (2007)
45. Pretto A., Loro V.L., Baldisserotto B., Pavanato M.A., Moraes B.S., Menezes C., Cattaneo R., Clasen B., Finamor I.A. and Dressler V., Effects of Water Cadmium Concentrations on Bioaccumulation and Various Oxidative Stress Parameters in *Rhamdia quelen.* Arch Environ Contam Toxicol, 60: 309–318 (2011)
46. Saglam D., Atli G., Dogan Z., Baysoy E., Gurler C., Eroglu A. and Canli M., Response Of antioxidant System of Freshwater Fish *(Oreochromis niloticus)* Exposed to Metals (Cd, Cu) in Differing Hardness. Turkish Journal of Fisheries and Aquatic Sciences 14: 43-52 (2014)
47. Barata C., Varo I., Navarro J.C., Arun S., and Porte C., Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran Daphnia magna exposed to redox cycling compounds.Comp Biochem Physiol C Toxicol Pharmacol, 140(2): 175-86 (2005)
48. Srikanth K., Pereira E., Duarte A.C. and Ahmad I., Glutathione and its dependent enzymes' modulatory responses to toxic metals and metalloids in fish—a review. Environ Sci Pollut Res, 20: 2133–2149 (2013)
49. Monteiro D.A., Almeida de J.A., Ratin F.T., Kalinin A.L., Oxidative stress biomarkers in the freshwater characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp Biochem Physiol C Toxicol Pharmacol 143(2): 141-9 (2006)
50. Atli G. and Canli M., Responses of metallothionein and reduced glutathione in a freshwater fish *Oreochromis niloticus* following metal exposures. Environ Toxicol Pharmacol,25: 33–38 (2008)
51. Berntssen M.H.G., Lundebye A.K. and Hamre K., Tissue lipid peroxidative responses in Atlantic salmon (*Salmo salar* L.) parr fed high levels of dietary copper and cadmium. Fish Physiol Biochem,23: 35–48 (2000)
52. Firat O., Cogun H.Y., Aslanyavrusu S. and Kargin F., Antioxidant responses and metal accumulation in tissues of Nile tilapia *Oreochromis niloticus* under Zn, Cd and Zn + Cd exposures. J Appl Toxicol, 29(4): 295-301 (2009)
53. Ventura-Lima J., de Castro M.R., Acosta D., Fattorini D., Regoli F., de Carvalho L.M., Bohrer D., Geracitano L.A., Barros D.M., Marins L.F., da Silva R.S., Bonan C.D., Bogo M.R. and Monserrat J.M., Effects of arsenic (As) exposure on the antioxidant status of gills of the zebrafish

Danio rerio (Cyprinidae). Comp Biochem Physiol C Toxicol Pharmacol, 149(4): 538-43 (2009)

54. Meister A. and Anderson M.E., Glutathione. Annu Rev Biochem, 52: 711–760 (1983)
55. Lima P.L., Benassi J.C., Pedrosa R.C., Dal Magro J., Oliveira T.B. and Wilhelm Filho D., Time-course variations of DNA damage and biomarkers of oxidative stress in tilapia *(Oreochromis niloticus)*exposed to effluents from a swine industry. Arch Environ Contam Toxicol, 50: 23–30 (2006)
56. Cao L., Huang W., Liu J., Yin X. and Dou S., Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. Comp Biochem Physiol C Toxicol Pharmacol 151: 386–392 (2010)
57. Jia X., Zhang H. and Liu X., Low levels of cadmium exposure induce DNA damage and oxidative stress in the liver of Oujiang colored common carp *Cyprinus carpio* Var. color. Fish Physiol Biochem, 37: 97–103 (2011).
58. Chaplen F.W., Upson R.H., Mcfadden P.N. and Kolodziej W., Fish chromatophores as cytosensors in a microscale device: detection of environmental toxins and bacterial pathogens, Pigment Cell Res, 15(1): 19-26 (2002)
59. Fujii R., Cytophysiology of fish chromatophores. Int Rev Cytol, 143: 191–225 (1993)
60. Bajpai S. and Tripathi M., Alternation in the pigmentation after fluoride exposure in stinging catfish, *Heteropneustes fossilis* (Bloch). Cibtech Journal of Zoology, 1(2): 47-52 (2012).
61. Kaur R. and Dua A., Fish scales as indicators of wastewater toxicity from international water channel Tung Dhab drain. Environ Monit Assess,184(5): 2729-2740 (2012).