J. Ecophysiol. Occup. Hlth. 11 (2011) 205-211

Assessment of cadmium induced alteration in superoxide dismutase and catalase activities of *Labeo rohita* (Hamilton, 1822)

Anurag Dabas, N. S. Nagpure*, R. M. Mishra[1], Ravindra Kumar, Basdeo Kushwaha, and Rashmi Srivastava

Molecular Biology and Biotechnology Division
National Bureau of Fish Genetic Resources
Canal Ring Road, P.O. Dilkusha, Lucknow – 226 002 (UP), India
[1]Department of Environmental Biology
Awadhesh Pratap Singh University, Rewa, Madhya Pradesh, India

Abstract: *The present study investigated the effects of cadmium chloride on the activities of antioxidant enzymes, viz. superoxide dismutase (SOD) and catalase (CAT), in liver and kidney tissues of cyprinid fish, Labeo rohita. The test specimens were exposed to three concentrations of cadmium chloride namely, 33.55, 67.09 and 100.64 mg L^{-1}, for 96 h duration. A significant elevation in SOD activity and reduction in CAT activity was observed in a dose- and time-dependent manner in both the tissues. The results suggest the essential role of SOD and CAT in antioxidant defense system for protecting fish species from oxidative stress; hence, it can be considered as a sensitive biomarker of the antioxidant defense system.*

Key Words: Antioxidant enzymes, Cadmium, *Labeo rohita*, Oxidative stress

Introduction

Cadmium (Cd), a non-essential element, is a potent industrial hazard, arising primarily from battery, electroplating, pigment, plastic and fertilizer industries. It is a widespread environmental pollutant, recognized as one of the most deleterious heavy metals (Stoeppler, 1991). Cd is regarded as a potential hazard for the fish and other aquatic lives. It's exposure to fish, even at lower concentrations, may result in several toxic effects, including tissue damage, vertebral alterations, respiratory changes and ultimately death (Sorensen, 1991). It is readily absorbed by the organisms directly from the water in its free ionic form Cd^{2+} (AMAP, 1998). At cellular level, Cd exposure can have a number of effects, like production of reactive oxygen species (ROS), DNA damage as well as repair processes and formation of denatured or abnormal proteins (Waisberg *et al*., 2003). The adverse effects of Cd on the reproductive, respiratory and hematological systems in many fish species have been reported (Risso-de Faverney *et al*., 2004) with the highest level detected in the kidney, liver and gills (Olsson *et al*., 1996). Cd produces inhibitory effect on mitochondrial electron transport and, as a result, leads to an enhancement in the ROS formation, which causes damage in the liver, kidney and gills (Stohs *et al*., 2000).

Oxidative stress is an adverse reaction resulting from the exposure of molecules, cells, or tissues to excess levels of free radical oxidants, especially ROS (Lesser, 2006). Aquatic organisms have evolved antioxidant defense mechanisms that prevent and intercept

*E-Mail: nagpurens@yahoo.co.in

ROS as well as repair mechanisms for oxidized components. The ROS are detoxified by antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) which protect macromolecules against oxidative damage (Ozmen *et al*., 2004). SOD catalyzes the conversion of superoxide anion radical to molecular oxygen and hydrogen peroxide (Vutukuru *et al*., 2007), whereas CAT catalyses the conversion of hydrogen peroxide to water and molecular oxygen and protect the biological systems against ROS (Romeo *et al*., 2000). The antioxidant enzymes have been shown to work in a cooperative or synergistic manner to protect against oxidative stress and tissue specific damage. Some studies revealed that exposure to contaminants, including Cd, in aquatic ecosystems can enhance intracellular formation of ROS, which could cause oxidative damage to biological systems (Ferreira *et al*., 2005). These changes in the level of antioxidant enzyme activities can be used as biomarkers in different aquatic organisms (Livingstone, 2003).

Labeo rohita is a teleost fish belonging to family Cyprinidae. It is commonly found in rivers, ponds and freshwater lakes in and around South Asia and South-East Asia. It can serve as an excellent indicator of water quality and environmental pollution in aquatic system (Vutukuru *et al*., 2007). The present study was undertaken to assess tissue-specific alterations in SOD and CAT activities in *L. rohita* exposed to three sub-lethal concentrations of Cd and to evaluate the relationship.

Materials and methods

Experimental animal and chemical

Freshwater fish *L. rohita* were procured from local outlets for the experiment. After transportation to the laboratory, the specimens were given prophylactic treatment by bathing them twice in 0.05% $KMnO_4$ solution for 2 min to avoid any dermal infections. The specimens had an average (±S.D.) wet weight and length of 21.0 ± 2.5 g and 13.0 ± 1.2 cm, respectively. The specimens were acclimatized in the laboratory condition for one month. Every effort, as suggested by Bennett and Dooley (1982), was made to maintain optimal conditions during acclimatization.

Technical-grade cadmium chloride, monohydrate, A.R. ($CdCl_2$; 98.0% EC), manufactured by HiMedia Laboratories Pvt. Ltd. Mumbai, India, was procured and used in the present study.

Determination of sub-lethal concentrations

The 96 h LC_{50} value of cadmium chloride for the test species was estimated as 134.18 mg L^{-1} using probit analysis. Based on the 96 h LC_{50} value, three test concentrations of cadmium chloride *viz*., sub-lethal concentration I (SL-I; 1/ 4^{th} of LC_{50} = 33.55 mg/L), II (SL-II; $1/2^{th}$ of LC_{50} = 67.09 mg/L) and III (SL-III; $3/4^{th}$ of LC_{50} = 100.64 mg/L) were estimated for *in vivo* exposure experiment.

In vivo exposure experiment

Acclimatized fish specimens were randomly selected and exposed to three afore-mentioned test concentrations. The exposure was continued up to 96 h and the tissue sampling was done at 24, 48, 72 and 96 h post-exposure at the rate of five specimens per sampling duration. Fish maintained in water without the test chemical were used as control. This study was conducted following the OECD guideline No 203 in the static test conditions (OECD, 1992). The physico-chemical properties of test water, namely temperature, *p*H, conductivity, dissolved oxygen, chloride, total hardness and total alkalinity, were analyzed by standard methods (APHA, AWWA, WPCF, 2005).

Biochemical estimations

Liver and kidney tissues were homogenized in chilled sodium phosphate buffer (0.1 M, *p*H 7.4) using a Potter-Elvehjem homogenizer. The

homogenate was centrifuged at 10,000 g for 10 min at 4°C using Sigma refrigerated centrifuge (model 3K30) and the resulting supernatant was centrifuged again at 12,500 g for 10 min at 4°C to prepare post-mitochondrial supernatant (PMS), which was further used for various biochemical analyses.

SOD activity of tissues was analyzed using the method of Kakkar *et al*. (1984). The SOD activity was expressed as units/min/mg of protein. The activity of CAT was measured according to the method of Aebi (1984) and expressed as µM H_2O_2 decomposed/min/mg protein. Protein content in tissue samples was determined using colorimetric method and BSA as standard (Lowry *et al*., 1951).

Statistical analyses

One way analysis of variance (ANOVA) was applied to compare the means obtained for parameters at different concentrations and durations. A *p*-value less than 0.05 were considered statistically significant. For biochemical constituents, control values considered as 100% when compared with exposed concentrations of cadmium. The values for all the biomarkers are expressed as mean ± S.E. (n=5). Regression analysis was performed to analyze relationship among different response parameters using MS Excel.

Results and Discussion

Cd stress can induce the ROS generation and interfere with the antioxidant enzymatic defense system in bivalves, such as in clam *Ruditapes decussatus* (Geret *et al*., 2002) and the oyster *C. gigas* (Jo *et al*., 2008). The present study explored cadmium induced alteration in superoxide dismutase and catalase in the liver and kidney tissues of *L. rohita.* The physico-chemical characteristics of the test water, measured during experimentation, were: water temperature 28.6–29.4 °C, dissolved oxygen 6.6–7.2 mg/L and pH 7.4–7.6. The conductivity of the test water ranged from 252–284 µM cm^{-1}, while the chloride, total hardness and total alkalinity varied from 42–48, 160–170 and 220–240 mg/L as $CaCO_3$, respectively (Table 1).

SODs are a group of metallo-enzymes that play a crucial antioxidant role and constitute a defense system against the natural or chemically induced production of ROS (Deviller *et al*., 2005). In view of its key role in countering the oxidative stress, SOD is considered as the first line of defense against ROS. It is a versatile biomarker, especially with respect to the pollution by toxic chemicals, including heavy metals (Cho *et al*., 2006). In our study, significant elevation of SOD activities in liver and kidney tissues were observed at all three test concentrations of cadmium chloride as compared to control group, except at SL-I concentration and 24 h post-exposure (Table 2). SOD activity increased from 116.32 to 153.72% in liver and from 112.36 to 149.60% in kidney of *L. rohita* with respect to control. The elevation in SOD activity was found to be dose and time-dependent. Significantly higher elevation in SOD activity with respect to control was in liver

Table 1. Physico-chemical properties of the test water

Characteristics	Unit	Mean	Range
Water temperature	°C	29.1	28.6–29.4
Dissolved oxygen	mg/L	6.9	6.6–7.2
pH	-	7.5	7.4–7.6
Conductivity	µM/cm	266	252–284
Chloride	mg/L	45	42–48
Total hardness	mg/L	166	160–170
Total alkalinity	mg/L	232	220–240

Table 2. Effect of cadmium chloride on superoxide dismutase (SOD) enzyme activities in liver and kidney of *L. rohita*

Tissues	Conc.	Exposure Time (h)			
		24	48	72	96
Liver	Control	4.78 ± 0.26^{a1}	4.85 ± 0.34^{a1}	4.89 ± 0.31^{a1}	4.84 ± 0.32^{a1}
	SL-I	5.56 ± 0.22^{a12}	6.05 ± 0.28^{ab2}	6.26 ± 0.19^{b2}	6.51 ± 0.18^{b2}
	SL-II	5.92 ± 0.25^{a2}	6.34 ± 0.27^{ab2}	6.57 ± 0.25^{ab2}	6.95 ± 0.25^{b23}
	SL-III	6.17 ± 0.27^{a2}	6.61 ± 0.16^{a2}	6.93 ± 0.28^{ab2}	7.44 ± 0.21^{b3}
Kidney	Control	3.64 ± 0.21^{a1}	3.71 ± 0.25^{a1}	3.79 ± 0.24^{a1}	3.77 ± 0.22^{a1}
	SL-I	4.09 ± 0.14^{a12}	4.49 ± 0.24^{ab12}	4.63 ± 0.21^{ab2}	4.91 ± 0.23^{b2}
	SL-II	4.41 ± 0.22^{a23}	4.86 ± 0.18^{b2}	5.08 ± 0.26^{b23}	5.32 ± 0.21^{b23}
	SL-III	4.77 ± 0.18^{a3}	5.12 ± 0.21^{ab2}	5.29 ± 0.17^{ab3}	5.64 ± 0.15^{b3}

Values with different alphabet (lowercase) superscripts differ significantly between exposure durations within concentration. Values with different numeric superscripts differ significantly between concentrations within exposure duration.

Table 3. Effect of cadmium chloride on catalase (CAT) enzyme activities in liver and kidney of *L. rohita*

Tissues	Conc.	Exposure Time (h)			
		24	48	72	96
Liver	Control	362.41 ± 8.33^{a1}	367.86 ± 10.28^{a1}	361.63 ± 9.61^{a1}	356.18 ± 8.35^{a1}
	SL-I	347.96 ± 6.27^{a12}	338.12 ± 7.49^{ab2}	327.22 ± 8.73^{ab2}	316.38 ± 6.63^{b2}
	SL-II	336.49 ± 5.32^{a2}	325.63 ± 6.56^{a23}	315.39 ± 7.52^{ab2}	303.15 ± 5.62^{b23}
	SL-III	327.37 ± 7.42^{a2}	316.59 ± 5.34^{a3}	304.92 ± 6.75^{ab2}	292.01 ± 8.11^{b3}
Kidney	Control	192.29 ± 5.37^{a1}	195.73 ± 7.14^{a1}	191.41 ± 4.68^{a1}	187.36 ± 6.37^{a1}
	SL-I	179.81 ± 6.48^{a12}	168.46 ± 5.79^{ab2}	160.32 ± 3.36^{ab2}	151.45 ± 5.14^{b2}
	SL-II	167.68 ± 4.68^{a2}	160.37 ± 3.87^{ab2}	151.88 ± 4.69^{bc23}	140.63 ± 3.91^{c23}
	SL-III	161.13 ± 526^{a2}	152.22 ± 4.13^{ab2}	139.63 ± 3.84^{bc3}	131.37 ± 4.71^{c3}

Values with different alphabet (lowercase) superscripts differ significantly between exposure durations within concentration. Values with different numeric superscripts differ significantly between concentrations within exposure duration.

(153.72%) followed by kidney (149.60%) at SL-III concentration and 96 h post-exposure. Elif *et al*. (2000) reported an increase in the hepatic SOD activity in Nile tilapia, exposed to Cd, which could be due to induction of free radicals and ROS/RNS by the heavy metal. The concentration- and time-dependent increase in SOD activity in tissues, observed in the present study, could be an indicator of compensatory tissue response to convert the superoxide radical to H_2O_2 induced by metal exposure.

Hydroxide free radical is produced from H_2O_2 via the Fenton reaction (Evans and Halliwell, 1994). CAT is the primary enzyme for scavenging H_2O_2, when CAT activity is inhibited; more H_2O_2 is available for production of hydroxide free radical. Significant (p<0.05) decline in CAT activity was observed as 92.81 and 87.20% in liver and kidney, respectively, at SL-II concentration and 24 h post-exposure (Table 3). CAT activity decreased from 96.01 to 81.98% in liver and from 93.51 to 70.12% in kidney at different concentrations of cadmium

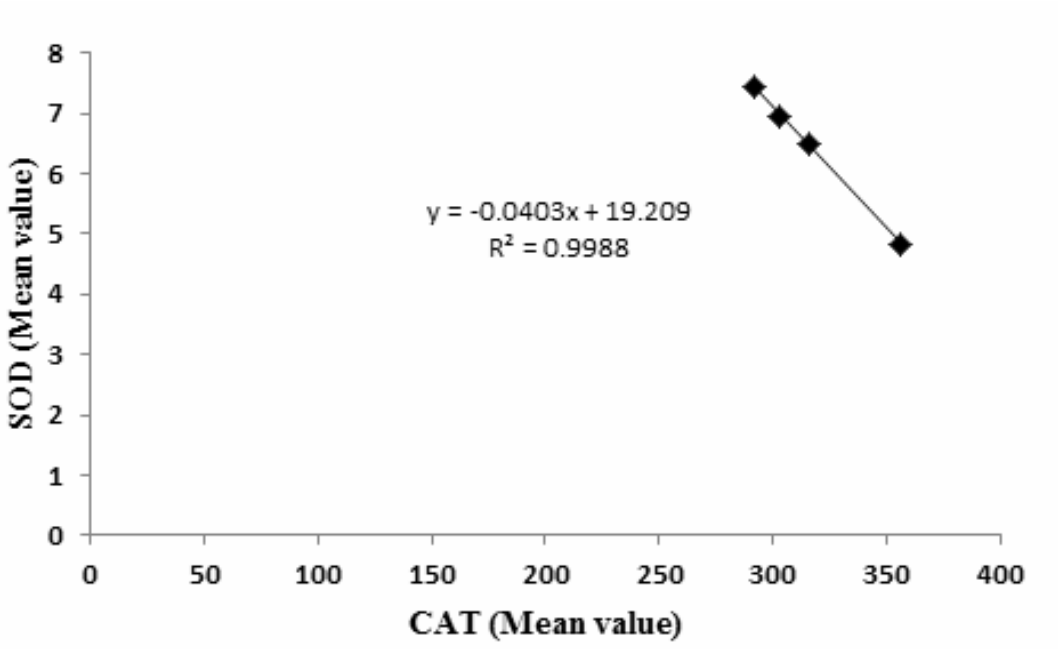

Fig. 1. Regression analysis of mean data of superoxide dismutase (SOD) and catalase (CAT) in liver of cadmium chloride exposed fish. Values comprise control and exposed groups at 96 h post exposure.

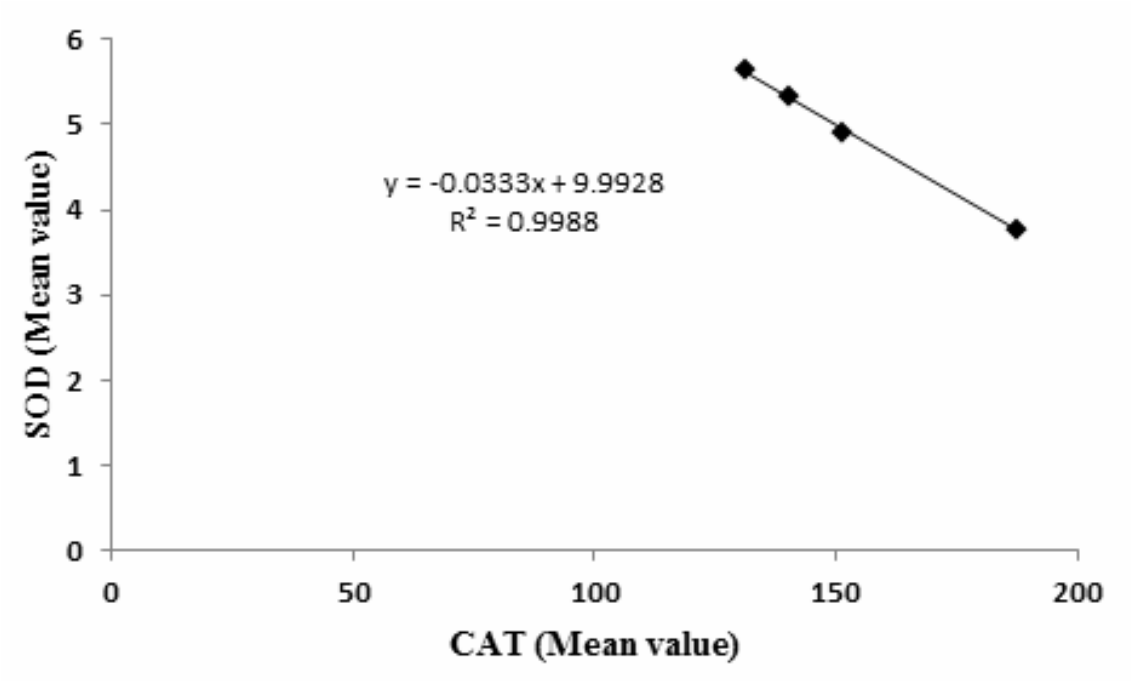

Fig. 2. Regression analysis of mean data of superoxide dismutase (SOD) and catalase (CAT) in kidney of cadmium chloride exposed fish. Values comprise control and exposed groups at 96 h post exposure.

chloride when compared with control group. In both tissues, CAT activity depleted in a dose-dependent manner and the highest depletion was observed at SL-III test concentration and 96 h post-exposure. The maximum depletion in CAT activity was in kidney (81.98%) followed by liver (87.20%) at SL-III concentration on 96 h post-exposure.

In this study, CAT activity decreased in liver and kidney tissues of *L. rohita*, together with the increase of SOD activity. The reduction in CAT activity by Cd exposure may be related to the direct binding of metal to –SH groups of the enzyme molecule. Pandey *et al*. (2001) suggested that the heavy metals are responsible for over-production of superoxide anion radical in treated specimens and these free radicals inhibit CAT activities. Our results are also supported by other findings (Soares *et al*., 2008; Osman *et al*., 2009) who worked on cadmium induced oxidative stress in various fish species. A significantly negative correlation among mean values of SOD and CAT in both tissues was observed in cadmium exposed fish specimens and control groups at 96 h post-exposure (Figs. 1 and 2). Regression analysis between SOD and CAT indicated that cadmium might be directly responsible to suppress CAT activity and induced production of hydroxyl radicals, to facilitate SOD activity elevation for neutralization of this kind of free radicals. The negative correlation between the increased activity of SOD and decreased activity of CAT indicated that the excess amount of superoxide radicals was neutralized by SOD to protect the biological system from free radicals.

The present study concludes that cadmium prompts generation of reactive oxygen species in liver and kidney tissues of *L. rohita* and the antioxidant defense enzymes seem to counteract with ROS. The exposure of Cd is responsible for elevation in SOD activity in both tissues, while the CAT activity reduced in all cadmium exposed groups. Additionally, data obtained from this study could be used as predictive biomarkers in monitoring and management of Cd in the aquatic environment and combined approaches using these assays will help in broad perspective in aquatic toxicology.

Acknowledgements

The authors are thankful to the Director, National Bureau of Fish Genetic Resources, Lucknow, India for providing support, suggestion, materials and laboratory facility for conducting this study.

References

Aebi, H. (1984) Catalase *in vitro*. In: Packer L (ed.) Methods in enzymology. Academic Press Orlando **105**, 121–126.

AMAP (1998) Assessment report: Arctic pollution issues Arctic Monitoring and Assessment Programme. Oslo.

APHA, A.W.W.A. and W.P.C.F. (2005) Standard methods for the examination of water and wastewater, 21st ed. American Publication of Health Association, Washington, DC.

Bennett, R. O. and Dooley, J. K. (1982) Copper uptake by two sympatric species of killifish *Fundulus heteroclitus* (L.) and L. majalis (Walkbaum). *J. Fish Biol*., **21**, 381–398.

Cho, Y. S., Choi, B. N., Kim, K. H., Kim, S. K., Kim, D. S., Bang, I. C. and Nam, Y. K. (2006) Differential expression of Cu/Zn superoxide dismutase mRNA during exposures to heavy metals in rockbream (Oplegnathus fasciatus). *Aquaculture,* **253**, 667–679.

Deviller, G., Palluel, O., Aliaume, C., Asanthi, H., Sanchez, W., Franco, N. M. A., Blancheton J.P. and Casellas, C. (2005) Impact assessment of various rearing systems on fish health using multibiomarker response and metal accumulation. Ecotoxicol. *Environ. Saf*., **61**, 89–97.

Elif. O. O. and Üner, N. (2000) Combined effects of 2, 4-D and azinphosmethyl on antioxidant enzymes and lipid peroxidation in liver of *Oreochromis niloticus. Com. Biochem. Physiol. Part C*., **127**, 291–296.

Evans, P. J. and Halliwell, B. (1994) Measurement of iron and copper in biological systems: Bleomycin and copper– phenanthroline assays. *Methods Enzymol.,* **233**, 82-92.

Ferreira, M., Moradas-Ferreira, P. and Reis-Henriques, M. A. (2005) 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**, 39–48.

Geret, F., Serafim, A., Barreira, L. and Bebianno, M. J. (2002) Effect of cadmium on antioxidant enzyme activities and lipid peroxidation in the gills of the clam *Ruditapes decussatus*. *Biomarkers,* **7**, 242–256.

Jo, P. G., Choi, Y. K. and Choi, C. Y. (2008) Cloning and mRNA expression of antioxidant enzymes in the Pacific oyster, Crassostrea gigas in response to cadmium exposure. *Comp. Biochem. Physiol.,* **C 147**, 460–469.

Kakkar, P., Das, B. and Viswanathan, P. N. (1984) A modified spectroscopic assay of superoxide dismutase. *Ind. J. Biochem. Biophys.,* **21**, 130–132.

Lesser, M. P. (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. *Ann. Rev. Physiol*., **68,** 253–278.

Livingstone, D. R. (2003) Oxidative stress in aquatic organisms in relation to pollution and aquaculture. *Rev. Med. Vet.,* **154**, 427–430.

Lowry, O. H., Rosenbrough, N. M., Farr, A. L. and Randall, R. J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem*., **193**, 265-275.

Olsson, P. E., Larsson, A. and Haux, C. (1996) Influence of seasonal changes in water temperature on cadmium inducibility of hepatic and renal metallothionein in rainbow trout. *Mar. Environ. Res.,* **42**, 41–44.

Organization for Economic Cooperation and Development, OECD (1992) Guideline for the testing of chemicals: Fish, acute toxicity test, Document 203.

Osman, H. A. M., Ibrahim, T. B., Ali, A. T. and Derwa, H. I. M. (2009) Field Application of Humic Acid Against the Effect of Cadmium Pollution on Cultured Tilapia *Oreochromis niloticus*. *World Appl. Sci. J*., **6,** 1569-1575.

Ozmen, I., Bayir, A., Cengiz, M., Sirkecioglu, A. N. and Atamanalp, M. (2004) Effects of water reuse system on antioxidant enzymes of rainbow trout (*Oncorhynchus mykiss* W., 1792). *Veterin. Med. Czech*., **49**, 373–378.

Pandey, S., Ahmad, I., Pervez, S., Hafeez, B., Haque, R. and Raisuddin, S. (2001) Effect of endosulfan on antioxidants of freshwater fish *Channa punctatus* Bloch. 1. Protection against lipid peroxidation in liver by copper pre-exposure. *Arch. Environ. Contam. Toxicol.,* **41**, 345–352.

Risso-de Faverney, C., Orsini, N., de Sousa, G. and Rahmani, R. (2004) Cadmium induced apoptosis through the mitochondrial pathway in rainbow trout hepatocytes: involvement of oxidative stress. *Aquat. Toxicol.,* **69**, 247–258.

Romeo, M., Bennani, N., Gnassia-Barelli, M., La, Faurie, M. and Girard, J. P. (2000) Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. *Aquat. Toxicol*., **48**, 185–194.

Soares, S. S., Martins, H., Gutiérrez-Merino, C. and Aureliano, M. (2008) Vanadium and cadmium in vivo effects in teleost cardiac muscle: Metal accumulation and oxidative stress markers. Comparative Biochemistry and Physiology, **Part C 147**, 168–178.

Sorensen, E. M. (1991) Cadmium. *In* "Metal Poisoning in Fish,'' pp. 175-234. CRC Press, Boston.

Stoeppler, M. (1991) Cadmium. *In* "Metals and Their Compounds in the Environment. Occurrence, Analysis and Biological Relevance'' (E. Merian, Ed.), pp. 804-851. VCH, Weinheim.

Stohs, S. J., Hagch, D., Hassoun, E. and Bagshi, M. (2000) Oxidative mechanisms in the toxicity of chromium and cadmium ions. *J. Environ. Pathol. Toxicol. Oncol*., **19**, 201–213.

Vutukuru, S. S., Prabhath, N. Arun., Raghavender, M. and Yerramilli, A. (2007) Effect of Arsenic and Chromium on the Serum Amino-Transferases Activity in Indian Major Carp, *Labeo rohita*. *Int. J. Environ. Res. Public Health,* **4**, 224-227.

Waisberg, M., Pius, J., Hale, B. and Beyersmann, D. (2003) Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicol.,* **192**, 95–117.