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ACID BLACK-1 AND ZINC INDUCED CHANGES IN THE ZINC DYNAMICS IN THE TISSUES OF *LABEO ROHITA* (HAMILTON)

Ramandeep Kaur and Arvinder Kaur*
Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab, India, 143005.
*Corresponding author email- arvinder99@yahoo.co.in

ABSTRACT
Aim of the present study was to investigate changes in the dynamics of Zn (an essential metal) in blood, liver, kidney, gill and muscle of a fresh water major carp *Labeo rohita*. On exposure to two important constituents of textile dye effluent *viz*. azo dye Acid Black-1(AB-1) and Zinc (Zn). The fishes were exposed in triplicate to 0,2,4,6,8 and 10 mg/L AB-1, 0.25, 50, 55 and 60 mg/L Zn and 2 mg/L AB-1+0, 15, 20 and 25 mg/L Zn (AB-1+Zn). The concentration for the mixture were selected on the basis of a preliminary bioassay in which 100% mortality of fish even for few hours was observed at > 2.5 mg AB-1 and > 27 mg Zn in combination. Exposure to AB-1 induced a concentration dependent decline in Zn in all the tissues after 96 h exposure. However content of Zn increased during the recovery period but was less than control in all the tissues of exposed fish. During the recovery period the accumulation of Zn was more in all the tissues on exposure to AB-1+Zn (2+25 mg/L) in comparison to Zn alone. It was almost equal to the level at 50 mg/L Zn. The results clearly show that mixture of AB-1 and Zn entering the aquatic bodies with textile effluents will be more harmful, therefore there is a strong need to check entry of such chemicals in aquatic bodies as fish is an important link in the food chain of man.

KEYWORDS: Acid Black-1, Zinc, flame atomic absorption spectrophotometer (FAAS), *L. rohita.*

INTRODUCTION

Textile industry releases treated and untreated effluents directly or indirectly into the running water bodies and is one of the major sources of aquatic pollution (Ghoreishi & Haghighi, 2003). Dyes, heavy metals, hazardous wastes (Sharma *et al*., 2009; Khayatzadeh & Abbasi, 2010) are the major pollutants released by this industry. Most of these being non biodegradable, cause serious threat to aquatic biodiversity (Mathur & Bhatnagar, 2007). Azo dyes are the largest and most versatile class of dyes of which 2000 different azo dyes are used by industries (Puvaneswari *et al*., 2006). Several of these dyes have been reported as toxic, carcinogenic and mutagenic in nature (Mathur & Bhatnagar, 2007; Jagruti, 2015). Along with dyes presence of heavy metals such as copper, zinc, chromium, cadmium in the effluents is a cause of concern for aquatic life (Woodling *et al*., 2001) because of their persistent nature, toxicity, tendency to accumulate and to undergo food chain amplification (Gupta & Srivastava, 2006). Among these heavy metals, zinc is widely used in textile industries as ZnO to enhance functionality of clothes due to its antibacterial, UVA and UVB property (AbdElhady, 2012; Rimbu *et al*., 2013). It is also used in cationic dyes, medicines, galvanization of iron, plumbing, paints and mining (Zeiner, 2007; Authman, 2015). Though, Zn is an important trace element, acts as a structural component and used as a cofactor *(Murugan et al*., 2008) but due to excessive discharge in water bodies, the Environment Protection Agency (EPA) has listed Zn as one of most common pollutants(Athar & Vohora, 2001). Aim of current study was to investigate the effect of an azo dye Acid Black 1(AB-1, CI: 20470, ~ 100% purity) and Zn on the level of Zn (separately and in combination) in blood, liver, kidney, gill and muscle in the food fish, *L. rohita* after 96 h exposure and during a post exposure period of 90 days. The study holds importance because excess as well as deficiency of Zn has been reported to cause damaging effects in the organisms (Nussey *et al*., 2000; Powell, 2000). To the best of our knowledge no such report is available till date on the combined effect of dye and zinc on fish till date.

MATERIALS & METHODS

The AR grade chemicals and $ZnSO_4$ (Product No. 28985) were procured from Himedia, SRL and Fisher Scientific, while the Azo dye Acid Black-1was purchased from the local market of Amritsar, Punjab. Fingerlings of *L. rohita* (13.87cm average length and 11.70 g average weight) were obtained from the Government fish farm, Rajasansi, Amritsar, Punjab. The fingerlings were disinfected by giving dip treatment in 0.1% $KMnO_4$ solution for 2-3 minutes and acclimated to laboratory conditions in tap water for 21 days in plastic pools of 200 L capacity. During the acclimation and experimental period, the fish were fed on '*Toya*' floating pellets @ 2% of their body weight, except for 24 h preceding and during the exposure. The exposure were given separately for AB-1, Zn and AB-1+Zn according to the Standard methods for examination of water and waste water APHA (2012). De-chlorinated tap water was used as a diluent and control, the test water was refreshed every 24h. The fish were exposed in triplicate to 0, 2, 4, 6, 8 and 10 mg/L AB-1, 0, 25, 50, 55 and 60 mg/L Zn and 2mg/L AB-1 + 0, 15, 20 and 25 mg/L Zn (AB-1+Zn). These concentrations were selected on the basis of preliminary bioassays. Live fish from each

concentration were kept for a recovery period of 90 days (post exposure) to observe prolongation of the stress of exposure. The amount of zinc was determined in blood, liver, kidney, gill and muscles after 96 h of exposure and on 45th and 90th day of recovery period with the help of flame atomic absorption spectrophotometer (FAAS, model: Agilent 240 FSAA). The standard solution of zinc was obtained from Agilent (1000 mg/L) and was run after every ten samples (0.5 mg/L, 1.0 mg/L and 1.5 mg/L).

To collect blood, heart of the fish was punctured with heparin rinsed syringe, the tissues were dissected, oven dried (70°C and 10-12 h) and powdered in mortar and pestle. Thereafter, 0.5 g sample was digested with 15 ml of diacid mixture (concentrated HNO_3 and $HClO_4$ in 4:1 v/v) in a digestion chamber until it became colorless. This mixture was then diluted to make a final volume of 50 ml with double distilled water in a volumetric flask and used for analysis. The MINITAB 14 was used to determine the extent of variation among observations under the influence of the treatment (ANOVA) and ASSISTAT was used for estimating the difference between the mean values (Tuckey's Test). The results have been reported as mean ± SE with a statistical significance at $p < 0.001$ and $p < 0.05$.

RESULTS

Variation in the content of Zn in blood, liver, kidney, gill and muscle after 96 h exposure and on 45th and 90th day of recovery period after exposure to AB-1, Zn and AB-1+Zn is depicted in figures1-6. The fish exposed to 10 mg/L dye could not survive after 55th day after 96 h exposure so data could not be calculated for this concentration on the 90th day of recovery period. Tissues of the fish exposed to AB-1, Zn and AB-1+Zn showed a variable trend of Zn accumulation after 96 h exposure and during the post exposure period.

After 96h exposure to AB-1 (Fig. 1 and 2), a significant decrease over control ($p < 0.001$) was observed in the concentration of Zn in all the tissues of fish except for blood and muscle. Maximum decrease over control in the content of Zn was observed in blood (16.12-89.77%), followed by liver (4.83-84.68%), kidney (6.67- 67.99%), gill (0.62-62.77%) and muscle (6.76-38.70%) after 96 h exposure to AB-1. On 45th day of recovery period, a significant increase over control ($p < 0.001$) in Zn was observed in blood while in liver, kidney, gill and muscle there was an increase in the content of Zn over 96 h values but it remained less than control in all the concentrations. On 90th day of recovery period, decrease over control in the content of Zn was less in all the tissues ($p < 0.001$) except for blood where a non-significant ($p < 0.891$) increase was observed in the content of Zn.

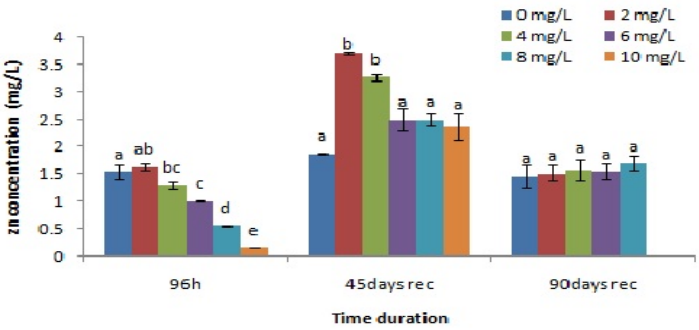

FIGURE 1. Variation in the concentration of Zinc (mg/L, mean ± SE) in blood of *L.rohita* after 96 h exposure to AB-1 and on the 45th and 90th day of recovery period.

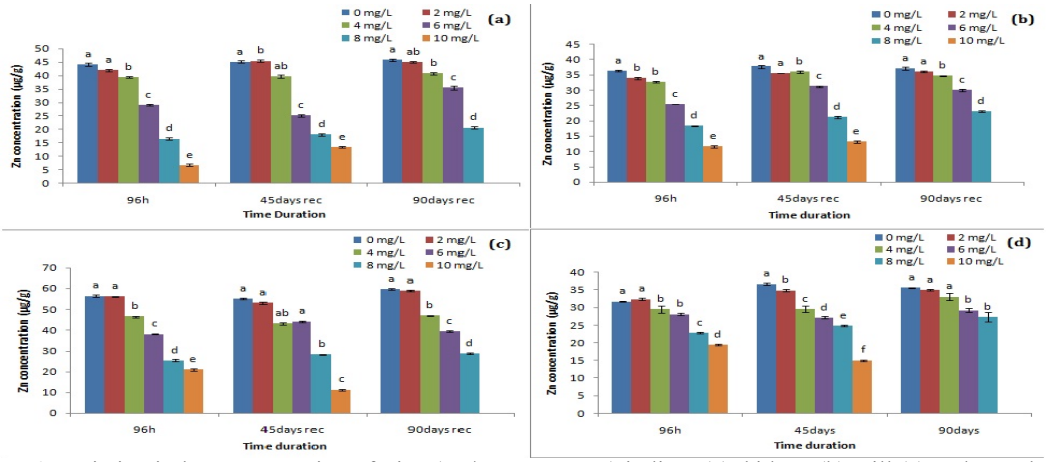

FIGURE 2. Variation inthe concentration of Zinc (µg/g, mean ± SE) in liver (a), kidney (b), gill (c) and muscle (d) of *L.rohita* after 96 h exposure to AB-1 and on the 45th and 90th day of recovery period.

On exposing the fish to Zn for 96 h, a significant ($p < 0.001$) increase over control was observed in Zn accumulation in all the tissues (Fig. 3 and 4). Blood (30.10- 157.99 %) showed maximum Zn accumulation followed by liver (55.01- 113.64%), kidney (7.07-100.30%), gill (5.52-72.25%) and muscle (4.34-34.71%), however, at 25 mg/L Zn there was 3.17% decrease over control in the concentration of Zn in blood. On 45th day of recovery period, marked increase in accumulation of Zn was observed over 96 h values in blood and muscle while it decreased significantly over 96 h values in liver, kidney and gill but overall there was an increased over control in the content of Zn in all the tissues. On 90th day of recovery period, high concentration of Zn still persisted in all tissues and maximum increase in Zn was found in blood while minimum increase was observed in gills.

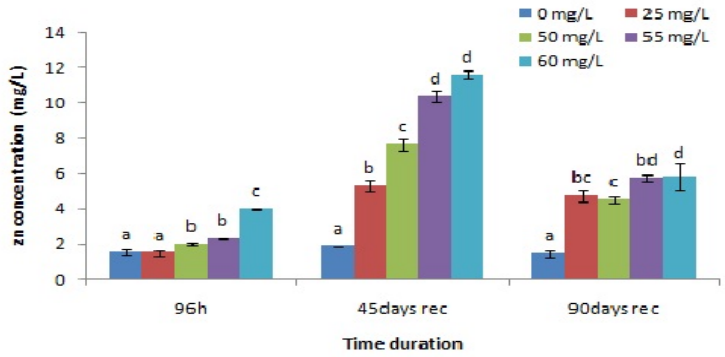

FIGURE 3. Variation in the concentration of Zinc (mg/L, mean ± SE) in blood of *L.rohita* after 96 h exposure to Zn andon the 45th and 90th day recovery of period.

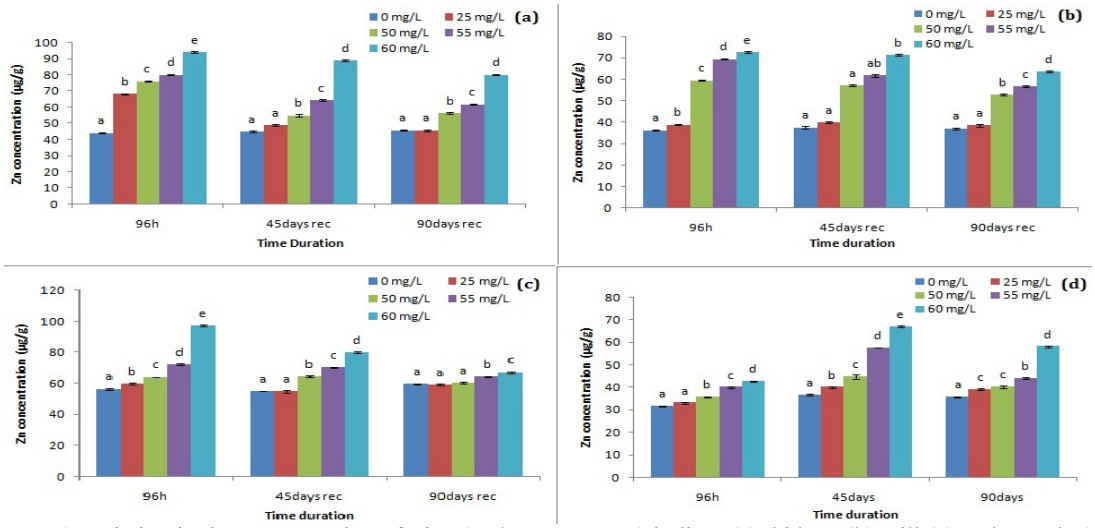

FIGURE 4. Variation in the concentration of Zinc (µg/g, mean ± SE) in liver (a), kidney (b), gill (c) and muscle (d) of *L.rohita* after 96 h exposure to Zn and on the 45th and 90th day of recovery period.

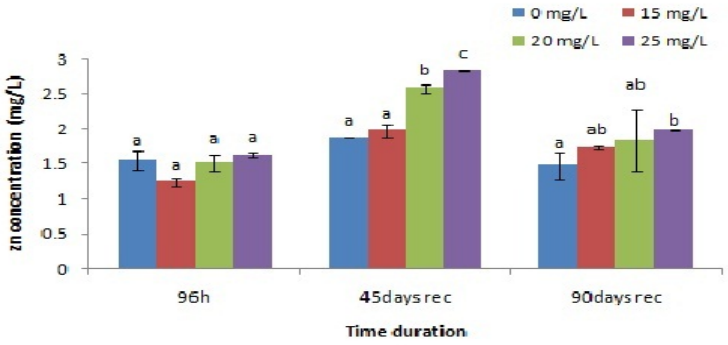

FIGURE 5. Variation in the concentration of Zinc (mg/L, mean ± SE) in blood of *L.rohita* after 96 h exposure toAB-1+Zn and on the 45th and 90th day of recovery period.

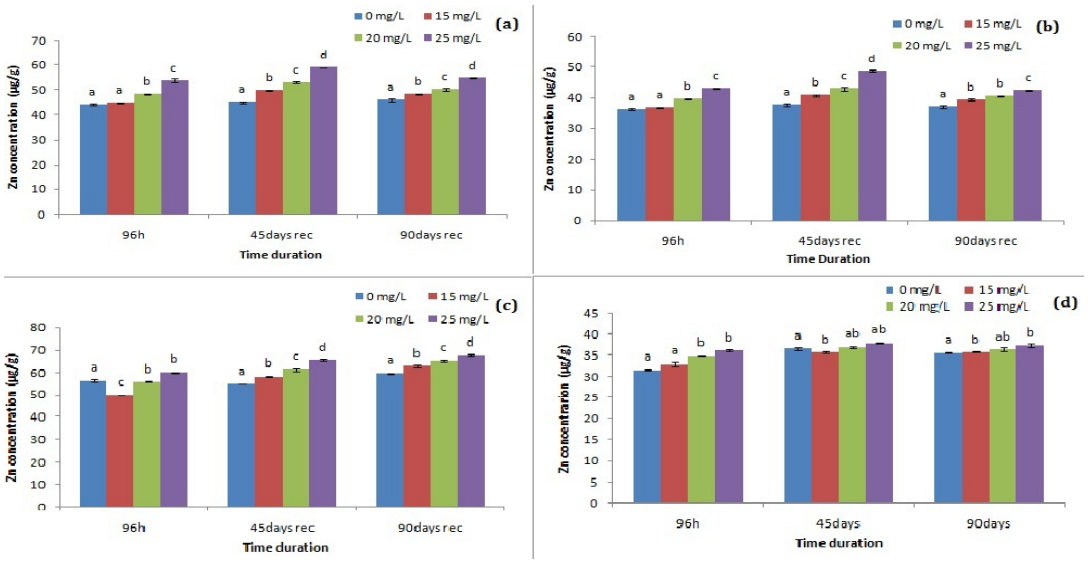

FIGURE 6. Variation in the concentration of Zinc (µg/g, mean ± SE) in liver (a), kidney (b), gill (c) and muscle (d) of *L.rohita* after 96 h exposure to AB-1+Zn and on the 45th and 90th day of recovery period.

When the fish was exposed to AB-1+Zn, a significant increase over control ($p < 0.001$) was observed in Zn accumulation after 96 h in all the tissues except for blood (increase only at 2+25 mg/L AB-1+Zn) (Fig. 5 and 6). Percent increase over control in concentration of Zn was 21.65% in liver, 18.50% in kidney, 14.52% in muscle,6.58% in gill and 5.57% in blood at 2+25 mg/L AB-1 +Zn. An increase in the level of Zn in blood and muscle after 45 days was followed by a decline over 96 h values on the 90th day of recovery period. However, in the other tissues content of Zn declined over 96 h values till the end of recovery period.

DISCUSSION

A concentration dependent decline over control in the level of Zn in all the tissues after 96 h exposure to AB-1 and its prolongation till the 90th day of recovery period hints towards a strong health damaging effect of AB-1. Several azo dyes have been reported to be genotoxic/ cytotoxic in nature (Matsuoka *et al*., 2001; Biswas & Khuda-Bukhsh, 2005; Rituparna & Ghosh, 2012). The present dye may have caused cytotoxic damage in the exposed fish because on the 45th day of recovery period the decline in Zn in liver, kidney, gill and muscle was accompanied with an increase over control in the content of Zn in blood. Another reason for increase in blood could be that blood flows through all the tissues and accumulates toxicants in return. Deficiency of Zn is known to lower digestibility of proteins and carbohydrates and to cause lens cataract and erosion of fins and skin (Ogino & Yang, 1979; Hughes, 1985). It is also known to induce alternations in antioxidant enzymes and to increase susceptibility of cells to oxidative damage (Powell, 2000). Toxic effect of the dye lasted till the 90th day of recovery period as the content of Zn in the dye exposed fish remained less than control in all the tissues. Dyes and their products pass through the membrane and accumulate in the cells (Tonogai *et al*., 1979). Long lasting effect of AB-1 on the antioxidative enzymes of fish has been reported by Kaur & Kaur (2015). In the present study also colour of the dye was evident on gills and body of the fish till the 45th day of recovery period at 6 and 8 mg/L dye. Higher decline in the Zn content of blood in the present study could be due to dye induced reduced affinity of haemoglobin towards oxygen that made erythrocytes more brittle and porous as suggested by Sharma *et al*. (2007).

On the other hand, exposure to Zn and AB-1+Zn, concentration of Zn increased over control in all the tissues during exposure as well as the recovery period of 90 days. However the concentration of Zn in all the tissues of the fish was less on exposure to AB-1+Zn (2+25 mg/L) in comparison to its concentration at 25 mg/L Zn. Being an essential element Zn is easily accumulated by the tissues of fish (Irwandi & Farida, 2009; Rejomon *et al*., 2010). Highest concentration of Zn in blood in both Zn and AB-1+Zn exposure could have been due to an increased number of immature RBCs in circulation as immature RBCs have been reported to have more content of Zn in comparison to mature RBCs (Lin *et al*., 2011). Exposure to heavy metals has been reported to induce a significant elevation in the RBCs of fish by Vinodhini & Narayanan (2009) also. Prolonged elevation of Zn in the tissues of present fish with maximum level in blood is corroborated by the findings of Witeska & Kościuk (2003) who also observed a considerably high level of Zn in blood of common carp (*Cyprinus carpio*) even after removal of the metal from water. They suggested that it was due to adsorption and deposition of Zn in the blood of fish. Blood being in contact with all the tissues was responsible for adsorption and accumulation leading to highest content of Zn in blood while liver cells have more affinity for metallothioneins(Kendrick *et al*., 1992; Ikem *et al*., 2003) and accumulate metals via blood from other parts of body (Kent, 1998). Production of metallothionein proteins by organisms increases under stress of metals to reduce their toxicity (Powell, 2000). Kidney is a gateway for heavy metal detoxification and gills regulate ions and osmosis (Jezierska *et al*., 2001; Jayakumar & Vattapparumbil, 2006). Lowest variation in the muscle on exposure to AB-1, Zn as well as AB-1 +Zn could be due to

less metabolic activity in this tissue (Adhikari *et al*., 2009) in comparison to liver, kidney, gill and blood. Dural *et al*. (2007) also reported that metabolically more active organs like liver, kidney and gill accumulated more amounts of heavy metals than muscle of *Dicentrarchus labrax and Mugilcephalus* captured from heavy metal polluted Tuzla lagoon. Prevalence of abnormal Zn in the tissues of exposed fish in comparison to control till the end of recovery period suggests that a longer period may be required after exposure to AB-1 and Zn (separately and in combination) for reversal of Zn to normal levels. Gupta & Srivastava (2006) also reported that stress of metals altered certain biodegradation mechanisms which required a long period to fall within normal levels after exposure. In the present study accumulation of Zn was almost same in the tissues after 96 h exposure to 25 mg/L Zn separately and 2+25 mg/L AB-1+Zn in combination. But during the recovery period content of Zn was more in all the tissues of fish exposed to AB-1+Zn. Only one concentration of dye was taken for AB-1+Zn in the present study as in the preliminary test, fish could not survive more than 2 mg/L AB-1 in combination with Zn. Therefore it is clear that in combination AB-1 enhances and prolongs accumulation of Zn in the body which can be more harmful for the fish.

CONCLUSION

The data clearly show that AB-1 and Zn are more toxic to fish in combination. Stress of 96 h exposure to AB-1 and Zn separately as well as in combination prolonged till the 90^{th} day of recovery period. Therefore there is a strong need to regulate the amount of such pollutants in aquatic ecosystems.

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REFERENCES

AbdElhady, M.M. (2012) Preparation and Characterization of Chitosan/ Zinc Oxide Nanoparticles for Imparting Antimicrobial and UV Protection to Cotton Fabric. *Int. J. Carbohydr. Chem.***2012**, 1–6.

Adhikari, S., Ghosh, L., Giri, B. S. & Ayyappan, S. (2009) Distributions of Metals in the Food Web of Fishponds of Kolleru Lake, India. *Ecotoxicol. Environ. Saf.* **72**, 1242–1248.

APHA (2012) *Standard Methods for the Examination of Water*, 21st ed. Washington D.C.: American Public Health Association.

Athar, M. & Vohora, S.B. (2001) *Heavy Metals and Environment*. New Age International Publishers.

Authman, M.M. (2015) Use of Fish as Bio-Indicator of the Effects of Heavy Metals Pollution. *J. Aquac. Res. Dev.***6**, 1–13.

Biswas, S.J. & Khuda-Bukhsh, A. R. (2005) Cytotoxic and Genotoxic Effects of the Azo-Dye P-Dimethylaminoazo benzene in Mice: A Time-Course Study. *Mutat. Res. Toxicol. Environ. Mutagen.***587**, 1–8.

Dural, M., Göksu, M.Z.L. & Özak, A.A. (2007) Investigation of Heavy Metal Levels in Economically Important Fish Species Captured from the Tuzla Lagoon. *Food Chem.***102**, 415–421.

Ghoreishi, S.M. & Haghighi, R. (2003) Chemical Catalytic Reaction and Biological Oxidation for Treatment of Non-Biodegradable Textile Effluent. *Chem. Eng. J.***95**, 163–169.

Gupta, P. & Srivastava, N. (2006) Effects of Sub-Lethal Concentrations of Zinc on Histological Changes and Bioaccumulation of Zinc by Kidney of Fish Channa Punctatus (Bloch). *J. Environ. Biol.***27**, 211–215.

Hughes, S.G. (1985) Nutritional Eye Diseases in Salmonids: A Review. *The Progressive Fish-Culturist***47**, 81–85.

Ikem, A., Egiebor, N.O. & Nyavor, K. (2003) Trace Elements in Water, Fish and Sediment from Tuskegee Lake, Southeastern USA. *Water. Air. Soil Pollut.* Kluwer Academic Publishers 2003, 51–75, doi:10.1023/A: 1025694315763.

Irwandi, J. & Farida, O. (2009) Mineral and Heavy Metal Contents of Marine Fin Fish in Langkawi Island, Malaysia. *Int. Food Res. J.***16**, 105–112.

Jagruti, B. (2015) Evaluation of Azo Dye Toxicity Using Some Haematological and Histopathological Alterations in Fish Catla Catla. *Int. J. Biol. Biomol. Agric. Food Biotechnol. Eng.***9**, 436–439.

Jayakumar, P. & Vattapparumbil, I. P. (2006) Patterns of Cadmium Accumulation in Selected Tissues of the Catfish Clarias Batrachus (Linn.) Exposed to Sublethal Concentration of Cadmium Chloride. *Vet. Arh.***76**, 167–177.

Jezierska, B., Witeska, M. & Norris, D.O. (2001) *Metal Toxicity to Fish*. Springer Netherlands.

Kaur, S. & Kaur, A. (2015) Variability in Antioxidant/ detoxification Enzymes of Labeo Rohita Exposed to an Azo Dye, Acid Black (AB). *Comp. Biochem. Physiol. Part - C: Toxicol. Pharmacol.***167**, 108–116.

Kendrick, M. J. (Maureen J. ., May, M. T., Plishka, M. J., Robinson, K. D. & Harrison, C. C. (1992) Metals in Biological Systems. *J. Chem. Technol. Biotechnol.***58**, 405–406.

Kent, C. (1998) *Basics of Toxicology*. Wiley.

Khayatzadeh, J. & Abbasi, E. (2010) The Effects of Heavy Metals on Aquatic Animals. *1st Int. Appl. Geol. Congr. Dep. Geol. Islam. Azad Univ. Branch, Iran***1**, 26–28.

Lin, T. Y., Chen, Y. H., Liu, C. L. & Jeng, S. S. (2011) Role of High Zinc Levels in the Stress Defense of Common Carp. *Fish. Sci.***77**, 557–574.

Mathur, N. & Bhatnagar, P. (2007) Mutagenicity Assessment of Textile Dyes from Sanganer (Rajasthan). *J. Environ. Biol.***28**, 123–126.

Matsuoka, A., Tada, A., Terao, Y., Nukaya, H., Önfelt, A. & Wakabayashi, K. (2001) Chromosomal Effects of Newly Identified Water Pollutants PBTA-1 and PBTA-2 and Their Possible Mother Compounds (AZO DYEs) and Intermediates (Non-ClPBTAs) in Two Chinese Hamster Cell Lines. *Mutat. Res.-Genet. Toxicol. Environ. Mutagen.* **493**, 75–85.

Murugan, S., Karuppasamy, R., Poongodi, K. & Puvaneswari, S. (2008) Bioaccumulation Pattern of Zinc in Freshwater Fish Channa punctatus (Bloch.) After Chronic Exposure. *Turkish J. Fish. Aquat. Sci.***8**, 55–59.

Nussey, G., Van Vuren, J. H. J. & Du Preez, H. H. (2000) Bioaccumulation of Chromium, Manganese, Nickel and Lead in the Tissues of the Moggel, Labeo Umbratus (Cyprinidae), from Witbank Dam, Mpumalanga. *Water SA***26**, 269–284.

Ogino, C. & Yang, C. (1979) Requirement of Carp for Dietary Zinc. *Bull. Jap. Soc. Sci. Fish***45**, 967–969.

Powell, S. R. (2000) The Antioxidant Properties of Zinc. *J. Nutr.* **130**, 1447–1454.

Puvaneswari, N., Muthukrishnan, J. & Gunasekaran, P. (2006) Toxicity Assessment and Microbial Degradation of Azo Dyes. *Indian J. Exp. Biol.***44**, 618–626.

Rejomon, G., Nair, M. & Joseph, T. (2010) Trace Metal Dynamics in Fishes from the Southwest Coast of India. *Environ. Monit. Assess.***167**, 243–255.

Rimbu, C., Vrinceanu, N., Broasca, G., Farima, D., Ciocoiu, M., Campagne, C., Suchea, M. P. & Nistor, A. (2013) Zinc Oxide Application in the Textile Industry: Surface Tailoring and Water Barrier Attributes as Parameters with Direct Implication in Comfort Performance. *Text. Res. J.***83**, 2142–2151.

Rituparna, S. & Ghosh, A. R. (2012) Metanil Yellow - an Azo Dye Induced Histopathological and Ultrastructural Changes in Albino Rat (Rattus Norvegicus). *The Bioscan***7**, 427–432.

Sharma, K. P., Sharma, S., Sharma, S., Singh, P. K., Kumar, S., Grover, R. & Sharma, P. K. (2007) A Comparative Study on Characterization of Textile Wastewaters (Untreated and Treated) Toxicity by Chemical and Biological Tests. *Chemosphere***69**, 48–54.

Sharma, S., Sharma, S., Upreti, N. & Sharma, K. P. (2009) Monitoring Toxicity of an Azo Dye Methyl Red and a Heavy Metal Cu, Using Plant and Animal Bioassays. *Toxicol. Environ. Chem.***91**, 109–120.

Tonogai, Y., Ito, Y., Iwaida, M., Tati, M., Ose, Y. & Sato, T. (1979) Studies on the Toxicity of Coal-Tar Dyes II. Examination of the Biological Reaction of Coal-Tar Dyes to Vital Body. *J. Toxicol. Sci.***4**, 211–219.

Vinodhini, R. & Narayanan, M. (2009) The Impact of Toxic Heavy Metals on the Hematological Parameters in Common Carp (Cyprinus Carpio L .). *Iran. J. Environ. Health. Sci. Eng***6**, 23–28.

Witeska, M. & Kościuk, B. (2003) The Changes in Common Carp Blood after Short-Term Zinc Exposure. *Environ. Sci. Pollut. Res. Int.***10**, 284–286.

Woodling, J. D., Brinkman, S. F. & Horn, B. J. (2001) Nonuniform Accumulation of Cadmium and Copper in Kidneys of Wild Brown Trout (Salmo Trutta) Populations. *Arch. Environ. Contam. Toxicol.***40**, 381–385.

Zeiner, M. (2007) Analytical Methods for the Determination of Heavy Metals in the Textile Industry Analytical Methods for the Determination of Heavy Metals in the Textile Industry. *Kem. u Ind.***56**, 587–595.

Metric system:
Mg (milligram); L (liter); µg (microgram); g (gram)
Symbols:
% percent