ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2016; 4(4): 08-13

www.fisheriesjournal.com
Received: 13-05-2016
Accepted: 14-06-2016

Sunaina
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur – 273009, India.

A Kumar Bhardwaj
Jagatpur Inter College, Jagatpur, Varanasi-221302, Uttar Pradesh, India.

B Alam Ansari
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur – 273009, Uttar Pradesh, India.

Correspondence
Sunaina
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur – 273009, India.

Oxidative stress biomarkers in assessing arsenic tri oxide toxicity in the Zebrafish, *Danio rerio*

Sunaina, A Kumar Bhardwaj and B Alam Ansari

Abstract
Heavy metals and their salts are considered as very important group of environmental pollutant which in small quantities may be essential nutrients that protect our health, yet in larger quantity it being toxic and dangerous to all living organisms including aquatic organisms. One of the major mechanisms behind heavy metal toxicity has been attributed to oxidative stress. Heavy metals are important inducers of oxidative stress in aquatic animals, promoting formation of reactive oxygen species (ROS). ROS are an unenviable part of aerobic life. During the present study, arsenic effect was studied at two doses (0.006 mg/l and 0.03 mg/l) for 7, 14 21 and 28 days in liver and ovary of Zebrafish *Danio rerio*. This study included the antioxidant profile namely catalase (CAT), lipid peroxidation (LPO) and reduced glutathione (GSH). A notable decrease was observed in the activities of catalase and reduced glutathione in the levels of other metabolite together with a significant increase in lipid peroxidation after arsenic exposure.

Keywords: Heavy metals, ROS, CAT, LPO, GSH and *Danio rerio.*

1. Introduction
Heavy metals play a crucial role in various biological functioning and remain present in trace amount in the body that is not exceeding 1 µg/g. However only slight increase in the concentration lead to high level of toxicity in different organs. Some heavy metals e.g. arsenic, cobalt, iron, aluminum, manganese, molybdenum, nickel, selenium, tin and zinc are essential heavy metals for some organisms. But industrial effluents containing toxic and hazardous substance, including heavy metals, consequently lead to the pollution of aquatic ecosystem, (Gbem *et al*., 2001) [1]. In aquatic ecosystem, heavy metals are considered as the most important pollutants, since they are present throughout the ecosystem and are detectable in critical amounts. The carcinogenicity for heavy metals is the generation of reactive oxygen species, (Flora *et al*., 2008) [2] which resulted in cellular damage like enzymes depletion and DNA damage (Chen *et al*., 2001) [3]. Heavy metals are considered as the most important pollutants, in aquatic ecosystem, since they are present throughout the ecosystem and are detectable in some critical amounts (Authman *et al.*, 2015) [4]. Due to the fact that even trace amount of some heavy metals can exhibit high toxicity to aquatic biota and human, these and increasing interest in studying interaction of these metals in the aquatic environments. The quality of freshwater has been changed due to the presence of different chemicals including metal pollutants, industrial effluents and pesticides. Aquatic ecosystems are exposed to a number of pollutants that are mainly released from industrial discharges from industries, sewage treatment plants and drainage from urban and agricultural areas and this type of pollutants cause serious damage to aquatic animals (Karbassi *et al*., 2006) [5]. Metal pollution is one of the most dangerous consequences of industrial activities to the aquatic environment (Saxsena and Garg 2010) [6]. Metals are the largest and most widespread groups of contaminants, because of their cumulative nature and slowly eliminate from environmental compartment (Minghetti *et al.*, 2008) [7], (Cirillo *et al.* 2012) [8].
Aquatic system is an ultimate sink of heavy metal pollutants and since aquatic animals tend to accumulate heavy metals from various sources including sediment soil erosion and runoff, air depositions of dust and aerosol and discharge of waste water (Goodwin *et al.,* 2003) [9], they provide the insights of toxicity mechanisms induced by heavy metals. Among these metals cadmium (Cd), Arsenic (As), Lead (Pb), copper (Cu) and Chromium (Cr) pose major problems to aquatic life.

As is one of the most common contaminants found in surface and ground water and is released in the environment through natural processes such as weathering, and may circulate in natural ecosystem for long time. Arsenic has been recognized as strong biological poison because of their persistent in nature and accumulative action. This heavy metal is able to accumulate in large amount in the sediments on the bed of water courses and reservoirs, and in aquatic organisms (Svobodova *et al.*1993) [10]. Arsenic compounds in the third (III) oxidation state (arsenites) are absorbed fairly rapidaly in to fish and are more toxic than arsenic compounds in the oxidation state V (arsenates) (Liao et al. 2004) [11] this is also a non-essential metal, have no biological function requirement and their concentration in fishes are generally low.

Fishes are excellent subject for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. Human`s are also exposed to arsenic in the environment primarily through the investigation of food and water (EFSA, 2009) [12], (WHO 2001) [13], (Carbonell- Barachina 2009) [14]. Fish are an important component of human nutrition, and those from contaminated sites present a potential risk to human health.

Due to the high sensitivity of the aquatic life towards dissolving toxicant, the fish has been utilized as the biological measurement (Biomarker) to indicate the existence of toxicant exposure and/or the impact towards the evaluation of molecular, cellular to physiological level (Sabullah *et al.,* 2015) [15]. Since fish occupy the top of the aquatic food chain, they are suitable bioindicators of metal contamination. Metals are well-known inducers of oxidative stress, and assessment of oxidative damage and antioxidant defenses in fish can reflect metal contamination of the aquatic environment (Livingstone, 2003) [16]. In present study we used Zebrafish for biomonitoring of environmental contamination. Therefore, examining the change in activity of antioxidant enzymes such as SOD, CAT and GSH should be an effective method of denoting oxidative stress and changes in their activity.

2. Materials and Methods

Zebrafish were collected from local ponds, acclimatized for 15 days, stocked and bred under laboratory conditions. The aquaria were continuously aerated through stone diffusers connected to a mechanical air compressor. Water temperature was 25±2ºC and pH was maintained between 6.6 and 8.5. The fish were fed twice daily alternately with egg, goat liver and raw brine shrimp pellets. The experimental fishes were exposed to different concentrations viz, 20% and 80% of 96 hour LC_{50} values i.e. 0.006 and 0.03 mg/l of arsenic calculated earlier (Sunaina and Ansari, 2014) [17]. 20 fishes for each concentration of metal test were used. In the experimental aquaria water was replaced daily with fresh treatment of metal. After the end of the experimental periods (7, 14, 21 and 28 days), required number of treated fish was taken out from experiment and control groups and their tissues were dissected. Six replicates for each concentration of arsenic were arranged.

3. Biochemical Estimation

3.1 Lipid peroxidation (LPO)

LPO levels were estimated with thio-barbituric acid (TBARS) and color reaction for malondialdehyde (MDA) according to procedure by (Placer *et al.*, 1966) [18]. Tissues were homogenized in chilled 0.15 M KCl using a Teflon pestle to obtain 10% w/v homogenate. One ml of homogenate was incubated at 37 ºC (± 0.5) for two hours. To each sample, 1 ml of 10% w/v trichloro acetic acid (TCA) (s. d. fine chem. Ltd; Mumbai) was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 minutes 1 ml of supernatant was taken with equal volume of 0.67% w/v TBA (thio-barbituric acid) and kept in boiling water bath for 10 minutes, cooled and diluted with 1 ml of distilled water. The absorbance pink color was observed, which measured at 535 nm against a blank. The concentration of MDA was read from a standard calibration curve plotted using 1, 1, 3, 3` tetra-methoxypropane (Sigma –Aldrich Co., St. Louis, USA) and the results were expressed as µmol of MDA formed 30 min^{-1} mg $protein^{-1}$.

3.2 Reduced glutathione (GSH)

The GSH levels was estimated according to procedures (Paglia *et al.,* 1975) [19], with which it is determined by its reaction with 5,5`- dithiobis-2-nitrobenzoic acid (DTNB) to produce/yield a yellow chromophore that was measured spectrophotometrically at 412 nm. The results were expressed as GSH mg per mg $protein^{-1}$. The protein contents of tissues were assayed using the Lowery *et al*., (1951) [20] method with bovine serum albumin as the standard.

3.3 Catalase (CAT)

The CAT activity was estimated according to procedures by (Sinha, 1972) [21]. This method is based on the fact that in acetic acid dichromate is reduced to chromic acetate when heated in presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromic acetate is measured colorimetrically at 620 nm. The catalase preparation is allowed to split H_2O_2 at different time intervals by the addition of a dichromatic acetic acid mixture and remaining H_2O_2 is determined colorimetrically. The results were expressed as µmol H_2O_2 utilized min^{-1} mg $protein^{-1}$.

Two way analysis of variance (ANOVA) was applied to test the significance of data. All the data are expressed as means (n=6) ± standard deviation (SD) and differences were considered significant at $P<0.05$.

4. Results and Discussion

Catalase activity of arsenic was significantly decreased in liver and ovary after the exposure periods. Changes of catalase activity in tissues of zebrafish is responses to concentrations viz, 20% and 80% of 96 hour LC_{50} i.e. 0.006 and 0.03 of arsenic and exposed for 7, 14, 21 and 28 days. After in 7 days of exposure to 20% 96 hour LC_{50} of arsenic, CAT activity reduced from 159.88 ±0.25 (93%) to 135.08±0.46 (90%) in liver and ovary, respectively. After 28 days of exposure to 80% 96 hour LC_{50} of arsenic, the activity was found to be 91.20±0.27 (59%) and 80.76±0.63 (53%) in liver and ovary which shows reduction in concentration, where the reduction in percent was 41% and 47% in comparison to control (100%) (Fig. 4.1 and 4.2).

The effect of sub lethal concentrations of arsenic on GSH content was estimated after scheduled time intervals of arsenic exposure to the Zebrafish *Danio rerio.* GSH content was also significantly reduced during all the exposure periods. In 7 days exposure, 20% 96 hour LC_{50} of arsenic, GSH level found to be 4.30±0.37 (93%) and 3.73±0.52 (90%) in comparison to control (100%) in liver and ovary, respectively. A maximum reduction was observed during 28 days 20% of 96 hour LC_{50} 2.91±0.46 (60%) in liver and decreased 2.15±0.68 (54%) in ovary in comparison to control (Fig.4.3 and 4. 4).

Elevation in LPO levels were observed significantly in the test tissues liver and ovary of Zebrafish exposed to arsenic for 7, 14, 21 and 28 days' time intervals. The MDA concentrations in these tissues, which exposed to arsenic, induced from 9.15±0.45 (102%) to 14.48±0.49 (110%) in 7 days 20% 96 hour LC_{50} of arsenic. However, in 80% 96 hour LC_{50} for 28 days exposure, MDA level further induced to 14.52±0.89 (143%) in the liver and 23.06±0.56 (173%) in the ovary in comparison to control (Fig.4.5 and 4. 6).

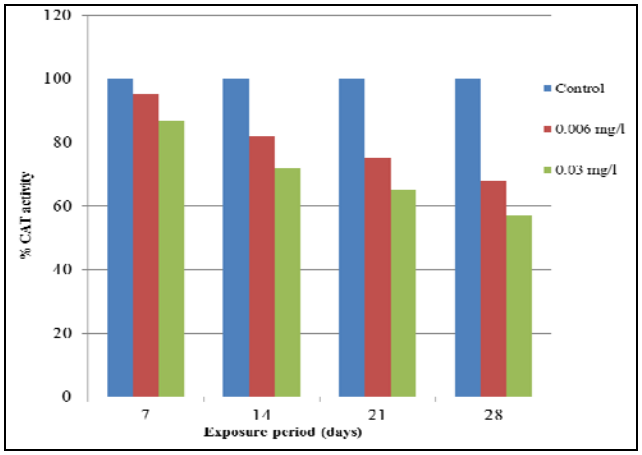

Fig 4.1: Effect of Arsenic on CAT in the liver of *Danio rerio*.

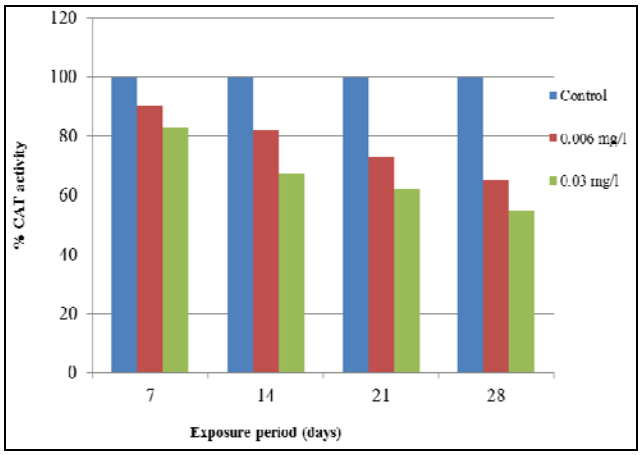

Fig 4.2: Effect of Arsenic on CAT in the Ovary of *Danio rerio*.

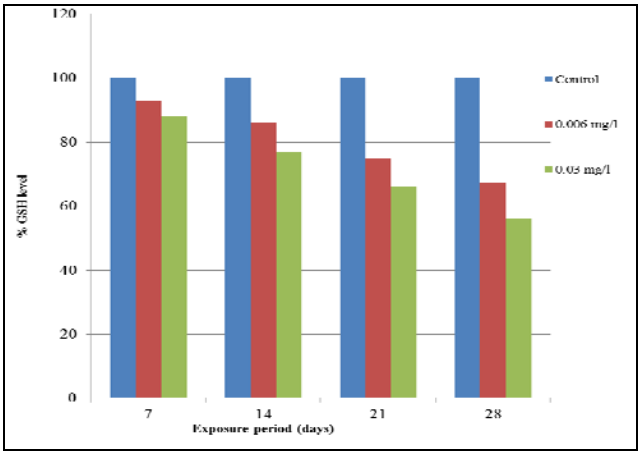

Fig 4.3: Effect of Arsenic on GSH in the Liver of *Danio rerio*.

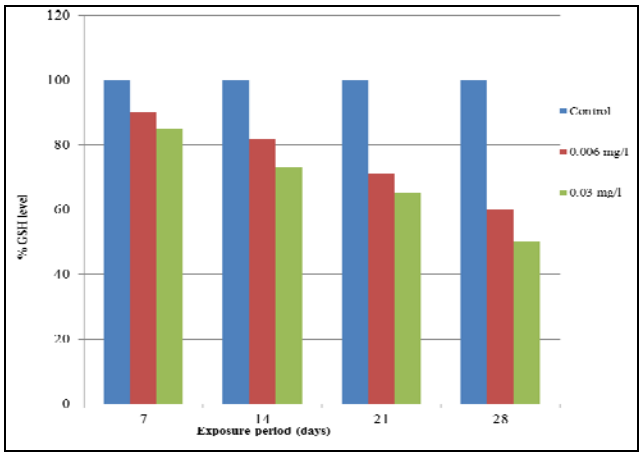

Fig 4.4: Effect of Arsenic on GSH in the ovary of *Danio rerio*.

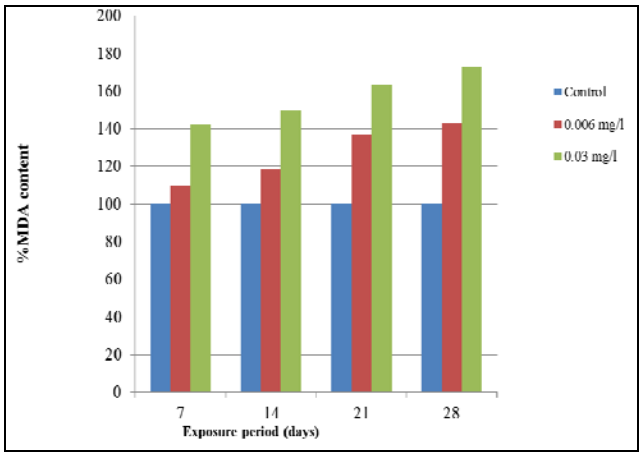

Fig 4.5: Effect of Arsenic on LPO in the Liver of *Danio rerio*.

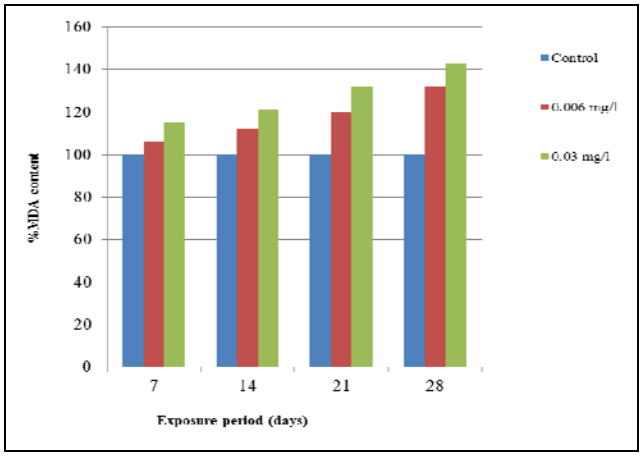

Fig 4.6: Effect of Arsenic on LPO in the ovary of *Danio rerio*.

Arsenic, heavy metal, an environmental toxicant and ubiquitous trace element is found naturally in ground water and as a contaminant from industrial and agricultural use. Inorganic arsenic has been classified by IARC (International Agency for Research on Cancer, Geneva) as group I carcinogen. Chronic exposure to humans has been associated with the disorders of the peripheral vasculature, diseases of cardiovascular system, diabetes and reproductive failure. Arsenic has also been known to be neurotoxic. Arsenic forms inorganic and organic complexes in the environment. Arsenite (III) and arsenate (V) are inorganic forms that can be

methylated. The trivalent arsenite is biologically more active than pentavalent arsenate. Alterations in biochemical composition on exposure to heavy metals are studied by many workers. Oxidative damage primarily occurs through production of reactive oxygen species (ROS) that damaged lipids, proteins and DNA contributing to loss of activity and structural integrity of enzymes and may active inflammatory processes, (Ozyurt *et al.,* 2004) [22]. Mostly the abnormal generation of ROS is considered as an important signal of oxidative damage, which can result in significant damage to cell structure (Brazilai *et al.* 2004) [23]. Oxidative stress is elevated which results of the three factors (a) an induction in oxidant generation, (b) a reduction in antioxidant protection, and (c) failure to repair oxidative damage, (Das *et al.,* 2010)[24]. Superoxide (O^{2-} one of the parental forms of intracellular ROS), is a very reactive molecule, but it can be converted to H_2O_2 by superoxide dismutase (SOD) and then to oxygen and water by several enzymes including catalase (CAT) and glutathione reductase (GR). Therefore, examining the change in activity of antioxidant enzymes such as SOD, CAT, and GR shall be an effective method of denoting oxidative stress and changes in their activity and other biomarkers could be the possible tools in aquatic toxicological research.

High concentration of metals in fish tissues can lead to redox reactions, generating free radicals, especially reactive oxygen species e.g. oxygen superoxides, peroxides, hydroxyl radical and hydrogen peroxides, (Patra *et al.,* 2011) [25]. The highly reactive compounds, molecules or ions formed by the incomplete reduce oxygen, may induce alteration and change some physiological responses of fish (Palacios *et al.,* 2000)[26], (Varanka 2004) [27], (Brucka- Jasterjebaska, 2010) [28].

To alternate the negative effects of ROS, fish possess an antioxidant defense system like other vertebrate that utilizes enzymatic and no enzymatic mechanisms. Oxidative state or any imbalance between production and degradation of ROS in animal tissues may cause inactivation of enzymes, LPO and plasma membrane alternations (Vinodhini and Narayanan, 2009) [29]. Arsenic and iron induced toxic effects on *Tilapia mosambica* (Kulkarni *et al*,. 2005) [30]. Oxidative stress-induced apoptosis (Seok *et al.,* 2007) [31] as a possible mechanism of arsenic toxicity in zebrafish *Danio rerio* liver cell line. The majority of total arsenic in fish tissue is present as arsenobetaine (Harkabusova *et al.,* 2009) [32], (Ciordullo *et al*., 2010) [33].

In present study the zebrafish was exposed to As_2O_3 for a period of 7.14,21 and 28 days at suitable concentrations that is 20% of 96 hours (0.006 mg/l) and 80% of 96 hours (0.03 mg/l) and recorded a significant reduction in CAT (Catalase) and GSH (reduction was recorded in GSH and CAT at the higher concentration 20% of 96 hours LC_{50} as compared to the lower concentration of 80% of 96 hrs and maximum induction of LPO was recorded at the 80% of 96 hours as compared to the higher concentration of 20% 96 hours. These observations revealed that the decline and upgrade in CAT, GSH and LPO level in different tissues was directly proportional to concentration of arsenic.

Glutathione is an electron donor in the reduction of arsenate to arsenite. Arsenic cell metabolisms generate ROS, although the mechanisms are not clear. Interaction of toxic metals with GSH metabolism is an essential part of toxic response of many metals (Hultberg *et al.,* 2001) [34]. When GSH is depleted by any metal, GSH synthesizing systems start making more GSH from cystiene via the Y-glutamyl cycle. GSH is usually not effectively supplied, however, if GSH depletion continues because of chronic metal exposure (Stohs *et al.,* 1993) [35], (Hultberg *et al.,* 2001) [36], (Quig *et al.,* 1998) [37]. The role of GSH in arsenic toxicity was described in several studies, (Allen *et al*., 2004) [38] biochemical toxicity of arsenite in *Channa punctatus*. In the present study, GSH level has been significantly decreased in liver and ovary of zebrafish *Danio rerio* to sublethal concentrations 0.03 and 0.006 which is 20% and 80% of 96 hours LC_{50} for 7, 14, 21 and 28 days of experimentation. GSH protection role in arsenic toxicity has been widely documented. In vitro studies showed that different cell lines have different antioxidant levels (GSH, GR and GST) (Snow *et al*., 2005) [39], which helps explain differential arsenic cytotoxicity in various cell lines. The depletion of GSH has been found to promote oxidative stress in various model systems (Yeh *et al*., 2002) [40].

In the present study a decreased level of Catalase activity was observed during the 7, 14, 21, and 28 days of exposure in the liver and ovary of zebrafish from As_2O_3 to sublethal concentrations 0.006 and 0.03 which is 20% and 80% of 96 hrs $LC_{50.}$ NADPH is necessary for the maintenance of CAT activity, since the reduced CAT activity may be associated with the observation of diminished level of NADPH in fish. Decreased CAT activity in $As_2O_{3,}$ exposed fish is in contrast to observation in *C. batarachus* where the increase in CAT activity was reported after arsenic exposure, (Bhattacharya and Bhattacharya, 2007) [41]. Catalase (H_2O_2: H_2O_2 oxidoreductase; EC 1. 11. 1. 6), a hydrogen peroxide scavenger, catalyze the breakdown of hydrogen peroxide to water and molecular oxygen to protect cells against the toxic effects of hydrogen peroxide (Chance *et al.,* 1979) [42].

The induction of LPO, an increased GSH ratio and excess production of hydrogen peroxide were observed in the cat fish *Clarias batarachus* exposed to non-lethal doses of arsenic for 10 days (Bhattacharya and Bhattacharya, 2007) [43]. The authors explained the elevated concentration of hydrogen peroxide as arsenic – induced alteration of peroxisome. Perturbation of glutathione content in cardiac tissue was reported earlier. A report indicate (Muthumani 2013) [44] that short- term arsenic toxicity in rats produces a significant decrease in cardiac GSH concentration associated with increased LPO. The same pattern of elevation in LPO and reduction in GSH and CAT in Zebrafish gills and livers, exposed to dimethoate reported, (Ansari and Ansari, 2014) [45].

In the present study we observed, LPO level has been significantly increased in liver and ovary of zebrafish *Danio rerio* at 7, 14, 21 and 28 days exposure period. The findings revealed that heavy metal, arsenic create harmful effects by generating reactive oxygen species that damage the cells by disturbing the fluidity balance.

5. Conclusion

The above mention findings suggested that oxidative stress induced by arsenic is an important issue in aquatic ecosystems. The present observations on the induction of oxidative stress and antioxidant system would make it clear that arsenic has a high degree of impact on antioxidant system in liver and ovary of zebrafish particularly during the exposures to arsenic would normally affect and alter the fish health.

6. Acknowledgements

The authors are thankful to the Rajiv Gandhi National Fellowship (F1-17, 1/2012-13/RGNF 2012-13-Sc-UTT-18032/SA- III/ (Website) for financial support and to Prof.

Rajendra Singh, Head of Department of Zoology, D. D. U. Gorakhpur University for providing laboratory facilities.

7. References

1. Gbem TT, Balogun JK, Lawal FA, Annune PA. Trace metal accumulation in *Clarias gariepinus* (Tuegels) exposed to sublethal levels of tannery effluent. Sci. the total environ. 2001; 1(3):1-9.
2. Flora SJS, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. Ind. Medi. Res.2008; 4:501-523.
3. Chen F, Ding M, Castranova V, Shi X-L. Carcinogenic metal and NF- $_k$B activation. Mole. and Cell. Biochem. 2001; 1(2):159-171.
4. Authman MMN, Zaki MS, Khallaf EA, Abbas HH. Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution. J. of Aqua. Res. Develop. 2015; 6:328..doi:10.4172/2155-9546.
5. Karbassi R, Bayati I, Mottar F. Origin and chemical portioning of heavy metals in river bed sediments,Int. J. Environ. Sci. Tech. 2006; 1:35-42.
6. Saxena R, Garg P. Vitamin provides protection against In Vitro Oxidative stress due to pesticide (Chlorphrifos) in goat RBC GERF. Bull. Biosci. 2010; 1:1-6.
7. Minghetti M, Leaver MJ, George SG. Copper transporter I metallothionein and glutathione reductase genes are differentially expressed in tissue of sea bream (*Sparus aurata*) after exposure to dietry or waterborne copper. Comp. Biochem. Physiol. 2008; 147:450-459.
8. Cirillo T, Amodio Cocchieri R, Fasano A, Lucisano A, Tafuri S, Ferrante E, et al., Cadmium accumulation and antioxidant responses in *Sparus aurata* exposed to waterborne cadmium. Arch. Envion. Contam. Toxicol. 2012; 62:118-126.
9. Goodwin TH, Young AR, Holmes MG. The temporal and spatial variability of sediment transport and yields within the Bradford Beck catchment, West Yorkshire. Sci. of the Total Environ. 2003; 314-316:475-494.
10. Svobodová Z. Water Quality and Fish Health. FAO, Rome, EIFAC technical 1993, 54, 67.
11. Liao CM, Tsai JW, Ling MP, Laing HM, Chou YH. Organspecific toxicokinetics and dose-response of arsenic in tilapia *Oreochromis mossambicus*. Arch. Environ. Contam. Toxicol. 2004; 47:502-510.
12. European Food Safety Authority Scientific opinion on arsenic in food. EFSA Journal. 2009; 7:1351. http://www.efsa.europa.eu/en/ scdocs/scdoc/1351.htm.
13. World Health Organization. Arsenic and Arsenic Compounds. Environmental Health Criteria, Geneva, Switzerland. 2001; 224.
14. Ángel A, Carbonell-Barrachina J, Antonio, Signes-Pastor, Vázquez-Araújo L, Francisco Burló, et al. Presence of arsenic in agricultural products from arsenic-endemic areas and strategies to reduce arsenic intake in rural villages. Mole. Nutri. & Food Res. May 2009, 531-541.
15. Sabullah M K, Ahmad SA, Shukor MY, Gansau AJ, Syed MA, Sulaiman MR, et al., Heavy metal biomarker: Fish behavior, cellular alteration, enzymatic reaction and proteomics approaches. Intern. Food Res. J. 2015; 22(2): 435-454
16. Livingstone DR. Oxidative stress in aquatic organism in relation to pollution and agriculture. Reve. de Medi. Veterin. 2003; 154:427-430.
17. Sunaina, Ansari BA. Acute toxicity of copper, cadmium and arsenic to zebrafish *Danio rerio* (Cyprinidae). Tre. Bio. 2014; 7(17):2357-2360.
18. Placer ZA, Cushman L, Johnson BC, Estimation of product of lipid peroxidation (Malonyldialdehyde) in biochemical systems Anal. Biochem, 1966; 16:359-364.
19. Paglia DE, Valentine WN, Dahlgren JG. Effects of low level lead exposure on pyrimidine 5' nucleotidase and other erythrocyte enzymes. Possible role of pyrimidine 5' nucleotidase in the pathogenesis of lead induced anemia. J of Clin. Invest. 1975; 561164-1169.
20. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J. Biol. Chem. 1951; 193:265-275.
21. Sinha AK. Colorimetric assay of catalase- Anal. Biochem 1972; 47:389-394.
22. Ozyurt H, Sogut S, Yildiri Z, Kart L, Iraz M, Armutcu F. Inhibitory effect of caffeic acid phenethyl ester on bleomycine-induced lung fibrosis in rats. Clinica Chimica Acta, 2004; 339:65-75.
23. Brazilai AKI, Yamamoto. DNA damage responses to oxidative stress. DNA Repair 2004; 3(8-9):1109-1115.
24. Das JS, Ravikanth VV, Sujatha M. Nitric oxide as a major risk factor for oxidative stress in coronary artery disease: a preliminary investigation. Sci. and Cult. 2010; 76(5-6):174-175.
25. Patra RC, Rautray AK, Swarup D. Oxidative stress in lead and cadmium toxicity and its amelioration. Vete. Medi. Intern, doi 2011; 10, 4061/2011/457327.
26. Paris- Palacios S, Biagannati- Rosbourg S, Vernet G. Biochemical and ultrastructural hepatic perturbation of *Brachydanio rerio* (Teleostei, Cyprinidae) exposed to two sublethal concentration of copper sulphate. Aquat. Toxicol. 2000; 50:109.
27. Varanka Z, Rojik I, Nemcsok J, Abraham M. Biochemical and morphological changes in carp (*Cyprinus carpio* L.) liver following exposure to copper sulfate and tannic acid. Comp. Biochem. Physiol. 2004; C:128-467.
28. Ewa Brucka-Jastrzebaska. The effect of aquatic cadmium and lead pollution on lipid peroxidation and superoxide dismutase activity in freshwater fish. Polish J. of Environ. Stud. 2010; 19(6):1139-1150.
29. Vinodhini R, Narayanan M. Biochemical changes of antioxidant enzymes in common carp (*Cyprinus carpio* L.) after heavy metal exposure. Turk. J Vet. Anim. Sci. 2009; 33(4):273-278.
30. Kulkarni AS, Venkateshwaran K, Wavde PN. Toxic effects of arsenic and iron on fresh water chichild fish Tilapia mosambica. Bioinfonet.2005; 2(3):189-192.
31. Seok SH, Baek MW, Lee HY, Kim DJ, Na YR, Noh KJ, et al., Arsenite-induced apoptosis is prevented by antioxidants in zebrafish liver cell line. Toxicology in Vitro, 2007; 21:870-877.
32. Harkabusova V, Macharakova B, Celechovska O, Vitoulova E. Determination of arsenic in the rainbow trout muscle and rice samples. Czechecoslovakia J of Food Sci. 2009; 27:S404-S406.
33. Ciardullo S, Aureli F, Raggi A. Cubadda FArsenic speciatipon in freshwater fish: focus on extraction and mass balance, Talanta. 2010; 81:213-221.
34. Stohs SJ, Bagchi D. Oxidative mechanism in toxicity of metal ions. Free radic. Biol. Med., 1993; 18:321-336.
35. Hultberg B, Andersson A, Isaksson A. Interaction of metals and thiols in cell damage and glutathione distribution: potentiation of mrercury toxicity by

dithiothreitol. Tox. 2001; 156:93-100.
36. Quig D. Cystiene metabolism and metal toxicity. Alter. Med. Rev.1998; 3:262-270.
37. Allen T, Singhal R, Rana SV. Resistance to oxidative stress in a freshwater fish Channa punctatus after exposure to inorganic arsenic. Biol. Trace Ele. Res. 2004; 98:63-72.
38. Snow ET, Sykora P, Durham TR, Klein CB. Arsenic, mode of action at biologically plausible low doses: what are the implications for low dose cancer risk? Toxicol Appl Pharmacol. 2005; 207:5557-5564.
39. Yeh, JY, Cheng LC, Ou BR, Whanger DP, Chang LW. Differential influences of various arsenic compounds on glutathione redox status and antioxidative enzymes in porcine endothelial cells. Cell Mol. Life Sci., 2002; 59:1972-1982.
40. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 1979; 59:527-604.
41. Muthumani M. Tetrahydrocurcumin Potentially Attenuates Arsenic Induced Oxidative Hepatic Dysfunction in Rats. J Clin Toxicol. 2013, 168. doi:10.4172/2161-0495.100016
42. Bhattacharya A, Bhattacharya S. Induction of oxidative stress by arsenic in *Clarias batarachus*. Involvement of peroxisomes, Ecotoxicol. and Environ. Safety 2007; 66:178-187.
43. Ansari S, Ansari BA. Temporal variations of CAT, GSH and LPO in gills and livers of zebrafish, *Danio rerio*, exposed to dimethoate. Arch. Pol. Fish. 2014; 22:101-109.