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Responsiveness of Total Plasma Protein After Administration of Some Toxic Heavy Metals in an Indian Teleost *(Clarias batrachus L.)*

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Abstract: Heavy metals of acetate salts of lead, zinc, copper and mercury cause serious toxic effects on protein biosynthesis. These metal salts reduce the plasma protein content. A dose-response relationship is found to occur in this blood parameter in the experimental fish in comparison to the control group of fishes. Both groups are fed. Higher dose of those chemical agents are much responsive to cause harmful effects on fish plasma protein amount. All the heavy metals toxicity except zinc toxicity are shown to be continued (reduced) upto day 42 but the decrease in the said parameter is more marked with zinc salt on day 7 only. The present investigation is an attempt to evaluate the relationship of this haematological parameter with the physiological status among the heavy metal polluted fishes during chronic treatment.

Keywords: Environmental Pollution, Metal Toxicity, Fish, Plasma Protein, Long Term Treatment

1. Introduction

All the living creatures are the inhabitants of Earth which make up our surroundings and affect our ability to live in the Earth. The Earth supports many life forms in its hydrospheres (water bodies) and lithosphere (land area) under the atmospheric cover providing conditions more or less favourable for life to sustain of which change is referred as pollution of the environment [1]

There are three possible ways of metal pollution through which metals enter the body of fish, the gills, digestive tract and body surface. High concentrations of heavy metals are found in fish river, kidney, gills [2, 3]. Low concentration of some heavy metals like NI, Cu, Cr, Hg, Zn, Cd, Pb etc. induce changes also in morphology, physiological and biochemical parameters in fish.More than three decades ago, growth of industries, urbanization and modernization gradually leads to environmental pollution. For the quest development and improvement of the society as well as the country industrialization is essential in the world. But the use of modern and new technologies for the industry are destroying the precious natural resources. Water is principal component of biosphere but fresh water is limited.Metals generally enter into the ecosystem in a relatively non-toxic form. Heavy metals are the most noxious pollutant owing to their diverse effects. Some of them through the environmental reactions involving various microorganisms and non-biological pathways are converted into toxic form. Examples can be cited. Such biological reactions are methylation of inorganic mercury, lead, selenium, arsenic, tin etc. The methylated metallic compounds have generally been found to be more toxic than their inorganic forms. Inorganic metals like lead, zinc, cadmium, nickel, copper, arsenic, mercury are very toxic to the animals, plants and human also.

Heavy Metal toxicity is one of the serious hazards to cause deleterious effects on animals, plants, humans, soils of the environment. Heavy metals are vital inducers of oxidation stress in aquatic species [4].Our environment comprising air,

water and land is being polluted by ourselves, knowingly or unknowingly, to meet the need of us. When civilisationstarted, the most developed mammalian creature, human being used some metallic tools, utensils, etc., for maintaining their domestic life activities. Pollution can be occurred physically, chemically and biologically resulting health hazards to man, animals, birds, wildlife, fish or aquatic life or to plants. Disposal of various waste products (inorganic and organic), such as, municipal wastes from lavatories, bathrooms, kitchens, laundries, laboratories, etc., industrial wastes from battery and paint manufacture and burning of fuels (lead, mineral acids), chrome-tanning and aluminium anodizing (chromium), ceramic plants and glass etching and transistor factories (fluorides), copper pickling and plating (copper and mineral acids), DDT manufacture (mineral acids), dye manufacture (phenolic compounds), explosive factories (nitrocompounds), galvanizing industries, rayon manufacture and rubber processing (zinc), metal refineries and cleaning (cyanides and fluorides), petrochemical industries and synthetic rubber, resin factories (hydrocarbon, phenol, fats, oils, formaldehyde, etc.), paper pulp, oil refining, plastic, and drug industries (mercury), tanneries (sulfides, chromium, phenols, tannic acids) etc., automobiles (dust particles, gases like carbon monoxide, carbon dioxide, vapour, etc.), and agricultural product (fertilizers, pesticides, animal wastes etc.) cause environmental pollution leading to degradation of aquatic ecosystem and adverse impacts on inhabiting flora and fauna [5-15]. If pollution becomes sustained for a long time, we cannot breath fresh air, drink clean water and take pure food to survive ourselves in our society. Moreover, pollution can adversely affect or becomes toxic to fish and other aquatic animals in sea, rivers, lakes and ponds which are toxic due to accumulation of heavy metals by the human activities and by industrialization [10, 16-26]

Water pollution has a great relevance with respect to health due to uses of contaminant water. There are incidences of number of diseases. Aquatic pollution has become a global problem; continued anthropogenic input of heavy metal toxicants through the agricultural land degradation into the aquatic environment dictates a continued assessment of their species - specific effect on representative of the ecosystem and biodiversity [23].

India is a vast country with a total geographical area of 329 million sq. km. India is also fortunate to have numerous rivers, big and small. Through our country's major water resources were relatively free from pollution upto some year back, marked deterioration in water quality has come to the notice in a number of location. Even the water of Ganga river, once considered the purest of all and regarded as sacred, is getting polluted by discharge of untreated sweage and industrial wastes like organo-chlorine pesticides and heavy metals [18, 21, 24- 26].

Toxic metals like lead, arsenic, silver, copper, zinc, chromium, mercury, cadmium etc. as mutagenic, teratogenic and carcinogic have been detected about the permissible levels in some rivers, lakes and coastal waters of Bombay, India and other countries also because of the vast release of industrial effluents from the nearby processing industries [1, 12, 16, 18, 27]. Surveys at the coastal waters of Bombay have revealed high content of mercury in the fish at the level of 80 - 120 µg/gm, fishes from Thane and Mumba Creek have the mercury level above 500 µg/gm due to effluents containing mercury from the nearby chlor-alkali plants and paper pulp industries [16] inorganic and organic mercury contained other water system like lakes, river, sea etc. have polluted fish as an aquatic animal [28-33].

The discharge from sewers, night soil, discharges from ship including oil, insectisides and fungicides and other chemical pollutants such as, acids, alkalies, free chlorine, sulphides, heavy metals, phenols, cyanides and even ammonia from a large number of factories and mills pollute the water of Hooghly river of West Bengal [8]. These substances directly or indirectly by a chain of chemical reactions seem to greatly damage the crabs, hilsa and other fishes and other animals also.

It may be also pointed out that water pollution inflicts immense damage to fisheries which the main source of protein in many countries of India. Many rural people also earn their liveligoods from the fisheries. So It is very important problem for these people to maintain their lives due to gradual pollution of water system and reduction of fish population by water pollutions caused by heavy metal toxicity as indicated in figure1. But human activities are responsible for leading to accumulation of heavy metals in aquatic environment [10].

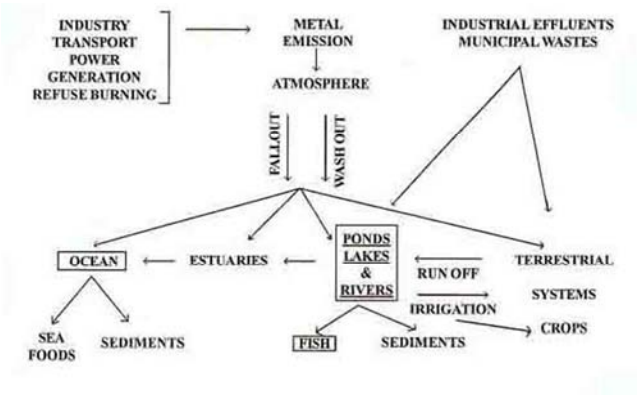

Figure 1. *Dispersion of Metals in Environment (Water system and land or Soil).*

The concept of heavy metal toxicity in experimental animals and human beings may be greatly modified and indeed modulated by the nutritional status of the animal or person. Toxicity of heavy metals cannot be assessed without definitive control of dietary intake and nutritional status of either the experimental animal or human subject [20, 30, 34].

Metal toxicity in fish is often characterised by gill damage and hypersecretion of mucus, ensuing mortalities are related to secondary physiological respiratory disturbances, i.e. ion regulatory and acid-base balance disturbances of which extent depends upon uptake and bio-accumulation of metals [13]. It is also reported that 80µg. of arsenic and 40µg of lead

per 100 ml of blood cause poisoning in adult, children (brain damage) and also in fish respectively, small increase in mercury level can damage to aquatic life, i.e., plants (algae), and animals (fish) of which mucous secretions of gills are precipitated resulting the arrest of interlamellar spaces and the movement of gill filaments thereby preventing respiration and also metabolic upsetin some organs due to accumulation of Zn, Cd, Hg, Cu in gills and also in liver, kidney, blood [2, 3, 13, 18, 28,35-39].

In mammalian system and fish organs metals cause early mortality, growth retardation, impaired reproduction with mortality of offspring, depression of physiologic parameters, derangement of cell membrane permeability and antimetabolic activity; those metals also can interact with a protein leading to an allosteric effect, or with DNA or RNA to stop normal metabolism as well as to reduce the nucleic acid contents even to a change in an ecological system, i.e. at the molecular level toxic effects are observed in changes in rates of catalytic decomposition of essential metabolites, enzyme inhibition, and irreversible conformational changes in macromolecular structure [35, 40-44].

In Channa punctatus a sublethal concentration (Immersion) of lead nitrate causes an inhibition of the activities of several peptidase and lipases of the digestive system [13]. Heavy metals at high concentrations can cause harmful effects on the bio-chemical system of fishes and this causes long term ecotoxicological effects on the organism that eat them [45]. Experiments on toxicity of mixtures of different heavy metals to a teleost are observed by the interaction of the concentration of copper, zinc and nickel [46]

It is observed that the lowering of delta ALA-D activity occurs in some visceral tissues like lung, heart, kidney and blood of rat by zinc [47]. In case of intoxication with lead, mercury and cadmium, these metals are accumulated in gills, liver and kidney of Coho salmon and other fishes and affect some blood picture and hematological indices of fish and these are primarily associated with proteins and carbohydrate metabolism as well as bioaccumulation of nickel and vanadium occurs also in fish organs [48-58].

Continuous accumulation of lead, zinc, mercury, copper, cadmium etc., can interfere with the enzyme, lipid, protein, and nucleic acids to form metal complexes and to cause huge defects on organs and blood level resulting functional disturbances in organs and structural changes in aquatic ecosystem [59-65].

After exposure to lead, chromium, copper, cadmium, mercury etc., enzyme value, nucleic acid, protein, lipid and carbohydrate contents show reduction in liver, muscleand kidney of rat, fish and other animals [37, 66-75].

Blood cholinesterase, deaminase, ferrochelatase, acid phosphatase, aminolevulinic acid dehydratese, ATPase and other enzyme activitiesand serum or plasma and tissue protein content as well as other biochemical parameters are shown to be inhibited or increased or altered by several metallic salts of lead, copper, nickel, zinc, cadmium, mercury, etc. in fish and other animals [15, 41-43, 65, 76-83].

2. Materials and Methods

The Magur fishes,*(Clarias batrachus L.)* a type of fresh water scaleless teleost of comparatively medium size, are abundantly available in West Bengal (India) and are good sources of protein. The varying sizes ranging from 10 or 15 g to about 200 to 300 gm are usually available in the local market in all the seasons. These fishes can easily be maintained in the laboratory with less mortality. They can survive in air for a considerable length of time because of the presence of accessory respiratory organs suitable for air breathing.

The magur fishes, irrespective of sex, of 40 to 60gm body weight were collected from the local market. They were maintained in tap water in big polythene tray in the laboratory as 25°± 1°C. The fishes were fed adlibitum with *Tubifex tubifex*. After adaptation of the fished for 4 to 5 days in the laboratory conditions they were used for the experiments

2.1. Acetate

2.1.1. Cupric acetate

It was the product of British Drug House (BDH), England. This was dissolved in distilled water and diluted in this medium at the desired concentration for injection.

2.1.2. Zinc acetate

It was the product of Sarabhai M. Chemicals, Bombay, India. This was dissolved in distilled water and diluted in this medium at the desired concentration for injection.

2.1.3. Mercuric acetate

It was the product of LobaChemieIndoaustranal Co., Bombay, India. This was dissolved in distilled water and diluted in this medium at the desired concentration for injection.

2.1.4. Lead acetate

It was the product of E. Merck A.G. Darmstadt, Germany. This was dissolved in distilled water and diluted in this medium at the desired concentration for injection.

2.2. Chemicals and Reagents Used for the Estimation of Plasma Protein of MagurFish

2.2.1. Standard protein solution

Bovine serum albumin, cohn fraction V (Sigma Chemical Company, St. Louis, USA) was used for the preparation of a standard protein solution. A standard containing 200 µg of protein per ml was prepared by dissolving the calculated amount in distilled water.

2.2.2. $^{2}/_{3}$ N H_2So_4 Solution

It was prepared by diluting 1.86 ml of conc. H_2So_4 [Glaxo Laboratories (India) Ltd.] to 100 ml by glass distilled water.

2.2.3. 10% Na-tungstate Solution

It was prepared by dissolving 10g of Na-tungstate [BDH, glaxoLaboratoires (India) Ltd.] in distilled water and made

the volume to 100 ml.

2.2.4. 2 M NaOH

It was prepared by dissolving 8g of N_aOH [Glaxo Laboratories (India) Ltd.] in distilled water and the volume was made upto 100 ml.

2.3. Reagent

2.3.1. Reagent A

500 mg of sodium potassium tartarate [BDH, Glaxo laboratories (India) Ltd.] and 20 g of sodium carbonate (Sarabhai Chemicals, India) and 4g of sodium hydroxide (E. Merck, India) were dissolved in 500 ml distilled water and then diluted with distilled water to one litre.

2.3.2. Reagent B

500 mg of cupric sulphate, 5 H_2O [BDH, Glaxo Laboratories (India) Ltd] was dissolved in 50 ml distilled water and then diluted to 100 ml with distilled water.

2.3.3. Regent C: Diluted Folin Reagent

The Folin reagent was prepared by refluxing gently for 10 hours a mixture consisting of 100 mg of sodium tungstate (E. Merck, India), 25 g of sodium molybdate (E. Merck A.G. Darmstadt, Germany), 700 ml of distilled water, 50 ml of 85% phosphoric acid and 100 ml of concentrated hydrochloric acid in 1.5 litre flask. It was allowed to boil and then 150 g lithium sulphate [Analar, Glaxo Laboratories (India) Ltd.], 50 ml of distilled water and few drops of bromine water were added; it was boiled for 15 minutes without condenser to remove excess bromine. This mixture was cooled anddiluted to one litre and filtered. The acid concentration of the reagent was determined by titration with 1 N NaOH to a phenolphthalein end point. Just before use the reagent was diluted accordingly so as to prepare a diluted Folin reagent of 1 N of acid strength.

2.4. Estimation of Plasma Protein

To 50 ml of plasma, 5 ml of 0.65% sodium chloride solution was added to dilute the plasma, 1 ml of 2/3 NH_2So_4 and 1 ml sodium tungstate were added to this diluted plasma and mixed thoroughly. It was then centrifuged. The precipitate was washed by suspension and sedimentation with 5 ml of absolute alcohol and finally it was dried with 5 ml of ether at room temperature. The dried protein thus obtained was dissolved in 5 ml of 2 N NaOH. The clear solution was suitably diluted and the protein was estimated by the method of Lowry et al., 1951[84]. The reading of the sample tubes was taken at 750 nm using 'Beckman Spectrophotometer' (USA).

2.5. Procedure

Fresh and healthy Magur fishes, (Clarias batrachus L.), irrespective of sex and of body weitht of 50 ± 10 g, were collected from a local supplier and maintained in the laboratory conditions at 25^0 C ± 1^0 C for 5 to 7 days before experiments. The fishes were distributed at random in different groups for experiments. They were fed *ad libitum* with *Tubifex tubifex*. The acetate salts of copper, zinc, mercury and lead were used for the treatments.

Since high doses of these metals caused mortality of most of the animals within a week, suitably tolerable doses of the acetate salts of copper, zinc, mercury and lead were first selected by series of trials in order to avoid mortality of the experimental aminals during the period of experiments. Therefore, the suitable doses were found to be: zinc acetate (Sarabhai M. Chemicals, Bombay, India)-5, 10, $20x10^{-9}$ mole/g (three consecutive days injections), lead acetate (E. Merck A.G. Darmstadt, Germany) -25, 50 75 x 10^{-9} mole/g (three consecutive days injections), cupric acetate (British Drug House, England) – 5, 10, 20 x 10^{-9} mole/g (single injection), mercuric acetate (LobaChemieIndoaustral Co., Bombay, India) – 5, 10, 20 x 10^{-9} mole/g (single injection). A single and two consecutive days injection(s) of zinc and lead acetate were ineffective and therefore, three consecutive days injections of the metal salts were given. Naturally 5, 10 and 20 x 10^{-9} mole/g of cupric, mercuric and zinc acetate and 25, 50, 75 x 10^{-9} mole/g of lead acetate were considered as lower, medium and higher effective doses respectively.

For investigating the plasma protein content, the animals were injected intraperitoneally with cupric acetate, zinc acetate, mercuric acetate and lead acetate, all dissolved in distilled water, at the doses as mentioned above. The injected volume did not exceed 180 µl. The control animals were injected with the same volume of distilled water. The day of first injection was taken as zero day. All the animals were fed. Blood was drawn from the cardinal vein with the help of a heparinised tuberculin syringe on days 7, 14, 28 and 42 for determining the plasma protein content. The plasma of heparinised blood was separated by centrifugation and plasma protein was estimated on days 7, 14, 28 and 42 by the method of Lowry et. al. (84) as mentioned before.

2.6. Statistical Analysis

In each case, all the data obtained from the experiments were statistically analysed. The mean data were the average from 10 to 15 animals. The results were expressed as mean ± S.E. The results were evaluated using Student's t-test. P value were considered statistically significant.

3. Results

Effect of different doses of cupric acetate, zinc acetate, mercuric acetate and lead acetate on plasma protein content of Magur fish, Clarias batrachus L. (Fig. 2, 3).

The total plasma protein content of control Magur fish ranged from 4.28 to 4.70 g per 100 ml of plasma. The plasma protein content declined after injection (s) of all the metals on different days of the treatment.

a. Changes after cupric acetate treatment (Figure2)

Compared to the control, different doses of cupric acetate (5, 10 and $20x10^{-9}$mole/g. single injection) significantly reduced the total plasma protein content to almost the same level on day 7. The lower dose ($5x10^{-9}$mole/g) of cupric

acetate could not prolong the decrease in plasma protein content after day 7, and thus the plasma protein content almost returned to the control level on day 14. Such unchanged level in comparison tothe control was also noted on days 28 and 42. But with the medium dose ($10x10^{-9}$mole/g) of cupric acetate the reduced plasma protein content, as observed on day 7, however, increased on day 14, but still the level was significantly less than the control. Almost such reduced level of plasma protein was found on day 28. But on day 42, the plasma protein content was not different from the control with the medium dose of cupric acetate. The higher dose ($20x10^{-9}$mole/g) of cupric acetate was found to be effective in sustaining the decreased level of plasma protein upto day 42. The data also showed that maximum decrease in plasma protein content occured on day 7, and in sustaining the lowering of plasma protein level for a longer period of time (upto day 42) the higher dose was most effective.

b. Changes after zinc acetate treatment (Figure 2)

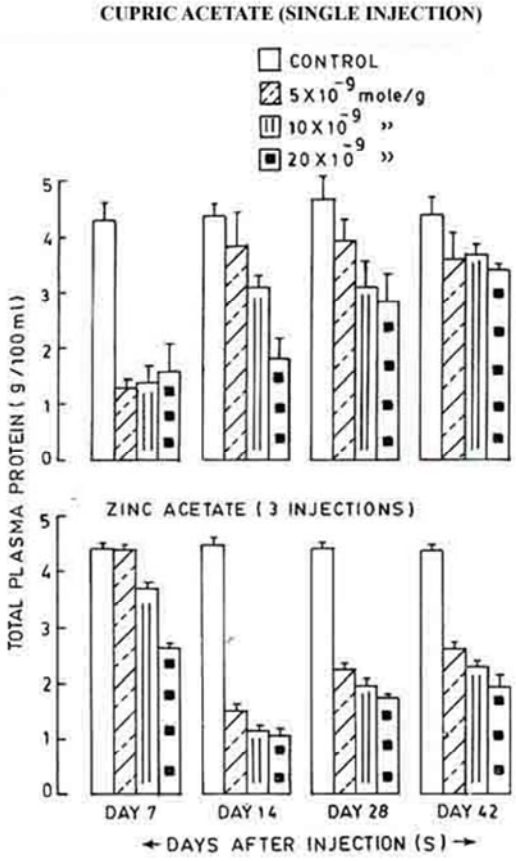

Figure 2. *Effect of a single injection of different doses (5, 10 and $20x10^{-9}$mole/g) of cupric acetate and of three consecutive days injections of different doses (5, 10 and $20x10^{-9}$mole/g) of zinc acetate on total plasma protein content of Magur fish, Clarias batrachus L. The fishes were fed during the experimental period, Blood was drawn on days 7, 14, 28 and 42 after injection(s). Each mean value was the average from 15 animals.*

In comparison to the control, all doses of zinc acetate (5, 10 and $20x10^{-9}$mole/g, three consecultive days injections), with one exception ($5x10^{-9}$mole/g, on day 7), significantly decreased the plasma protein content as observed on all the days of investigations (days 7, 14, 28, 42). Although any change in plasma protein content was not found on day 7 with the lower dose of zinc acetate ($5x10^{-9}$mole/g), a reduction was found on day 14 withthis dose; thereafter the plasma protein level increased on day 28 and day 42, but he levels on day 28 and day 42 were still less than the respective control. With the medium dose of zinc acetate ($10x10^{-9}$mole/g), more reduction in plasma protein content occurred on day 14 in comparison to that on day 7. An increase in the plasma protein level was observed on days 28 and 42 in comparison to that on day 14 with the medium dose, but the plasma protein level on day 28 and day 42 were significantly less than the control. Also with the higher dose of zinc acetate ($20x10^{-9}$mole/g) more decrease in plasma protein level took place on day 14 in comparison to that on day 7. Although an increase in plasma protein content occurred on day 28 and day 42 in comparison to that on day 14, the plasma protein levels on day 28 and day 42 were much less than the respective control. It was also evident from the data that the higher dose of zinc acetate exerted more marked effect on the plasma protein level than the lower or medium dose. This was particularly found on day 7.

c. Changes after mercuric acetate treatment (Figure3)

Marcuric acetate exerted comparatively less effect on the plasma protein level than cupric acetate and lead acetate. This was supported by the fact that the lower and medium doses (5 and $10x10^{-9}$mole/g, single injection) of mercuric acetate failed to cause any change in plasma protein content in comparison to the control. But the higher dose of mercuric acetate ($20x10^{-9}$mole/g, single injection) decreased the plasma protein content on day 7. Further reduction in plasma protein level was found on day 14. Although there was enhancement of plasma protein levelon day 28 and day 42, the plasma protein levels on these days were to be less than the control. Thus it was evident that the decreased plasma protein level with the higher dose of mercuric acetate sustained upto day 42.

d. Changes after lead acetate treatment (Figure 3)

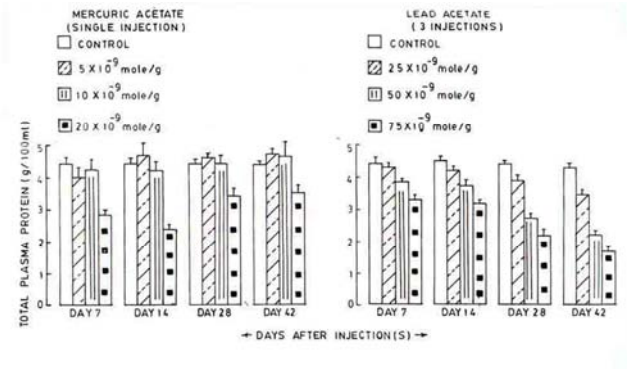

Figure 3. *Effect of a single injection of different doses (5, 10 and $20x10^{-9}$mole/g) of mercuric acetate and of three consecutive days injections of different doses (25, 50 and $75x10^{-9}$mole/g) of lead acetate on total plasma protein content of Magur fish, Clarias batrachus L. The fishes were fed during the experimental period. Blood was drawn on days 7, 14, 28 and 42 after injection(s). Each mean value was the average form 15 animals.*

It was revealed also that lead acetate affected the plasma protein content and thereby significantly decreased the level which continued upto day 42. This metal too had deleterious effect on the formation of plasma proteins and thus upsetting the maintainance of normal plasma protein level. All the doses of lead acetate used (25, 50 and $75x10^{-9}$mole/g, three consecutive days injections) decreased the plasma protein content on days 7, 14, 28 and 42, except in case of lower dose ($25x10^{-9}$mole/g) which failed to cause any change on day 7. The data also showed that with the advancement of the days of investigation the decreased level of plasma protein became more and more marked particularly with the medium and higher doses (50 and $75x10^{-9}$mole/g). It was apparent also that the higher dose of lead acetate exerted maximum effect and such greater influence was evident on all days of investigations (day 7 to day 42).

It might, therefore, be concluded that zinc acetate appeared to be the most powerful agent in altering (decrease) the plasma protein content of Magur fish. Then came cupric acetate in the second rank as a plasma protein-reducing agent, followed by lead acetate in the third rank, and mercuric acetate only at the higher dose occupied the fourth place for affecting the plasma protein content of fish.

4. Discussion

It is generally accepted that metal accumulation in tissues of aquatic animals is dependent upon the exposure, concentration and period as well as some other factors, such as salinity, temperature, interacting agents and metabolic activities of tissue concerned. According to the physiological mechanism transport of metals into the intra cellular compartment may be facilitated by either diffusion of the metal across the cell membrane or by active transport by a carrier protein.

Some selectivity or specificity in the effectiveness of different metal salts was also evident from the other physiological parameters investigated. It was observed that plasma protein of Magur fish was significantly reduced by cupric acetate, zinc acetate, mercuric acetate and lead acetate. But it is to be pointed out that zinc acetate was the most effective substance followed by cupric acetate, lead acetate and mercuric acetate in order of the extent of the change (decrease) in plasma protein content. The last named compound decreased the plasma protein content only with the higher effective dose, while the other three metal salts reduced the plasma protein content with lower, medium and higher effective doses. These results with respect to the decrease in plasma protein content reflect a significant deleterious influence of these substances on the liver which is supported to be the key organ in metabolism and the site of the synthesis of various kinds of proteins including plasma proteins. Thus it may be suggested that the synthesis of plasma proteins in liver was adversely affected by these metal salts. On the other hand, it was observed that changes in blood picture concerning the reduction in RBC count and Hb content are ample indications of the influence of these metal salts on the haemopoietic organ(s), particularly kidney. It seems, therefore, that these substances adversely affect the metabolic functions of kidney and liver by their impact on the organs of fishes leading to deterioration of health.So, it is clearly indicated that as environmental stressors heavy metals are known to alter the biochemical parameters of serum of plasma suggesting the biochemical indices could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of metal contamination and fish health [82].

It could be generally suggested that the decrease in plasma protein content might be due to some physiological hazards in protein metabolism and some cellular disturbances in an important metabolic organ liver and anhemopoietic organ, kidney in our experimental fishes. In our investigations, the following causes may be responsible for the above findings – due to heavy metal induced pathological processes including plasma dissolution, renal damage and protein elimination in the urine, decrease in liver proteinsynthesis, alteration in hepatic blood flow and / or hemorrhage into the peritonealcavity and intestine [85]. They have observed that the low level of total protein in plasma, muscle and liver of *Cyprinus carpio* reflects the capacity of protein synthesis and denotes the osmolarity of the blood and liver impairments (a valuable indicator in the diagnosis of toxicity in fish) because enzymes are necessary for normal cellular metabolism including that of the liver and the degenerative changes due to the combined metal toxicity exhibited in the liver alter the level of a number of its enzymes. As the protein synthesis is related to RNA and ribosome, so Muhammad et. al., 2006[86] has reported that RNA / DNA ratio could be due to decrease in ribosomal activity and heavy metals have an inhibitory effect on protein biosynthesis via the effect on RNA and ribosomal activity [87] Genetic information flows from DNA to RNA to protein. This sequence shows that DNA directs the synthesis of RNA which in turn directs protein synthesis. This is the basic information pathway. So it can be stated that DNA act as signalling factor for the protein synthesis through RNA. If the function of DNA is altered by affecting heavy metal interactions between them, the protein synthesis would be impaired. It has been reported that metal can damage DNA in fish in contaminated aquatic medium involving cell damage and functional disturbances [1, 88, 89].

The information that emerge from various reports from other laboratories are to some extent in conformity with the results obtained from our experiments. It has been reported that the resulting conformational distortion of the polypeptide chains accounts by lead, zinc, mercury etc.Forinhibition of many enzyme system [90, 91] as well as in fish also exposure of red blood cells to increased concentrations (0.05-0.3 mmol/l) of copper and mercury can initiate structural changes and can also induce conformational alteration of internal peptides and proteins[99, 100].These enzymes are essential for particular protein synthesis. Some authors suggest an inhibitory effect of lead on globin synthesis at the translational level [92]. Methylmercury, inorganic mercury,zinc, chromium,lead and cadmium can exert inhibitory effect on protein synthesis in mouse glioma and

rabbit plasma reticulocytes and also in fish [49, 65, 70, 79, 87, 94-96]. Haider, 1977 [76] has reported that serum proteins are changed after administration of lead acetate, cadmium chloride and cupric sulphate in rainbow trout. Lowering of total serum or plasma proteins are also found with prolonged administration of nickel, cadmium etc to rabbit and carp fish (97, 98). Metals are also known to be involved in affecting protein molecules by interactions between them. Several metal ions like lead, zinccopper, cadmium, nickel, mercury etc. can interact with protein molecule, from ligands by binding with -SH, $-NH_2$, -COOH groups, etc., and precipitate the protein as metal-protein complexes in mammals, fish and other animals [35, 83. 99-102]. It has been reported that mercury vapors exposure to workers increase the prevalence of subjects with excessive excretion of albumin, orosomucoid and immunoglobulin [77]. They have also reported that cadmium causes excretion of a greater amount of high mol. wt. proteins. Among the toxic effects of the four heavy metals, from our above findings it was clear that the maximum deduction in plasma protein content occurred in zinc acetate treated fish.

It is evident from the results that exposure of fish to this pollutants decreased the protein content in plasma. It can be concluded that the protein content and the fish quality are markedly affected by water pollution. Also, the heavy metal overload of such fish can cause severe health problems in man specially in liver and kidney.

5. Conclusion

The toxicity of metals salts to aquatic animals is modified by several environmental factors, particularly their hardness of the dilution of water, dissolved oxygen concentration and temperature. The resistance of aquatic animals to the metal poisoning varies with species. Metal distribution in various organs is also time-related. Accumulation of metals in the organs of fish is a function of uptake and elimination rates and metal concentrations in various organs may change during and after exposure, according to various patterns. In fish, concentrations of most metals are usually inversely related to the age and size. Various species of fish from the same water body may accumulate different amounts of metals. Interspecies differences in metal accumulation may be related to living and feeding habits.

Fish are the major part of human diet and it is not surprising that numerous studies have been carried out on metal accumulation in different species. Fish also have been popular targets of heavy metal monitoring programmes in marine environments because sampling, sample preparation and chemical analysis are usually simpler, more rapid and less expensive than alternative choices such as water and sediment. Metals can be taken up by fish from water, food, sediment and suspended particulate material. In recent years, such attention has been directed to the concentration of some inorganic elements in marine fish and other aquatic organisms. The commercial and edible species have been investigated in order to check for those hazards to human health [55].

Due to deleterious effects of metals on aquatic ecosystems, it is necessary to monitor their bioaccumulation in key species because this will give an indication of the temporal and spatial extent of the process, as well as assessment of the potential impact on organism health.

The world Environment Day theme for 2003 was water. This theme had been chosen to support the United Nations International year of Fresh Water.

Human intervention plays a role in the occurrence and severity of naturaldisasters such as water and soil pollution.

According to the United State Agency for International Development (USAID); an estimated 250,000 children in Pakistan die each year due to waterborne diseases. Infections from water including cholera, typhoid and dysentery, burden the public health system, with government reports stating 40 percent of hospital beds annually are taken up by people suffering from such diseases [96, 103].

Fish are considered as an important source of high quality animal protein as they contain large amount of essential amino acids. Humans need fish for their greater quality of life and protection from some diseases but humans now have a strong influence on almost every major aquatic ecosystem and their activities dramatically altered the quality of receiving waters worldwide.

It is now our greater responsibility to reduce future release of maximum extent of heavy metals in order to stop water pollution by contamination of global aquatic ecosystem and to prevent deleterious effects on fish and human.

Thus, there is continuous need to develop and apply novel and effective technologies to detect, manage and correct human induced degradation of aquatic systems.

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References

[1] Banerjea, D. (2016). Environmental Pollution: Causes, effects and control, Sc. Cult. 82, 69-83.

[2] Javed, M. and Usmani, N. (2011). Accumulations of heavy metals in fishes, a human health concern, Int. J. Environ. Sci., 2, 659-670.

[3] Javed, M. and Usmani, N. (2012). Uptake of heavy metal in fishes by Channa punctatus from Sewag fed aquaculture pond of Pancthi, Aligarh, Global J. Res. Eng (C), 12, 27-34.

[4] Sevcikova, M., Modra, H., Slaninova, Z. and Svobodova, Z. (2011). Metals as a cause of oxidative stress in fish, A review. VeterenarniMedicina, 56, 537-546.

[5] Manivasakam, N. (1989). In: 'Environmental Pollution', National Book Trust, New Delhi, India, pp. 1-76.

[6] Shukla, O.P. (1990). Biodegradation for Environmental Management, Everyman's Science, xxv, 46.

[7] Mondal, B. K., ChowdhuryT.R., Samanta, G., Mukherjee, D.P., Chanda, C.R., Saha, K.C. and ChakrabortyT.I., D. (1998). The Science of the Total Environment, 218, 185-201.

[8] Paul, S.C. (2001), Sectional Presidential Address on Environmental Hazards-A Bundle of Global Problems, 88th Ind. Sc. Cong. 3-39.

[9] Saifullah, S. M., Khan, S. H. and Sarwat, I. (2002) Distribution of nickel in a polluted mangrove habited of the Indus delta. Mar. Poll. Bull., 44, 551-576.

[10] Yang, H. and Rose, N. L. (2003). Distribution of Hg in the lake sediments across the U.K., SCI, Total Environ., 304, 391-404.

[11] Vutukuru, B. (2005). Acute effects of hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of the Indian major carp, Labeorohita, Int. J. Environ. Res. Public health, 2, 456-462.

[12] Begum, A., Amin, M. N., Satoshi, K. and Kiyohisa, O. (2005). Selected elemental composition of the muscle tissue of three species of fish, Tilapia nilotica, Cirrhona mrigala and Clarias batrachus from the fresh water Dhamnondi lake in Bangladesh, Food Chem., 93, 139-143.

[13] Shukla, V., Dhankar, M., Prakash, J. and Sastry, K. V., (2007) Bio accumulation of Zn, Cu and Cd in Channa punctatus, J. Env. Biol., 28, 395-397.

[14] Debarandara, S., Naddafi, K. Nazmara, A. and Ghaedi, H. (2010). Heavy metals (Cd, Cu. Ni, Pb) content in two fish species of Persian Gulf in Bushehr Port, Iran, African J. Biotech., 9, 6191-6193

[15] Panigrahi A., (2014) Heavy metal toxicity, Everyman's Science, XLVIII, NO 6, 418-423.

[16] Ray, P.K. (1984), Symposium on Metals, Environment and Safety, 71st Ind. Sc. Cong. 1-12.

[17] Paul, S.C., Dasmahapatra, A.K. and Medda, A.K. (1989). National Symposium on modern trends in Environmental Biology and seminar on Environment and Animal life, S-III, 38.

[18] Singha, D.K. and Mukherjee, A.D. (2000), Fundamentals of Environmental Studies, Visva-Bharati Pub., 12.

[19] Adhikari, S., Saha, R. and Gupta S.K. (2001). Heavy metal contamination of Sewage-fed fish, Laboerohita in eastern Calcutta,Sci. Cult., 67 (3-4), 115.

[20] Turkmen, A., Turken, M., Tepe, Y. and Akyurt, J. (2005). Heavy metals in three commercially valuable fish species from Iskenderun bay, Northern East Mediterranean Sea, Turkey, Food Chem. 91, 167-179.

[21] Karbassi, A. R., Bayati, I. and Moattar, F. (2006). Origin and chemical partitioning of heavy metals in riverbed sediment, Int. J. Environ. Sci. Tech., 3, 35-42.

[22] Chattopadhyay, M.K. and Grossari, H.P. (2010), Pollution by heavy metals: for-reaching consequences, Curr. Sc., 99, 1163.

[23] Chauhan, R. (2008), Water shortage "A Global Problem", p33.

[24] Abbasi, S.A. and Abbasi, N (1996). Water and Water Pollution, EnviroMedia,Karad, Maharashtra, India 8-30.

[25] John, W.E. (2006), Environmental problems and policies, Arise Pub.andDistb. New Delhi, India, 104-105.

[26] MondalR.B. (2006). Water Resource Management, Concept Publishing Company, New Delhi-59, 74, 75, 102.

[27] Palmer, C.D. and Pula, R.W. (1994). Natural attenuation of hexavalent chromium in ground water and soils, USEPA, Ground Water Issue, U.A. Govt. print Office, Washington D.C. 57-72.

[28] WHO (1986). World Health Organization, Review of potentially harmful substances-arsenic, mercury and selenium, Reports and studies,28, 172.

[29] WHO (1990), Methyl mercury, World Health Organization, Environmental Health Criteria, 101, Geneva.

[30] Barghigiani, C. and Ranieri, De, S (1992). Mercury Content in different sizes of important edible species of the northern Tyrrhenian sea, Mar. Pollut, Bull, 24, 114-116.

[31] Voight, H.R. (2003). Concentration of mercury and cadmium in some coastal fishes from the Finnish and Estonian parts of the Gulf Finland, Proc. Estonian Acad. Sci. Biol. Ecol, 52, 305-318.

[32] Pandey, S. Kumar, R. Sharma, S. Naghpure, N.S. Srivastava, S.K. and Verma, M.S. (2005). Acute foxicity bioassays of mercuric chloride malathione on air-breathing fish, Channa punctatus Eco. Toxicol, Env. Sci., 61, 114-120.

[33] Benson, N.U., Essien, J.P., Williams, A.B. and Bassey, D.E. (2007). Mercury accumulation in fishes from tropical aquatic ecosystems in the Niger Delta, Nigeria, Curr. Sc., 92, 781-784.

[34] Topcuoglu, S. Kurbasoglu, C. and Gungor, N. (2002). Heavy metals in organism and sediments from Turkish coasf of the black sea, Environ. Int, 1069, 1-8.

[35] Luckey, T.D. and Venugopal, B.S. (1977), Metal Toxicity in Mammals, Vol. 1, P. 1- 149.

[36] Kodama, M., Ogata, T. and Yammamori, K. (1982). Acute Toxicity in Zinc in To Rainbow trout, Salmo gairdneri, Bull. Jap. Soc. Fish, 48, 1055-1058.

[37] El Damendash, F.M. and Elagamy, E.I. (1999) Biological effects in Tilapia nilotica fish as indicator of pollution by cadmium and mercury Intl J. Envron Health Res. 9, 89-97.

[38] Abreu, S.N., Pereira, E., Vale, C and Duarta, A.C. (2000) Accumulation of mercury in seabass from a contaminated lagoon (Ria de Avero, Portugal), Mar. Pollut, Bull, 40, 293-297.

[39] Wepener, V. Van Vuren, J. H. J. and du Prez H.H. (2001). Uptake and distribution of a copper, iron and zinc mixture in gills, liver and plasma of fresh water teleost, Tilapia sparrmanii, Water, 27, 99-108.

[40] Panigrahi, A. (1992). Physiological studies on the effects of some Environmental Pollutants on Fish, Ph.D. Thesis, University of Calcutta, India.

[41] Karan V., Vitotovic, S.; Tutundzic, V. and Poleksic, V. (1998). Functional enzymes activity and gill history of carp after copper sulphate exposure and recovery, Ecotoxicol, Environ, Saf., 40, 49-55.

[42] Yang, J.L. and Chen, H.C. (2003). Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure, Zoological Studies, 42, 455-461.

[43] Abou El-Naga, E.H., El-Mosellhy, K.H. and Hamed M.A. (2005). Toxicity of cadmium and copper and their effect on some biochemical parameters of marine fish Mugilseheli, Egyptian J. Aquatic Res., 31, 60-71.

[44] Banerjee, T.K. and Devi, R. (2007). Toxicopathological impact of sub-lethal concentration of lead nitrate on the aerial respiratory organs of MurrelChanna striate (BlochPiscus), Iran J.Enrviron. Health, Sci, Eng, 4, 249-256.

[45] Strmack, M. and Braunbeck T. (2000). Isolated hepatocytes of Rainbow trout Oncorhynchus mykiss as a tool to discriminate between differently contaminated small river system, Toxicol In Vitro, 14, 161-377.

[46] Khangarot, B.S, Durve, V.S. and Rajbanshi, V.K. (1981). Toxicity of interactions of zinc-nickel, copper-nickel and zinc-nickel-copper to a freshwater teleost, Labistes reticulates (Peters), Acta.hydrochimhydrobiol., 9, 495-503.

[47] Abdullah, S., Javed, M. and Javid, A. (2007). Studies on acute toicity of metals to the fish (Labeorohita) Int. J. Agnicult, Biol., 9, 333.

[48] Revis, N. W., Major, T.C. and Horton, C.Y. (1980). The effects of calcium, magnesium, lead or cadmium on lipoprotein metabolism and atherosclerosis in pigeon, J. Environ. Pathol. Toxicol., 4, 293-304.

[49] Akahori, A., Gabryelak, T., Jozwiak, Z. and Gondko, R. (1999). Zinc induced damage to carp (Cyprinus carpio L.) erythrocyte in vitro, Biochem. Mol. Biol. Int., 47, 89-98.

[50] Karuppasamy, R. (2000) impact of phenylmercuric acetate (PMK)on the biomodal respiration in an air breathing fish Channa punctatus (Bloch), J. Environ. Pollut., 7, 287-293.

[51] Almeida, J., Novelli, E., Dal Pai Silva, M. and Jumor, R. (2001). Environmental Cadmium exposure and metabolic responses of the Nile tilapia Oreochromis niloticus, Environ. Pollut., 114, 169-175.

[52] Joshi P.K., Bose, M. and Harish, D. (2002). Hematological changes in the blood of Clarias batrachus exposed to mercuric chloride, J. Ecotox. Environ. Monit., 12, 119-122.

[53] Atli, G. and Canli, M. (2007). Enzymatic responses to metal exposure in a fresh water fish Oreochromis niloticus, Comp. Biochem. Physiol., C. Toxicol. Pharmacol., 145, 282-287.

[54] Zaki M. A.,Moustafa, S., Rashad, H. and Sharaf, N. (2008). Assesment of the hazardous effect of lead pollution on Oreochromis niloticus including haematological, biochemical and immunological parameters, Am. Eurasian J. Agric. Environ. Sci., 3, 91-95.

[55] Kamaruzzaman, B. Y., Ong. M. C. and Rina, S. Z. (2010). Concentration of Zn, Cu and Cd in some selected Marine Fishes onthe Pahang coastal waters, Malaysia, Am. Z. Appl. Sc., 13, 309-314.

[56] Hodson, P. V. (2011). Temperature effects on lactate-glycogen metabolism in zinc intoxicated Rainbow trout (Salmo gairdneri), J. Fish. Res. Board Can., 33, 1393-1397.

[57] Saroch, J. D., Nisar, H., Shrinivastav, R. Qurashi, T. A. and Manohar, S. (2012). Hematological studies of mercuric chloride affected fresh water catfish Clarias gariepinus fed with Spirulina, J. Chem. Bio. Phy. Sci. B, 2, 1862-1869.

[58] Cogun, Y. H. and Sahin, M. (2013). The effect of lead and zeolite on haematological and some biochemical parameters in Nile fish (Oreochromis niloticus), Curr. Prog. Biol. Res., 277-286.

[59] Gabryelak, T, Filipick, A. and Brichon, G. (2000). Effects of zinc on lipids of erythrocyte from carp (Cyprinus carpio L.) acclimated to different temperature, Comp. Biochem. Physio., Part C 127, 335-343.

[60] Vinodini, R. and Narayanan, M. (2008). Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (common carp), Int. J. Environ. Sci. Tech., 5, 179 -182.

[61] Vinodini, R. and Narayanan, M. (2009). Changes of antioxidant enzymes in common carp (Cyprinus carpio L.) after heavy metal exposure, Turk. J. Vet. Anim. Sci., 3, 273 -278.

[62] Panigrahi, A., Dasmahapatra, A. K. and Medda, A. K. (2011). Toxicological impact of some heavy metals on a serum enzyme activity, ICMSP 100, University of Calcutta, W.B., India, LL1, p21.

[63] Salman J. M., Al-Mamoori, A. M.J., Al-Saadi, A.H. and Al-Terchi, M.N. (2012). DNA damage in common carp fish (Cyprinus carpio L.) induced by acute exposure to copper and cadmium, World App. Sc., J., 20, 679-682.

[64] Javed, M. and Usmani, N. (2014). Impact of heavy metal toxicity on hematology and glycogen status of fish: A review, Proc. Natl. Acad. Sci., India, Sect B, Biol. Sci., 11 pages.

[65] Arya. A. (2014) Evaluation of biochemical and histochemical changes following the combined treatment of mercury and cadmium in a fresh water catfish, Clarias batrachus (Linn), Int. J. Pharmacy and Pharmaceutical. Sc., 6, 356-358.

[66] Mathers, E. M., Houlihan, D. F., Mc. Carthy, I. D. and Burren, I. J. (1993). Rates of growth and protein synthesis correlated with nucleic acid content in fry of rainbow trout, J. Anim. Physiol. Anim. Nutr., 88, 275-287.

[67] Canli M. (1996). Effects of mercury, chromium and nickel on glycogen reserve and protein levels in tissues of Cyprinus carpio, Turk. J. Zool., 30, 161-168.

[68] James, R.,Sampath, K. and Alagurathinam, S. (1996). Effects of lead on respiratory enzyme activity, glycogen and blood sugar levels of the teleost Oreochromis mosambicus (Peters) during accumulation and depuration, Asian Fish Sci., 9, 87-100.

[69] Kazlauskiene, N. and Vosyliene, M. Z. (1999). Peculiarities of the physiological responses of rainbow trout to copper, Acta. Zoo. Litua. Hydro., 9, 1392.

[70] Kakkar, P. and Jaffery, F. N. (2005). Biological markers for metal toxicity, Environ. Toxicol, Pharm., 19, 335-349.

[71] Bedii, C. and Kanan, F. (2005). The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of Cyprinus carpio (L. 1758), Turk. J. Vet.Anim. Sci., 29, 113-117.

[72] Abdel-Tawwb, M., Mousa, M. and Abbass, F. (2007). Growth performances and physiological response of African catfish, Clarias gariepinus (B) fed organic selenium prior to the exposure to environmental copper toxicity, Aqucult., 272, 335-345.

[73] Vinodini, R. and Narayanan, M. (2008). Effects of heavy metals induced toxicity on metabolic biomarkers in common carp (Cyprinus Carpio L.), Int., J. Sci. Technol., 2, 192-200.

[74] Ozgur, F., Cogun, H. Y., Yuzererogin, T.A., G. K. G. Ozge, Kargin, F. and Koleman, Y. (2011). A comparative studies on the effects of a pesticides (Cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus, Fish Physol. Biochem., 97, 657 666.

[75] Tripathi, S. Mishra, B. B. and Tripathi, S. P. (2012). Effect of heavy metal cadmium sulphate on the toxicity and biochemical parameters of reproductive cycle of ColisaFasciatus, Researcher, 4, 65-68.

[76] Haider, G. (1977). Damages to red blood picture by heavy metals, Methoden Toxizitaet Spruef. Fischen; Situaet. Beurteilung, Ber. Kollog., 113-116.

[77] Buchet, J.P., Lauwerys, R., Roels, H. and Bernerd, A. (1980). Relationship between exposure to heavy metals and prevalence of renal dysfunction, Arch. Toxicol., Suppl. 4, 215-218.

[78] Oskarsson, A. and Fowler, B. A. (1987).Alteration in renal heme biosynthesis during metal nephrotoxicity, Ann. N.Y. Acad. Sci., 514, (Mech. Chem, Induced prophyrinopathies), 268-277.

[79] Zikic, R. V., Stajn, A. S., Ognjanovic, B. I., Pavlovic, S. Z. and Saicic, Z. S. (1997). Activities of superoxide dismutase and catalase in erythrocyte and transaminase in plasma of carps (Cyprinus carpio L.) exposed in cadmium, Physiol. Res., 46, 391-396.

[80] Coppo, J. A., Mussort, N. B. and Fioranelli, S. A. (2001). Physiological variation of enzymatic activities in blood of bull frog, Rana catesbeiana, (Shaw, 1820), Rev. Vet., 12/13, 22 -27.

[81] Levesque, H. M., Moon, T.W., Campbell, P. G. C. and Hontela, A. (2002). Seasonal variations in carbohydrate, lipid metabolism of yellow perch, Percafiavescens: Chronically exposed to metals in the field, Aqua Toxicol., 60, 257-267.

[82] Oner, M., Atli, G. and Canli, M. (2008). Changes in serum biochemical parameters of fresh water fish Oreochromis niloticus following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposure, Environ. Toxicol. Chem., 27, 360-366.

[83] Said, A. K., Ferencz, A., Nemcsok, J. and Hermesz. E. (2010). Expression of heat shock and metallothionein, genes in the heart of common carp (Cyprinus carpio): Effects of temperature shock and heavy metal exposure, Acta. Biol. Hung., 61, 10-23.

[84] Lowry, O. H., Rosenbrouh, W. S., Farr, A. and Randall, T. (1951). Protein measurement with folin-phenol reagent, J. Biol. Chem., 193, 265-275.

[85] Rajamanickam, V. and Muthuswamy, M. (2008). Effect of heavy metals induced toxicity in metabolic biomerkers in common carp (Cyprinus carpapio L.) Mj. Int. J. Sci. Tech., 2, 192-200.

[86] Muhammad, A., Iqbal, R., Rana, S. A., Athar, M. and iqbal, F. (2006). Effect of feed, cycling on specific growth rate, condition factor and RNA / DNA ratio of Labeo rohita, African J. Biochem., 5, 1551-1556.

[87] Buckley, L.J. and Bulow, F. J. (1987). Techniques for the estimation of RNA, DNA and protein in fish, In:R.C. Summerfelt and G.E. Hall, Eds. The age and growth of fish, The Iowa State University Press, Ames, 345-354.

[88] Jia, X., Zhang, H. and Liu, X. (2011). Low level of cadmium exposure induce DNA damage and oxidative stress in the liver of Oujiang colored common carp Cyprinus carpio var. color., Fish Physiology Biochemistry, 37, 97-103.

[89] Cok, I., Ulutas, O. K.,Okusluk, O, Durmaz, F. and Demir, N. (2011). Evaluation of DNA damage in common carp (Cyprinus carpio L.) by Comet assay for determination of possible pollution in Lake Mogan (Ankara), The Scientific World Journal: TSW Environment, 11, 1455-1461.

[90] Garavini, C., Martelli, P., Seren, P., Caldeo, F. and Villani, L. (1979). Effect of lead and zinc on the erythropoiesis of cat fish,Rivista Di Biologica, 72, 83-90.

[91] Krzysztof. G. (1992). Structural changes of proteins in fish red blood cells after copper and mercury treatment, Arch. Environ. Contam. Toxicol., 33, 426-430.

[92] Haeinen, M.J. (1977). In 'Dyserythropoieses' (S.M. Lewis & R.R. Vermilghen, eds.). Acad. Press. London, New York, San Francisco, PP. 316-337.

[93] Nakada, S., Nomoto, A. And Imura, N. (1980). Effect of methylmercury and inorganic mercuryon protein synthesis in mammalian cells,Ecotoxicology and Environmental Sefety, 4, 184-190.

[94] Salem, M. F. I. (2003) Effects of cadmium, copper and lead contamination on growth performance and chemical composition of Nile Tilapia, Mansoura University Journal of Agricultural Science, 28, 7209-7222.

[95] Mary, J. R. A., John, M. M. C., Uthiralingam, M. and Azhaguraj, R. (2011). Quantitative variation of protein in tissues of a fresh water fish Clarias batrachus exposed to mercury and chromium, Int. J. Curr. Res., 13, 230-236.

[96] Habib, S. A. and AbouShehattaSamah, A. M. (2013). Effect of many metals pollution on protein biosynthesis in cat fish, J. Water Resource and Protection, 5, 555-562.

[97] Saxena, M., Saxena H. M., Sangha, G. K. and Kaur, K. (2008). Effect of heavy metal pollution of water on total plasma proteins and serum protein profiles of common carp fish (Cyprinus carpio). Internet J. Vet. Med., 5, 2.

[98] Bolawa, O. E. and Ghenle, G. O. (2013). Effect of consumption of heavy metals contaminated fish (Tilapia oreochromis) on metabolic parameters in Rabbits, Int. J. App. Biol. Pharmc., 317-321.

[99] Neff, J. M. (1985). Use of biochemical measurement to detect pollutant mediated damage to fish, ASTM Spec. Tech. Publ., 854, 155-183.

[100] Kapila, M. and Ragothaman, G. (1999). Mercury, Copper and Cadmium induced changes in the total protein level in muscle tissues of an edible estuarine fish boieoopthalmusdessumieri, Cuv. J. Environ. Biol., 20, 231-234.

[101] Adami, G. M. Beribieri, P., Fabiani, M., Piselli, S., Predonzani, S. and Reisenhofer, E. (2002). Levels of cadmium and zinc in hepatopancreas of reared Mytilusgalloprovincialis from the gulf of Trieste (Italy), Chemosphere, 48, 671-677.

[102] Filipovic, V. and Raspor, B. (2003). Metallothionein and metal levels in cytosol of liver, kidney and brain in relation to growth parameters of Mullussurmuletus and Liza aurata from the Eastern Adriatic Sea, Water Resource, 37, 3253-3262.

[103] Dixit, N. K. (2008). Water Shortage 'A Global Problem', PP.33.