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DETERMINING THE RELATIONSHIP BETWEEN METAL CONTAMINATION AND PEROXIDE ACTIVITY IN GRASS CARP FROM INDUS RIVER

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ABSTRACT

Heavy metals are important contaminants of aquatic system and can induce oxidative stress in fish. The aim of this research is to evaluate relationship between metals (Zn, Cu, Cr, Ni and Co) contamination and peroxidase activity in liver, gills, kidney heart and muscles in *Ctenopharyngodon idella*. Fish sample were collected from various sites of Indus River and brought to the laboratory for further study by preserving them in crushed dried ice. Water samples were also taken from the sampling sites to check physico-chemical parameters. Fish was dissected to get organs kidney, liver, gills, heart and muscles. For the enzyme assay organs were homogenized in phosphate buffer (PH 6.5 to 7). The extracted organs were digested on hot plate to determine the concentration of selected heavy metals by using atomic absorption spectrophotometer. Highly Significant difference was observed in peroxidase activity statistically at $p \leq 0.05$. The sequence of heavy metals found in riverine *C. idella* was Zn> Co> Cr> Ni> Cu, Zn> Ni> Cu< Cr> Co, Co> Zn> Cu> Ni> Cr, Zn> Cu> Co> Cr> Ni and Zn> Cu> Co> Cr> Ni for Liver> kidney> gills> heart> muscles, respectively. Metal concentration was observed lower in hatchery raised *C. idella.* This study could be potentially helpful in providing important information regarding how fish antioxidant systems are affected by the presence of heavy metals.

INTRODUCTION

Fish is an important group of vertebrates that benefit humans in a variety of ways. The nutritive and economical values of fish are attributed to its good and cheap source of protein, minerals, un-saturated fatty acids and Omega-3 that help in reduction of the blood cholesterol and prevent heart malfunction (arteriosclerosis). Freshwater fish Grass Carp (*Ctenopharyngodon idella)* belonging to family Cyprinidae is a popular fish throughout the world. Though it is relatively a resistant fish, natural populations in many rivers are diminishing due to saltwater intrusion (Jiang *et al.,* 2014).

Fresh water constitutes just 3% of total water on earth and yet this little amount is under enormous stress, rapid industrialization is one of the main causes for aquatic pollution (Mirza *et al*., 2012). Metals are discharged into aquatic habitat that can contaminate the water ecosystem and change the biodiversity because of their accumulative and toxic nature (Mansour *et al.,* 2002). Heavy metals such as Cu, Zn and Fe act as essential elements of enzymes and metabolism functions in fish whereas Cd, Pb and Hg are toxic and may adversely affect DNA and enzymatic processes. Chromium is identified for its low accumulation in fish bodies, but larger concentrations of Cr cause harm to the fish gills (Trevizani *et al.,* 2019).

An unavoidable component of aerobic life is oxidative stress. Oxidative stress has negative impact on physiological health, normal body functions, growth, reproduction and development of fish via a series of physiological, metabolic, and biochemical pathways as it produce ROS including superoxide radicals, hydrogen peroxide and hydroxyl radicals in body cells (Livingstone *et al.,* 2001). These reactive oxygen species can oxidize nucleic acids, lipids and proteins, causing cell destruction if not detoxified on time (Seebaugh *et al.* 2005).

Enzymes are biological compounds that sustain life by catalyzing millions of biochemical reactions in the bodies of living organism (Hansen *et al*., 2006). To neutralize heavy metal induced reactive oxygen species, organisms have developed non-enzymatic and enzymatic antioxidant defense mechanisms (Jiraungkoorskul *et al.,* 2003; Sanchez *et al.,* 2005). Antioxidant enzyme systems help to balance reactive oxygen species (ROS) and free radicals, keep safe the cell against free radical-induced damage.

Under normal physiological conditions, other pro oxidants and reactive oxygen species are continually reduced to less reactive species by the antioxidant defense system, which consists of antioxidant enzymes that protect the tissues and cells from the harmful effects of reactive oxygen species. If the production of reactive oxygen species overcomes the antioxidative capacity of cells, an inequity between the removal and generation of reactive oxygen species can induce oxidative stress, resulting in oxidative damage to multiple cellular targets (Pena-Liopis *et al*., 2001).

Peroxidase is a heme-containing enzyme that acts as a catalyst in many processes, primarily improving the oxidation of many substances. The peroxidase enzyme is comprised of many iso-forms which perform a variety of metabolic processes in organisms including cell adhesion, phagocytosis, antioxidants functions and immune cell function. Peroxidase is an oxido-reductases that catalyzed a wide range of processes, including the reduction hydrogen peroxide and the oxidation of inorganic and organic molecules (Kurutas *et al.,* 2009). It acts as the first line of defense against reactive oxygen species when oxidative stress develops. Peroxidase levels may vary as a result of heavy metals poisoning, during redox reactions, hydro-peroxides are reduced to hydroxyl molecules, whereas peroxides are converted to water. Peroxidase prevents red blood cells from damage by hydrogen peroxide (H_2O_2) as well as tissues from oxidative stress caused by lipid peroxidase. As a result, the amount of peroxidase might be used for determining heavy metal toxicity in fish (Farombi *et al*., 2007).

METHODOLOGY

3.1. Fish sampling and organs tissue Extraction

Fish samples of **Grass carp** (30 individuals) were randomly collected from five commercial fishing sites of Indus River. fish samples were also taken from fisheries research farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. Various fish organs (liver, gills, kidney, heart and muscle tissues) were taken out by dissecting fish samples on the sampling site.

Figure 1: Dissection of fish, *C. Idella*

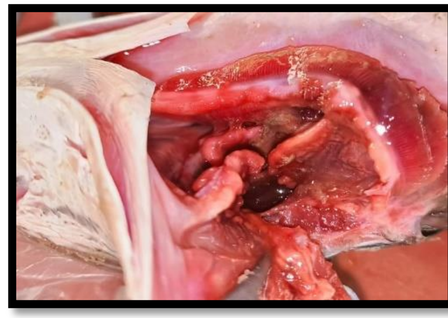

Figure 2: Extracted heart from *C. idella*

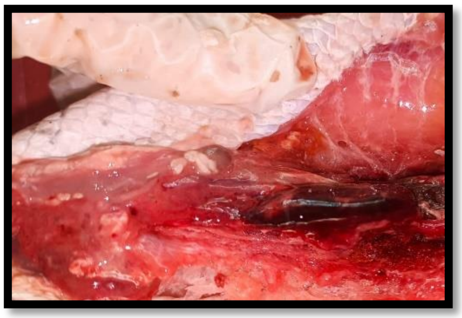

Figure 3: Extracted kidney from *C. idella*

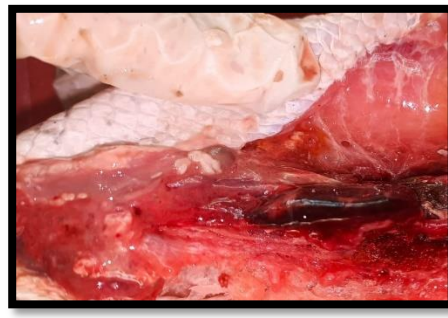

Figure 4: Extracted Gills from *C. idella*

Water Sampling

To analyze the physico-chemistry of Indus River, water samples were also taken from selected sites of the River Indus and brought to the Aquaculture Biotechnology Laboratory.

Determination of physico-chemical parameters

Processed for studying the Physiochemical parameters by following the procedure described by A.P.H.A (2012).

Homogenization of extracted organs from *Grass carp*

Liver, kidney, gills, heart and muscle tissues were processed to check the activity of Peroxidase. Selected fish organs (Liver, heart, gills, kidney and muscle) were weighed by using analytical weight balance. Phosphate buffer (pH=7) was added four times more than weight of organ i.e. 1:4. At 4 ºC, organs were homogenized by using pestle and mortar for 15minutes. Muslin cloth was used to filter the biomass from homogenized tissues. Muslin cloth was used to filter the biomass from homogenized tissue. Whatman's filter paper number 1 was used to filter the liquid obtained from muslin cloth. Filtrate obtained from Whitman's filter paper, centrifuged for 15 minutes at 10,000 rpm. Supernatants and sediments were preserved at 4 C till next procedure.

Figure 5: Homogenization of organs by using pestle and mortar

Figure 6: Muslin cloth to remove biomass

Figure 7: Filtration by using Whatman's filter paper number 1

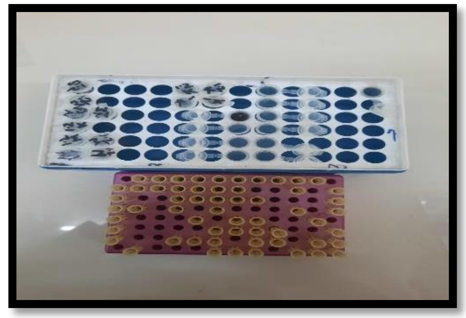

Figure 8: Separation of supernatant and sediments

Analysis for peroxidase activity

The peroxidase activity was checked by its capability to lower the concentration of H_2O_2 at 470nm adapting the methodology given by Chance and Mchaly (1977).

Detection of heavy metals by digestion method

1 g of samples (riverine) was weighted for the detection of metals. Kept the samples in conical flask. Concentrated Nitric acid (30 mL) was added to it, then flask was set on the hot plate. Perchloric acid (10 mL) was added to it when mixture started to boil. Kept the mixture on the hot plate, heated it until the colorless mixture of I mL was obtained. Diluted the removed flask by adding 100 ml. of distilled water to obtained crystal clear solution. Finally, mixture was filtered by using filter paper to remove all matter in the digestion solution prior to analysis of minerals (AOAC, 1990). At the end, metals were detected by using atomic absorption spectrophotometer after proper dilution.

Determination of heavy metals

Selected metals (Zn, Co, Cr, Cu and Ni) in processed samples were quantified by Hitachi Polarized Zeeman AAS, Z-8200, Japan following the methodology described in AOAC (1990).

Statistical analysis

The obtained data was analyzed by applying one way ANOVA to check the antioxidants enzyme (Peroxidase) activity as influenced by metal accumulation in Grass carp.

RESULTS

Comparison of peroxidase activity of enzyme in different tissue of farmed and riverine *C. idella*

The inference of this research indicated the showed activity of peroxidase in the liver of *C. idella* (185.33 ± 3.00), kidney (116.67 ± 3.00), gills (91.333 ± 2.00), heart (65.000 ± 1.00), (67.333±1.00), muscles collected from Guddu Barrage compared to other selected studied sites (Fig. 1). Highly significant difference ($p<0.05$) was examined for peroxidase activity in liver of fish samples. The value of peroxidase activity in *C. idella* samples obtained from various locations was found to vary in the following order, Guddu Barrage > Chashma Barrage > Taunsa Barrage > Jinnah Barrage > Ghazi Ghat > Hatchery.

Concentration of selected metal in liver of *C. idella* collected from different sites of River Indus and hatchery

In the present study Zn, Ni, Cr, Cu and Co were selected to measure the concentration in water samples from various sites at River Indus. The results revealed that concentration of Zinc from Ghazi Ghat reached higher as compared to other metals Ni, Co and Cu in liver, samples taken from Guddu Barrage, Chashma Barrage, Taunsa Barrage, Jinnah Barrage and hatchery.

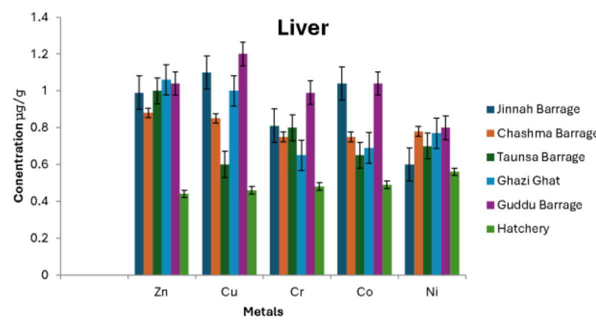

Figure 9: Graphical representation of metal concentration in liver of *C. idella*

Concentration of selected metal in kidney collected from different sites of River Indus and hatchery

In the present research Zn, Ni, Cr, Ni, Cu and Co were selected to measure the concentration in water samples from various sites at River Indus. The results revealed that concentration of Zinc from Jinnah Barrage reached higher as compared to other metals, Ni, Cr, Co and Cu in kidney samples collected from Guddu Barrage, Chashma Barrage, Taunsa Barrage, Jinnah Barrage, Ghazi Ghat and hatchery.

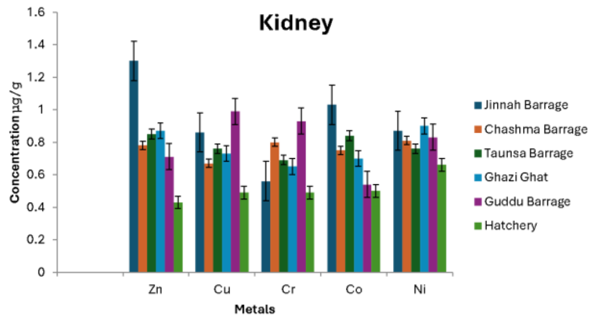

Figure 10: Graphical representation of metal concentration in kidney of *C. Idella* Concentration of selected metal in gills collected from different sites of River Indus and hatchery

In the present study Zn, Cu, Ni, Co and Cr were selected to measure the concentration in water samples from various sites at River Indus. The results revealed that concentration of Zinc from Guddu Barrage reached higher as compared to other metals Ni, Cu and Co in gills samples taken from Guddu Barrage, Chashma Barrage, Taunsa Barrage, Jinnah Barrage, Ghazi Ghat and hatchery.

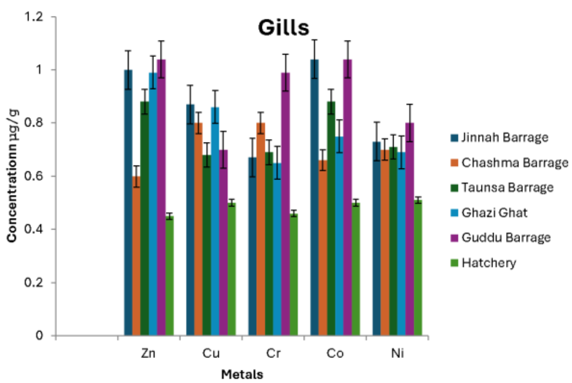

Figure 11: Graphical representation of metal concentration in gills of *C. Idella* Concentration of selected metal in heart collected from different sites of River Indus and hatchery

In the present study Zn, Ni, Cu, Cr and Co were selected to measure the concentration in water samples from various sites at River Indus. The results revealed that concentration of Zinc from Jinnah Barrage reached higher as compared to other metals, Ni, Cu and Co in heart samples taken from Guddu Barrage, Chashma Barrage, Taunsa Barrage, Jinnah Barrage, Ghazi Ghat and hatchery.

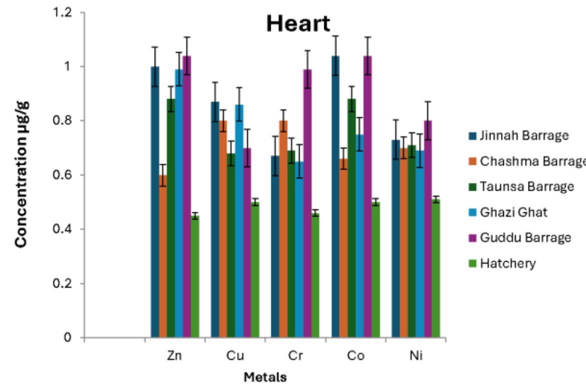

Figure 12: Graphical representation of metal concentration in heart of *C. Idella* Concentration of selected metal in muscles collected from different sites of River Indus and hatchery

In the present study Cr, Zn, Ni, Cu and Co were selected to measure the concentration in water samples from various sites at River Indus. The results revealed that concentration of Zn from Guddu Barrage reached maximum as compared to other metals Co Cr, Ni and Cu in muscles samples collected from Guddu Barrage, Chashma Barrage, Taunsa Barrage, Jinnah Barrage, Ghazi Ghat and hatchery.

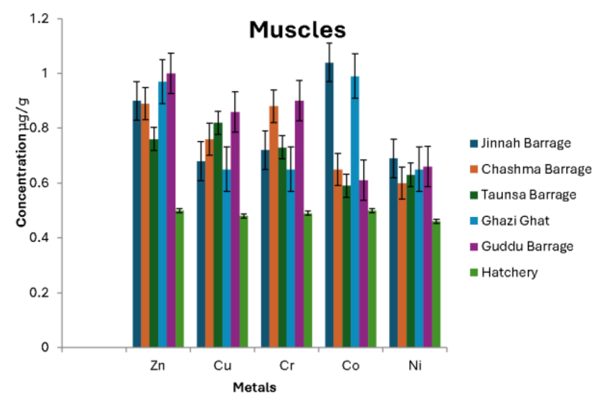

Figure 13: Graphical representation of metal concentration in muscles of *C. Idella* Detection of selected metals in water samples of River Indus and hatchery

In the present study the concentration of Zn, Ni, Cr, Cu and Co were measured in water samples from various sites at River Indus. The results revealed that concentration of Zinc was higher as compared to other metals.

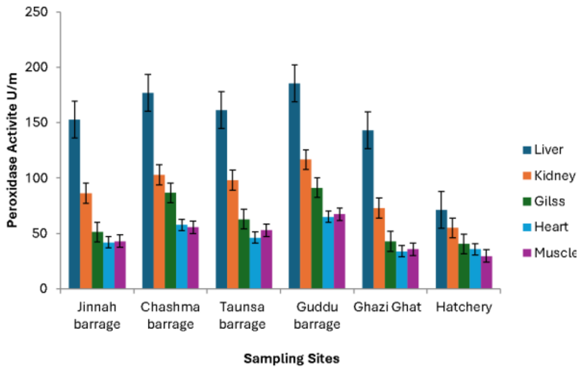

Figure 14: Comparative graphical representation of peroxidase activity in different tissues of *C. idella*

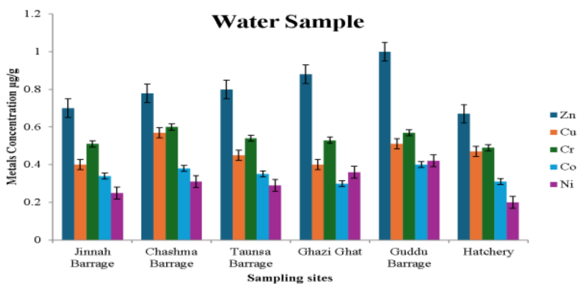

Figure 15: Graphical representation of metal concentration in water samples from different sites of River Indus and fish hatchery

DISCUSSION

The present study entitled **"Determining the relationship between metal contamination and peroxidase activity in grass carp from Indus River"** was conducted in Aquaculture Biotechnology lab. The details of results are as follows;

Fish can be used as bio-monitor to assess the metal pollution level in aquatic system. Several systems in fish keep antioxidant substances in balance that regulate oxidative response. Highly reactive metabolites produced during the metabolism of hydrocarbons are scavenged by the antioxidant enzymes (Kurutas *et al.,* 2009). Higher peroxidase activity in various organs was measured in *C. idella* captured from Guddu barrage as compared to other selected sites. The activity of enzymes is different in all sites because presence of varying level of aquatic pollution. Guddu Barrage is most affected site in this study and indicated elevated activity of ROS system. These results are comparable with Javed *et al.* (2020) that peroxidase activity increased in numerous body tissues due to metal contaminants in freshwater fish *C. idella* sampled from Indus River Pakistan.

The content of Zn, Cr, Cu, Ni and Co metals were examined in kidney, liver, Heart, muscles and gills through Atomic Absorption Spectrophotometer. The mean value of Zinc was recorded higher in liver, kidney gills, heart and muscles of *C. idella.* With context to sampling locations, Zn concentration was highest in *C. idella* collected from Chashma Barrage than other studied sites. Bioaccumulation of selected heavy metals in riverine *C. idella* was in following order: Zn> Co> Cr> Ni> Cu, Zn> Ni>Cu< Cr> Co, Co> Zn> Cu> Ni> Cr, Zn> Cu> Co> Cr> Ni and Zn> Cu> Co> Cr> Ni in Liver> kidney> gills> heart> muscles respectively, samples taken from different sites of River Indus. Similar findings were reported by Ali *et al*. (2019) who determined the copper and Zinc level in both water samples and *Channa gachu* from River Swat. They find out higher concentration of Zn in fish liver than copper. Vinodhini and Narayanan (2009), who investigated the varied metal buildup in several organs of *C. carpio*. Similar findings were reported by Eneji *et al*. (2011) they evaluated the concentration of Metals (zinc, copper, lead) in tissues of *Tilapia zilli* and *Clarias gariepinsus*. Similarly, elevated level of Zn was observed by Gupta *et al.* (2009), who found significant Zn concentrations in water samples taken from the River Ganges at Allahabad. Present study can also be relatable with Asante *et al.* (2014) who investigate the bioaccumulation of the heavy metals Mn, Cd, and Hg in the water, sediments, and tissues of a few particular fish from the Red Volta, Nangodi in the upper east of Ghana.

In the present study Peroxidase activity order was in following order liver>kidney>gills>heart>muscles at different site of River Indus and hatchery raised *C. idella.* Bano *et al.* (2017) determined the antioxidant enzyme and heavy metals assessment in various organs of fish from Pakistan's hatchery, farm and Indus River.

In this study, significant variations were seen in physico-chemical parameters of water collected from different sites of River Indus. The findings of present work are similar to those of Narayanan *et al.* (2008) who observed the variation in physico-chemical parameters of water sample due to high level of pollution in River Indus.

CONCLUSION

The present research findings concluded that determining the peroxidase activity in various fish organs is useful for determining the level of pollution in an aquatic environment. The present study's inferences further stated that the water pollution at the Indus River's Jinnah, Taunsa, and Ghazi Ghats met acceptable requirements for human consumption and was good for fish farming. However, it was advised that frequent monitoring be carried out due to the presence of metals Zn, Cr, Ni, Cr, and Co in different organs of *C. idella* from the riverine system in order to stop the accumulation of various heavy metals.

REFERENCES

Gupta, A., Rai, D. K., Pandey, R. S., & Sharma, B. (2009). Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. *Environmental monitoring and assessment*, *157*, 449-458.

Asante, F., Agbeko, E., Addae, G., & Quainoo, A. K. (2014). Bioaccumulation of heavy metals in water, sediments and tissues of some selected fishes from the Red Volta, Nangodi in the Upper East Region of Ghana. 4(4):

Vinodhini, R., and M. Narayanan. "Heavy metal induced histopathological alterations in selected organs of the Cyprinus carpio L.(Common Carp)." (2009): 95-100.

Jiang, G., J. Keller and P.L. Bond. 2014. Determining the long-term effects of H_2S concentration, relative humidity and air temperature on concrete sewer corrosion. Water Res. 65:157169.

Mirza, Z.S., M.S. Nadeem, M.A. Beg and M., Qayyum. 2012. Population status and biological characteristics of Common Carp, *Cyprinus carpio*, in Mangla Reservoir (Pakistan). J. Anim. Plant Sci. 22:933-938.

Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. Mar. Pollut. Bull. 42:656-666.

Mansour, S.A. and M.M. Sidky. 2002. Ecotoxicological studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate. Egypt. J. Chem. 78:15-22.

Trevizani, T. H., C. Domit, M.C. Vedolin, J.L.F. Angeli and R.C.L. Figueira. 2019. Assessment of metal contamination in fish from estuaries of southern and southeastern Brazil. Environ. mont. Assess. 191:1-16.

Seebaugh, D.R., D. Goto and W.G. Wallace. 2005. Bioenhancement of cadmium transfer along a multi-level food chain. Mar. Environ. Res. 59:473-491 Jiraungkoorskul, W., E.S. Upatham, M. Kruatrachue, S. Sahaphong, S. Vichasri-Grams and P. Pokethitiyook. 2003. Biochemical and histopathological effects of glyphosate herbicide on Nile Tilapia (*Oreochromis niloticus*). Environ. Toxicol. An. Int. J. 18:260-267.

Sanchez, W., O. Palluel, L. Meunier, M. Coquery, J.M. Porcher and S. Ait-Aissa. 2005. Copperinduced oxidative stress in three-spined Stickleback: Relationship with hepatic metal levels. Environ. Toxicol. Pharmacol. 19:177-183.

Asante, F., E. Agbeko, G. Addae and A.K. Quainoo. 2013. Bioaccumulation of heavy metals in water, sediments and tissues of some selected fishes from the Red Volta, Nangodi in the Upper East Region of Ghana. Br. J. Appl. Sci. Technol. 4:594-603.

Bano, Z., S. Abdullah, W. Ahmad, M.A. Zia and W. Hassan. 2017. Assessment of heavy metals and antioxidant enzyme in different organs of fish from farm, hatchery and Indus river of Pakistan. Paki. J. Zool. 49:2227-2233.

Eneji, I.S., R. Sha'Ato and P.A. Annune. 2011. Bioaccumulation of Heavy Metals in Fish (*Tilapia zilli* and *Clarias gariepinus*) organs from River Benue, North-Central Nigeria. Pak. J. Anal. Environ. Chem. 12:25-31

Pena-Llopis, S., Pena, J.B., Sancho, E., Fernandez-Vega, C. and M.D., Ferrando, 2001. Glutathione-dependent resistance of the European eel (*Anguilla anguilla*) to the herbicide molinate. Chemosphere. 45:671-681.

Kurutas, E.B., A. Sahan and T. Altun. 2009. Oxidative stress biomarkers in liver and gill tissues of Spotted Barb (*Capoeta barroisi Lortet,* 1894) living in Ceyhan River, Adana-Turkey. Turk. J. Biol. 33:275-282.

Farombi, E.O., O.A. Adelowo and Y.R. Ajimoko. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (*Clarias gariepinus*) from Nigeria Ogun River. Int. J. Environ. Res. Public Health. 4:158-165.

AOAC. 1990. Official Methods of Analysis. 15th Ed. Association of Official Analytical Chemist, Washington, USA.

APHA. 1998. Standard Methods for the Examination of Water and Wastewater. 20^{th} Ed. Water Environmental Federation, Washington, USA.

Javed, M., K. Abbas, T. Ahmed, S. Abdullah, H. Naz and H. Amjad. 2020. Metal pollutants induced peroxidase activity in different body tissues of freshwater fish, *Labeo rohita*. Environ. Chem. Ecotoxicol. 2:162-167.

Ali, H., W. Ali, K. Ullah, F. Akbar and H. Khan. 2019. Assessment of Cu and Zn in water, sediments and in the carnivorous fish, *Channa gachua* from River Swat and River Barandu, Malakand Division, Pakistan. Iran. J. Sci. Technol. 43:773-78.