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Assessment of Toxicity of Copper Sulphate Pentahydrate on Oxidative Stress Indicators on Liver of gold Fish (*Carassius auratus)*

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ABSTRACT
Copper sulphate is frequently used as a general biocide in the aquaculture industry and as a treatment option in household aquarium. In present investigation, influence of increasing concentrations of copper sulphate pentahydrate ($CuSO_4.5H_2O$) after 24 hrs exposures on liver of gold fish (Carassius auratus) was investigated to understand the oxidative stress produced during the treatment of biocide. Fish were exposed to copper sulphate pentahydrate (CuSP), oxidative stress indicators i.e. MDA levels, non-enzymatic indicators like glutathione (GSH) & total ascorbic acid (TAA) contents and enzymatic indicators like glutathione peroxidise (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) activities were measured after 24 hrs exposure at different doses (0.1, 0.5, 1.0 and 1.5). Results explicate that, at dosage level 0.1 ppm significant changes were obtained only in MDA levels, TAA contents, CAT, SOD, GPx activities and non-significant changes were achieved in rest of the parameters as compared to control. However, at dosage level 0.5-1.5 ppm all the parameters shows significant changes ($p \leq 0.05$) as compared to control, which confirmed that, CuSP causes significant oxidative stress in golden fish which may reduce its life span and lead to early death in household aquarium. Therefore, 0.1 ppm concentration of CuSP is comparatively safer than other high dose for the treatment in house hold aquarium.
Key Words: *Copper sulphate pentahydrate, toxicity, C. auratus, oxidative stress, MDA levels*

INTRODUCTION

Most living organisms depend on ATP generation by oxygen-based metabolism, but one consequence of oxygen dependence is the production of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$), mainly as by product of oxidative metabolism. The mitochondrial electron transport chain and a variety of cellular oxidases are the main sources of ROS generation [1]. ROS can attach multiple cellular constituents, including proteins, nucleic acids, and lipids. To cope with damaging actions of ROS, organisms have evolved multiple systems of antioxidant defence. So called low-molecular weight anitoxidants include metabolites such as glutathione, ascorbic acid, uric acid, etc. whereas high-molecular weight defences include enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidise (GPx), glutathione reductase (GRx) and glutathione-s-transferase (GST). These enzymatic and non-enzymatic constituents deal directly with radical species and protect against intoxication of any damage to the macro and micro molecules in body [2]. Copper pollution appears in the aquatic environment from natural and anthropogenic sources such as mine washing, agricultural leaching and direct application as algicide and molluscicide. Monitoring biochemical and histo-cytological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in laboratory and field studies. Moreover, liver is known to be the primary organ for copper storage in fish [3-4].

Copper sulphate pentoxide (CuSP) is available as a dust, wettable powder, or liquid concentrate. The effectiveness of CuSP as a therapeutant is reduced as the total alkalinity and/or total hardness (as mg/l $CaCO_3$) of waters increase. Additionally, the toxicity of this compound to fish is also decreases as pH, total alkalinity and total hardness increase, as copper (Cu) binds to inorganic or organic substrates [5]. To overcome with this problem, the current practice for the therapeutic use

of CuSP is to increase the application rate in direct proportion to the total alkalinity of the water. CuSP is usually not recommended as a therapeutant in waters having less than 50 mg/l alkalinity because of the acute toxicity [5]. Copper sulfate is highly toxic to fish. Even at recommended rates of application, this material may be poisonous to trout and other fish, especially in soft or acid waters. Fish eggs are more resistant than young fish fry to the toxic effects of CuSP. When CuSP dissolves in the pond, it breaks down into copper ions that are presumed to be toxic to algae, parasites and fish. In ponds that have high pH and alkalinity, copper ions quickly react with ions stay in water for such a long period that it is difficult to kill algae and parasites without killing the fish. Copper sulfate is toxic to aquatic invertebrates, such as crab, shrimp, and oysters. The 96-hour LC_{50} of copper sulfate to pond snails is 0.39 mg/L at 20^0 C. Acute severe toxicity of copper sulphate results in gastrointestinal injury with diarrhoea haemolysis with haemoglobinuria, haematuria and haemolytic anaemia, kidney and liver failure.

CuSP solution is the common treatment option for controlling flagellated and non flagellated algae related toxicity in ponds and aquarium to preserve ornamental as well as commercially important fishes. However, scientists had proven copper sulphate pentahydrate toxic rather than preservative to many of the ornamental and commercially high valued fishes. The mechanism of CuSP is partially known and still detailed study is required to understand its potential to produce oxidative stress at different dosage on liver of gold fish. Therefore, present investigation has been undertaken to throw light on the oxidative stress produced by CuSP to liver of common golden fish i.e. *C. auratus* after twenty four hours of exposure at different concentrations.

MATERIALS AND METHODS

Chemicals

Chemicals used in the entire study were procured from Hi Media Laboratories Pvt. Ltd.; Sisco Research Laboratories Pvt. Ltd., Mumbai, India. The optical density measurements were carried out on Systronics 118 UV-Vis Spectrophotometer.

Experimental Fish Specimens

Male Gold fish (*Carassius auratus*) weighing 8 – 15 g were bought from local aquarium shop (Bhuj, Kachchh) after conforming of not having exposure of copper sulphate pentahydrate in any form. Fish were acclimated for two weeks prior to the experiments. They were kept in fibreglass tank, with constantly aerated fresh water (1800 L), under 12:12 light-dark cycle, temperature of 22-25^{o}C, dissolved oxygen (DO) of 7.38 to 7.81 mg/L, pH 7.6 to 7.7, total hardness 310 mg/l, alkalinity 200 mg/l (desirable in drinking water or tap water), nitrite and ammonia lower than 0.05 and 0.01 mg/L. The fish were fed ad libitum with tropical fish chow (Purina Ltda, Campinas, SP, Brazil) with optimal protein concentration (38%) for Oreochromis. The above said condition try to recover any stress occurred on fish during transportation. To study the effect of CuSP, gold fish were carefully transferred to five separate glass aquarium (250 L) containing different dosage of CuSP ranging from 0.1 to 1.5 ppm and maintained at standard temperature and light-dark cycle with adequate oxygen supply. Ten fishes weighing 12 – 15 g were kept in each tank for twenty four hours exposure. Dosage was selected from the LC_{50} of goldfish i.e. 0.1 ppm for 96 hrs.

Control: No treatment has been given, ideal condition has been maintained as describe above.

Treatment: 0.1, 0.5, 1.0 and 1.5 ppm of copper sulfate pentahydrate were maintained in each tank. Ten gold fishes were kept in each tank.

Tissue Sampling

The fish specimens were exposed to the four aforementioned test concentrations of CuSP along with the control and the experiments continued for 24 h followed by transspinal dissection. The liver was dissected out carefully, washed in ice-cold saline, blotted and weighed. It was then homogenized in using a mortar pestle. The resulting homogenate was centrifuged at 10,000 g for 20 min in a refrigerated centrifuge at 4 ^{o}C. The clear supernatants collected were used for protein estimation and assaying the activity of enzymes.

Biochemical Assays

Lipid peroxidation (LPO)

The level of lipid peroxidation in the liver was measured by the method as described by Okhava et al., (1979) [7]. This method is based on the formation of a red chromophore that absorbs light at 532 nm following the reaction of thiobarbituric acid (TBA-1%) with products of lipid peroxidation

like malondialdehyde (MDA) and others collectively called as thiobarbituric acid reactive substances (TBARS). The results were expressed as n moles MDA formed/mg protein/60 min.

Glutathione (GSH) content

The glutathione content in liver was measured by the method of Grunert and Philips (1951) [8]. In saturated alkaline medium the GSH present in the tissues reacts with 0.067 M sodium nitroprusside and 1.5 M sodium cyanide mixture to give a red coloured complex which was measured at 520 nm. The glutathione content was expressed as µg/100 mg tissue weight.

Total ascorbic acid (TAA) content

Total ascorbic acid (TAA) content was estimated in the liver by the method of Roe and Kuether (1943) [9]. TAA is oxidized to dehydroascorbic acid (DHA) by Norit reagent in the presence of TCA. This couples with 2, 4-dinitrophenyl hydrazine (2%) in the presence of thiourea (10%) and sulphuric acid to yield a red coloured complex which was read at 540 nm. TAA content was expressed as mg/gm tissue weight.

Catalase activity (CAT)

Catalase (EC 1.11.1.6) activity was determined by measuring the decrease of hydrogen peroxide concentration at 240 nm according to Luck (1963) [10]. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7) and 10 mM H_2O_2 freshly added. The enzyme activity was expressed as µ moles H_2O_2 consumed/mg protein/min

Glutathione peroxidase activity (GPx)

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was measured by the method of Pagila (1967) [11]. The (GSSG) generated by GPx was reduced by GR, and NADPH oxidation was monitored at 340 nm using UV-Vis Spectrophotometer. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7), 1mM EDTA, 0.1 E.U/mL oxidised glutathione reductase (GSSG), 1 mM sodium azide, 1 mM reduced glutathione (GSG), 0.2 mM NADPH, and 25 mM H_2O_2. The enzyme activity was expressed as units/mg protein/min, where 1 unit of GPx equals to n moles of NADPH consumed/mg protein/min.

Glutathione reductase activity (GR)

Glutathione reductase (GR; EC 1.6.4.2) activity was assayed by the method described by Carlberg and Mannervik (1975) [12], with some modifications, by measuring the oxidation of NADPH at 340 nm. The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.63 mM NADPH, and 0.15 mM GSSG. The enzyme activity was calculated as n moles NADPH consumed/mg protein/min.

Glutathione-s-transferase activity (GST)

The glutathione-S-transferase activity was assayed by the method of Habig et al., (1974) [13]. The increase in absorbance was noted at 340 nm using 1-chloro-2, 4-dinitrobenzene (CDNB). The reaction mixture 0.3 mM phosphate buffer (pH 6.5), 30 mM 1-chloro-2, 4-dinitrobenzene (CDNB) and double distilled water. After pre incubating the reaction mixture at 37°C for 5 min, the reaction was started by the addition of homogenate and glutathione as substrate. The absorbance was followed for 5 min at 340 nm. The enzyme activity was calculated as µmoles CDNB conjugates formed/mg protein/min.

Superoxide oxide dismutase activity (SOD)

Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by Kakkar et al., (1984) [14]. The reaction mixture consisted of 0.052 M sodium pyrophosphate buffer (pH 8.4), 186 µM phenazine methosulphate (PMS), 30 µM nitroblue tetrazolium chloride (NBT) and 780 µM NADH. Activity is reported in units of SOD per milligram of protein. The activity was calculated wherein, one unit of the enzyme concentration required to inhibit 50% of the optical density of chromagen formed in one unit at 560 nm under the assay condition.

STATISTICAL ANALYSIS

All the data are expressed as means ± standard error mean (S.E.M.). The data were statistically analyzed using one-way Analysis of Variance (ANOVA) by SPSS 17th version followed by Tukey test. The level of significance was accepted with $p \leq 0.05$.

RESULTS AND DISCUSSION

MDA levels

The MDA levels (**Table 1**) significantly (p≤0.05) increases in the liver of goldfish as compared to control liver. At 24 h exposure of 0.1, 0.5, 1.0, and 1.5 ppm of CuSP, the MDA level increases from 13.27%, 18.60%, 25.41% and 36.64% respectively. The effect was not dose- dependent.

Table 1 Effect of different concentration of CuSP on oxidative stress parameters of liver of Gold fish

	Control	0.1 ppm	0.5 ppm	1.0 ppm	1.5 ppm
LPO (n moles of MDA/mg protein/60 min)	45.31 ± 0.20	51.58 ± 0.18^{acde}	54.38 ± 0.17^{abde}	57.6 ± 0.24^{abce}	61.73 ± 0.24^{abcd}
PROTEIN (mg%)	17.88 ± 0.43	14.91 ± 0.48^{acde}	11.56 ± 0.30^{abde}	8.6 ± 0.30^{abce}	6.24 ± 0.15^{abcd}
TAA (mg/gm protein)	65.52 ± 0.34	60.11 ± 0.48^{acde}	56.60 ± 0.28^{abde}	52.8 ± 0.48^{abce}	47.75 ± 0.39^{abcd}
SOD (U/mg protein)	49.37 ± 0.51	43.95 ± 1.35^{acde}	30.39 ± 1.42^{abde}	24.5 ± 0.94^{abce}	17.26 ± 1.39^{abcd}
CAT (U/mg protein)	34.34 ± 0.23	31.91 ± 0.16^{acde}	26.69 ± 0.41^{abde}	22.9 ± 0.82^{abce}	19.26 ± 0.58^{abcd}
GPx (n moles of NADPH consumed/mg protein/min)	51.36 ± 0.20	47.77 ± 0.47^{acde}	44.21 ± 0.22^{abde}	41.6 ± 0.36^{abce}	38.72 ± 0.34^{abcd}
GR (n moles of NADPH consumed/mg protein/min)	10.71 ± 0.27	10.25 ± 0.22^{cde}	7.37 ± 0.07^{abde}	6.0 ± 0.23^{abce}	4.49 ± 0.25^{abcd}
GST (µmoles CDNB conjugated formed/mg protein/min)	9.90 ± 0.19	9.58 ± 0.30^{cde}	4.85 ± 0.10^{abde}	3.70 ± 0.15^{abce}	2.79 ± 0.15^{abcd}
GSH (µg/100 mg tissue weight)	25.9 ± 0.13	25.11 ± 0.45^{cde}	21.06 ± 0.07^{abde}	18.6 ± 0.24^{abce}	16.63 ± 0.34^{abcd}

Values are mean ± SEM; n = 10

[a] As compared to group 1: p<0.05; [b]As compared to group 2: p<0.05; [c]As compared to group 3: p<0.05

[d]As compared to group 4: p<0.05; [e]As compared to group 5: p<0.05.

Total protein content

The total content of protein (**Table 1**) in liver of goldfish was reduced significantly (p<0.05) as compared to control. Concentration dependent decrease was found at 0.1, 0.5, 1.0, and 1.5 ppm of CuSP, as 16.61%, 35.35%, 52.01% and 65.10% respectively.

Non-Enzymatic (Low Molecular Weight) antioxidants

The concentration of total ascorbic acid content (Table 1) reduces as the dose of CuSP increases from 0.1, 0.5, 1.0, and 1.5 ppm by 8.26%, 13.61 %, 19.38% and 27.12% respectively in liver of goldfish. The reduction in content of TAA is significant as compared to control at p≤0.05 level. The effect was not found to be dose dependant. Glutathione content (Table 1) of liver of goldfish exposed to CuSP is found to be 3.05%, 18.69%, 28.03% and 35.39%. Significant reduction in GSH content was found to be at 0.5, 1.0 and 1.5 ppm concentration, however at 0.1 ppm non-significant reduction was found as compared with control.

Enzymatic (High molecular weight) antioxidants

Results revealed that CuSP exposure for 24 h at different concentrations decreases the activity of CAT, SOD, GPx, GR and GST activity (Table 1). The activity of CAT in control liver is 34.34 µ moles H_2O_2 consumed/mg protein/min which is decreases by 7.08%, 22.28%, 34.41% and 43.91% after twenty four hours exposure to CuSP at different doses like 0.5, 1.0, 1.5, and 2.0 ppm respectively. The activity of SOD (Table 1) decreases as the exposure of CuSP increases from 0.1 to 1.5 ppm for 24 h. Activity in control liver is around 49.37 U/mg proteins which reduced by 10.98 %, 38.44%, 50.39% and 64.84% respectively. Activity of GPx is reduced from 7%, 13.92%, 18.98% and 24.61% at the concentration from 0.1, 0.5, 1.5, and 2.0 ppm respectively. GR activity is also reduced from 4.3%, 31.19%, 43.7%, and 58.08% at different exposure from 0.1 to 1.5 ppm respectively. However, at 0.1 ppm reduction in the activity of GR found to be non-significant as compared to control. The effect was found to be dose dependent and significant (p≤0.05) as compared to

control. The activity of GST is reduces as the dose increases. The reduction is 3.23 % at 0.1 ppm which is not significant as compared to control. However, at 0.5 ppm reduction is 51.01%, 1.0 ppm reduction is 62.73% and 1.5 ppm reduction is 74.75% which is significant ($p \leq 0.05$) as compared to control.

Fish are often used as sentinel organisms for ecotoxicological studies because they play number of roles in the trophic web, accumulate toxic substances and respond to low concentration of mutagens therefore, the use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems [15]. Copper is present as a cofactor in various enzymes (e.g. Cu, Zn-superoxide dismutase, cytochrome oxidase etc.) but as a toxic substance it can able to bind the cysteine side chains of proteins and to form multidentate complexes with histidine and tryptophan side chains of proteins which is responsible for its toxicity [3]. This may be the reason of decrease in total protein content in goldfish liver exposed with CuSP after 24 h at different dose. CuSP might be bind with protein and form such type of complex thus it makes unavailable for protein requiring processes. Also, the reduction in protein content might be also due to the proteolysis process for energy production and utilization owing to the decreased food intake of fish under stress.

Free radicals also are formed by homolytic bond fission, which can be induced by electron transfer to the molecule (reductive fission). It can also generate hydroxyl radical ($OH^{\cdot}$), a free radical of paramount toxicological fission from hydrogen peroxide [16]. The Fenton reaction, which is catalyzed by transition metal ions, typically Cu (I) or Cr (V), Fe (II) is a major toxicity mechanism for HOOH and its precursor $O_2^{\cdot}$ as well as transition metals may be one of the major reasons for producing ROS in the present investigation [16/17]. Fenton reaction will not allow the conversion of hydrogen peroxide in to water molecule and instead of it produces ROS.

Oxidative stress may also be due to the depletion of cellular GSH content below the critical level which prevents the conjugation of xenobiotics like CuSP to GSH for detoxification and thus enables them to freely combine covalently with cell proteins. The results obtained in our study also show significant reduction in GSH content. The reduction might be due to faster rate of GSH utilization for the neutralization of produced ROS or degradation. Moreover, Cu is not conjugated to glutathione by GST, the decrease in hepatic GST activity may be explained by its antioxidative activity that means it is activated to defend the toxic effects of CuSP. GST induction is also related to decontamination processes of Cu-altered constitutive molecules of the cell (e.g. lipid or protein peroxides).

Cu is known to induce the formation of reactive form of oxygen which can produce enzymatic deactivation, lipid peroxidation and DNA damage. Cu has a great capacity to alter membrane structural lipids and could provoke membranous disruption [17]. Moreover, the elevated level of lipid peroxidation in the liver of *C. auratus* in response to the exposure to CuSP in the present investigation suggests that there is increased production of ROS. The liver is also noted as site of multiple oxidative reactions and maximal free radical generation [18]. Yuanyuan Sun et al, (2006) [19], also revealed the reduction in the activity of different oxidative enzymes like CAT, GPx, GR, GST, and SOD may reduce the secondary antioxidative defence mechanism in the hepatic cells of goldfish which is in accordance to the present investigation.

However, organisms are equipped with interdependent cascades of enzymes to alleviate oxidative stress and repair damaged macromolecules, produced during normal metabolism or due to exposure to xenobiotics. In this cascade, SOD and CAT are the major enzymes in eliminating ROS formed during bioactivation of xenobiotics in the hepatic tissues [20] and the induction of SOD/CAT system provides a first line of defence against ROS. SOD help to dismutase superoxide radical O_2^{-} to hydrogen peroxide (H_2O_2). CAT activity decreased after 24 h of exposure to CuSP, as the values obtained were significantly ($p \leq 0.05$) lower than control. Decrease in CAT activity could be due to decrease in the rate of reaction as a result of the excess production of H_2O_2 as CAT is responsible for the detoxification of H_2O_2 to water, conversion reduced as the CAT activity decrease in present investigation and leads to more production of ROS.

Therefore, present investigation revealed high risk of RSO production at different dosage like 0.1, 0.5, 1.0, and 1.5 ppm for 24 h exposures. Increase in ROS ultimately reduced primary and secondary antioxidant defend mechanism and leads to reduced life span in aquarium. However, 0.1ppm concentration of CuSP found to be comparatively safer than the other concentration.

Therefore, it is recommended that application of CuSP as biocides should not be more than 0.1 ppm for its safe use.

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REFERENCES

1. Zhang Y., Chen K., Zhang S., Ferguson I. (2003). The role of salicylic acid in postharvest ripening of kiwifruit. *Postharvest Biol Technol* 28: 67-74.
2. Hermes-Lima M., Storey K.B. (1993). Role of antioxidants in the tolerance of freezing and anoxia by garter snakes. *Am J Physiol* 265: R646-R652.
3. Hinton D.E., Lauren D.J. (1990). Liver structural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure. In: McCarthy, J.F., Shugart, L.R. (Eds.), Biomarkers of Environmental Contamination. Lewis, Boca Raton, pp. 17–57.
4. Toth L., Juhasz M., Varga T., Csikkel-Szolnoki A., Nemcsok J. (1996). Some effect of $CuSO_4$ on carp. *J Environ Sci Health* B31: 627–635.
5. Tucker C.S., Robinson E.H. (1990). Channel Catfish Farming Handbook. Van Nostrand- Reinhold, New York, NY, USA.
6. Oldenquist G., Salem M. (1999). Parenteral copper sulphate poisoning causing acute renal failure. *Nephrol Dial Transplant* 14:441-443.
7. Ohkawa H., Ohishi N., Yagi K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351-358.
8. Grunert R.R., Philips P.H. (1951). A modification of the nitroprusside method of analysis for glutathione. *Arch Biochem* 30: 217-225.
9. Roe J.H., Kuether C.A. (1943). The determination of ascorbic acid in whole blood and urine through the 2, 4, dinitrophenylhydrazine derivative of dehydroascorbic acid. *J Biol Chem* 147: 399-7.
10. Luck H. A (1963). Spectrophotometric method for the estimation of catalase. In : *Methods of Enzymatic Analysis*, Bergmeyer, H.U. (ed.), Academic Press, New York, , pp. 886-887.
11. Pagila D.E., Valentine W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70**:** 158-169.
12. Carlberg I., Mannervik B. (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J of Biol Chem* 250: 5475–5480.
13. Habig W.H., Pabst M.J., Jokoby W.B. (1974). Glutathione S-transferase: The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249: 7130-7139.
14. Kakkar P., Das B., Viswanathan P.N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys* 21:130-132.
15. Van Der Oost R, Beyer J, Vermeulen NPE. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharm* 13: 57–149.
16. Klaassen C. (2001). Casarett & Doull's Toxicology: The Basic Science of Poisons, 6th Edition Chap 3, pp. 35-64.
17. Segner H., Braunbeck T. (1998). Cellular response profile to chemical stress. *Ecotoxicology* 3: 521–69.
18. Atli G., Alptekin O., Tukel S., Canlin M. (2006). Response of catalase activity to Ag+, Cd+, Cr6+, Cu2+ and Zn2+ in five tissues of fresh water fish *Oreochromis niloticus*. *Comp Biochem Physiol C* 143: 218–224.
19. Sun Y., Yu H., Zhang J., Yin Y., Shi H. (2006). Bioaccumulation and depuration of phenanthrene in fish for exposure groups chemical pollutants. *Chemosphere* 63:1319-27.
20. Sk H.U., Bhattacharya S. (2006). Prevention of cadmium induced lipid peroxidation, depletion of some antioxidantive enzymes and glutathione by series of organoselenocyanates. *Environ Toxicol Pharmacol* 22: 298–308.