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Effects of heavy metal Cd on essential metal Zn levels in freshwater fish Channa gachua

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Abstract: Heavy metal pollution is a key threat to aquatic ecosystems and associated biodiversity. Current study highlights the heavy metal pollution (Cd) and its impacts on Zn level in snake head fish channa gachua. Total of 42 individuals were collected from river Barandu KP, Pakistan. Out of which n=36 individuals of fish were arranged into one control group and five experimental groups I, II, III, IV and V (each having n =6 individuals) were treated with different concentration of Cd solution 0.1, 0.5, 1.0, 2.5 and 5.0 ppm respectively for 90 days. After treatment, sample were analyzed for Cd bioaccumulation and Zn level in liver, gills and muscles tissues of fish. Highest Cd bioaccumulation and lowest Zn level were recorded in group V (at 5.0 ppm treatment); mean value of Cd concentration in liver 107.73±45.14 mg kg^{-1}, gills 36.40 ±0.435 mg kg^{-1} and in muscles 36.20 ±0.458 mg kg^{-1} and Zn level of 14.56 ± 15.07 mg kg^{-1}, 16.76 ± 1.35 mg kg^{-1}, and 4.60 ± 0.72 mg kg^{-1} in liver, gills and muscles respectively. While highest Zn level were recorded in Control group with mean value; liver 51.56 ± 23.04 mg kg^{-1}, gills 26.20 ± 2.81 mg kg^{-1} and muscles 15.10 ± 1.17 mg kg^{-1}. The Zn level were found declined with the bioaccumulation of Cd in different tissues of fish. The declined order of Zn level in liver were 51.56 mg kg^{-1} > 24.43 mg kg^{-1} > 23.03 mg kg^{-1} > 21.06 mg kg^{-1} > 16.80 mg kg^{-1} > 14.46 mg kg^{-1} in control group, I, II, III, IV and V respectively. The same order were found in gills and muscles tissues also. Current report indicated that Cd has considerable effect on trace element Zn level and would be valuable contribution on this regard.

Keywords ***Heavy metal; Freshwater fish; Cd bioaccumulation; Zn level; Pakistan***

1. INTRODUCTION

The agents causing pollution are pollutants, which are released into water ecosystems by various humans activities but the main causes are industrial waste discharge into rivers (Azizullah *et al*., 2011). These pollutants badly affect the aquatic life as *Tor putitora* endangered fish species declined rapidly due to pollution in river Kabul, Pakistan (Yousafzai & Shakoori, 2011). Among these toxic heavy metals are pollutants of serious environmental issue (Ali *et al*. 2019). Fish living in water contaminated with heavy metal bioaccumulate higher concentration as fish have high tropic position in food web (Siraj *et al.* 2014). Any natural metal whom atomic and mass number are (Z) >20 and (A) >5 g cm^{-3} respectively are known heavy metals. (Ali and Khan, 2018). After agricultural and industrial revolution heavy metal pollution are major environmental concern as they are very persistent and not degradable in nature, toxic and bioaccumulate in organisms through food chain (Hayat and Javed, 2008; Hashem *et al*. 2017). Among which copper, cadmium, mercury and lead are of great concern and even very low concentrations of these metals accumulate in sediments and living systems to attain significant concentration to cause heavy metal pollution (Namminga and Wilham, 1976; (Linnik & Zubenko, 2000). Further these metals can pollute underground resources of water as they leak underground from one point to another (Hayes *et al*.1995).

Heavy metals interact and disturb normal metabolic pathways, e.g in stomach metals gets their stable oxidation states (Cd++) then interact with proteins to alter their actions (M. O. Ogwuegbu & Ijioma, 2003). Cadmium is most threaten metal and when their concentration in living tissue is greater than threshold level causes cancer, mutation and also found to be teratogenic in many species (Singh *et al*. 2009; Degraeve, 1981). The general signs of poisoning effects of metals including "Cd, Pb, As, Hg, Zn, Cu and Al are gastrointestinal (GI) Disorders, hemoglobinuria, diarrhea, tremor, paralysis, stomatitis, ataxia, vomiting and convulsion, when volatile vapor and fumes are inhaled cause depression and pneumonia" (Mc Cluggage 1991). Furthermore it also impaired fertility, ailment of many organs and intoxication (Oliveira Ribeiro *et al*., 2000). Cadmium also mimic Zn and replace it and cause toxicity in living tissue (Bridges and Zalpus, 2005).

Different studies have been reported on Cd toxicity and have been placed in black list international Conventions of pollution due to their toxic nature in organisms (Taylor, 1983). Especially cadmium has great toxic effect on liver and kidneys in vertebrates. (Tjalve *et al*. 1986).Which has various poisonous effect on biochemistry and physiology of living beings and disturb them (WHO 1992b). Cd have been reported by researchers to cause reduction of thyroid hormones, inhibition of estrogen receptors, and interruption with growth hormones (Gue vel *et al*. 2000; Hontela *et al*. 1996; Le; jones *et al*. 2005). Calcium absorption are hindered by Cadmium which further impair behaviour and development, hypocalcaemia and growth retardedness, as well disrupt in reproduction (Dang and Wang 2009, McGeer *et al*. 2011). Many reports have been published on heavy metal toxicity and are available in literature (Callender, 2005; Salomon *et al.* 2012; Alloway, 2013; Carolin *et al.* 2017; Ali *et al.* 2018; Rehman *et al.* 2018; Bolisetty *et al.* 2019; Martin and Griswold, 2019; Rai *et al.* 2019; Fu and Xi, 2020; Jiang *et al*. 2020; Leong and Chang, 2020; Li *et al*. 2020; Sarkar, 2020). But few studies have been reported from KP, Pakistan on this regard. Therefore this is current need to assess and highlight the toxicity of heavy metals and their impacts on aquatic as well terrestrial life from this area which may be valuable contribution to literature of heavy metal pollution.

2. MATERIAL AND METHODS

2.1. Sampling location

Fish were sampled from river Barandu which originate from many springs and streams in different area of district Buner Khyber Pakhtunkhwa, Pakistan. River Barandu flow in tehsil Dagger and then enter to the tehsil Dagra. The river Barandu falls into river Indus at Kala Dhaka. (Khan *et al*., 2012).

2.2. Sampling method

The only one species of fish snakehead (*Channa Gachua*) were selected for the study and were collected (n=42) by using net. The collected fish were immediately stored in mobile aquarium and transported to the Laboratory. Identification of the fish for the scientific study was done through various taxonomic or systematic keys. For this purpose the keys used are. Pakistan Ki Machlian awr Mahipawari (Mirza and Bhatti, 1993).

2.3. Designing of experiment

After weighing and body length measurement, 36 samples of fish were grouped into total six (6) aquariums; control group and five were the experimental groups (I, II, III, IV and V). Each aquarium were filled of 40 L of water and air pumps were fixed for fresh air supply, before treatment fish were kept up to two weeks for acclimatization in laboratory.

2.3. Chemical Treatment

After 2 week of acclimatization, Cadmium Solution of different concentrations 0.1 ppm, 0.5 ppm, 1.0ppm, 2.5 ppm, and 5.0 ppm, were added in the aquaria I, II, III, IV and V respectively twice in week for 90 days to treat the fish with Cd metal. Water in aquarium was renewed twice a week to keep oxygen and other parameters at balance.

2.4. Sample preparation

Muscles, gills and liver tissues were separated and digested (on wet weight basis) in concentrated HNO_3 (75%) and $HClO_4$ (25%). The mixture were heated up to 80 ^oC on a hot plate for 30 minutes till the solution cleared and fumes stopped, after that the sample were left for cooling for few minutes and then were filtered using Whatmann filter paper and distilled water were added for dilution to 50 ml. Finally samples were collected into bottles with suitable labels and transported for analysis.

2.5 Chemical analysis

For chemical analysis of Cd and essential metal Zn Atomic Absorption Spectrometry was used as AAS having highly specific availability and selectivity therefore it is preferred commonly for metal detection (Garica and Baez, 2012). For this purpose samples were sent to Department of Physics, University of Peshawar, Pakistan.

2.6 Statistical analysis

SPSS18 (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc) and Origin Pro 2016 (OriginLab, Northampton, USA) were used for Anova, Pearson correlation and graphs respectively. Furthermore 95% Confidence level were used for analysis.

3. RESULTS AND DISCUSSION

3.1. Cd Bioaccumulation in muscle, gills and liver

The highest average concentration of Cd bioaccumulation were recorded in liver 107.73±45.14 mg kg^{-1} , gills 36.40 ±0.435 mg kg^{-1} and muscles tissues 36.20 ±0.458 mg kg^{-1} in group V (at 5.0 ppm Cd treatment solution). The bioaccumulation concentration order in liver was 107.73 mg kg^{-1} > 107.23 mg kg^{-1} >53.43 mg kg^{-1} > 29.50 mg kg^{-1} > 5.33 mg kg^{-1} > 0.87 mg kg^{-1} in groups V (5.0 ppm) , IV (2.5 ppm), III (1.0 ppm) , II (0.5 ppm), I (0.1 ppm) and control group (0.0 ppm) respectively. The same order were recorded in gills tissues as 36.40 mg kg^{-1} > 36.16 mg kg^{-1} > 8.30 mg kg^{-1} > 2.30 mg kg^{-1} > 1.30 mg kg^{-1} > 0.43 mg kg^{-1} in groups V (5.0 ppm) , IV (2.5 ppm), III (1.0 ppm) , II (0.5 ppm), I (0.1 ppm) and control group (0.0 ppm) respectively. In muscles tissue the order of Cd bioaccumulation were 36.20 mg kg^{-1} > 35.93 mg kg^{-1} > 2.33 mg kg^{-1} >2.05 mg kg^{-1} > 0.46 mg kg^{-1} > 0.16 mg kg^{-1} in groups in groups V (5.0 ppm) , IV (2.5 ppm), III (1.0 ppm) , II (0.5 ppm), I (0.1 ppm) and control group (0.0 ppm) respectively. The Cd bioaccumulation were highest in concentrated treatment solution (group V) and lowest in control group in all types of tissues liver gills and muscles (Figure 1).

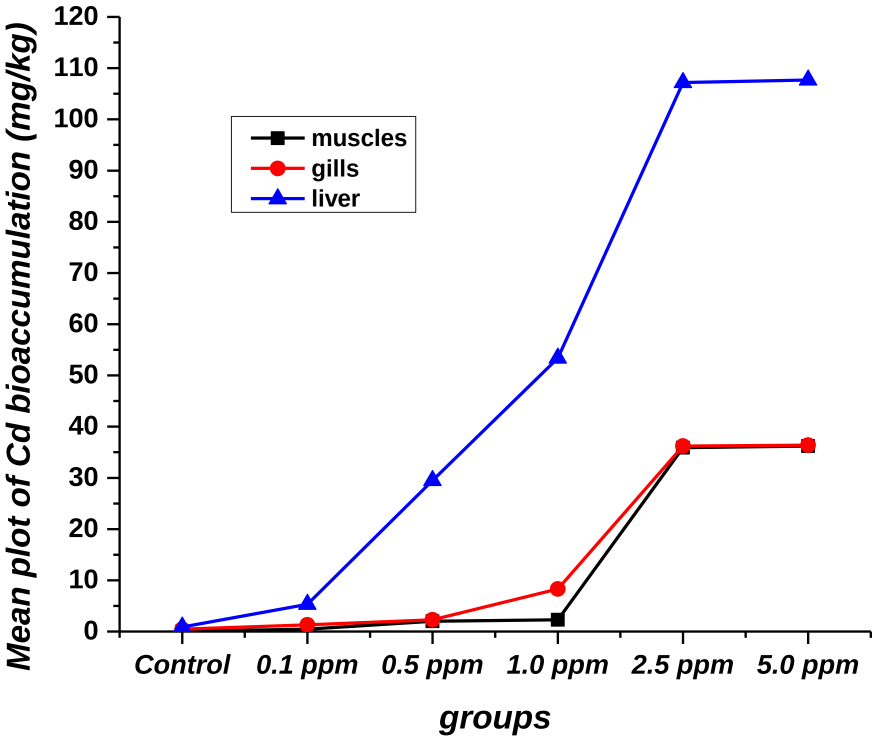

Figure 1 mean plots of Cd bioaccumulation in fish tissues after treatment

The result of two way Anova showed highly significant difference in bioaccumulation of Cd concentrations among all groups of treatment solutions *p < 0.05 (p=0.000)* as well highly significant difference in Cd bioaccumulation concentration among liver , gills and muscles tissues of fish *p < 0.05 (p=0.000).* Furthermore the Pearson correlation proved that there were moderate positive correlation between the concentration of Cd treatment solution and the Cd bioaccumulation in fish tissues. Pearson correlation coefficient were r =0.68 and were highly significant at 99% confidence level *p < 0.01 (p =0.002).* Which mean Cd concentration in solution has direct influence on Cd bioaccumulation in fish tissues. This study was under laboratory condition which reflect the Cd bioaccumulation and their effect on essential metal Zn in fishes within polluted rivers. Different studies across the globe have been reported heavy metal pollution in fish with different concentration from wild being caught directly from river which reflect the heavy metal pollution in corresponding rivers , the contribution from Pakistan e.g. Siraj *et al*. (2016) reported Cd concentration in *Wallago attu* 509.7 ± 386.1 (μg g−1 wet weight) from river Kabul Pakistan. While Cd concentrations of 66.7 ± 8.5 μg g−1 wet weight in *Labeo dyocheilus* , 53.3 ± 2.9 μg g−1 wet weight in *Cyprinus carpio* , 2.0 ± 0.37 μg g−1 wet weight in *Cirrhinus mrigala* , 71.7 ± 12. μg g−1 wet weight In *Ompok bimaculatus* ,by Ahmad *et al*. (2015) , Yousafzai *et al*. (2012) , Ali and Khan (2018a) , Siraj *et al*. (2014) respectively in different species of fishes from same river Kabul Pakistan. Tariq *et al*. (1993) reported 1.0 and 0.08 μg/g (wet weight) in *Notopterus* *chitala* and *Mastacem armatus* from Tarbela Reservoir Pakistan. While Malik and Maurya (2014) reported Cd concentration in fishes 20.8 µg /g wet weight in *Puntius ticto* and 42.4 µg/ g wet weight in *Heteropneustes fossilis* in kali river India. Tariq *et al*. (1993) reported 1.0 and 0.08 µg/g (wet weight) in *Notopterus chitala and Mastacem armatus* from Tarbela Reservoir Pakistan. While Malik and Maurya (2014) reported Cd concentration in fishes 20.8 µg g−1 wet weight in *Puntius ticto* and 42.4 µg g−1 wet weight in *Heteropneustes fossilis* in kali river India.The possible reason of higher concentration of Cd in fishes in present study is due to being treated in higher concentration of Cd solution as compared to wild river water conditions.

3.2. Concentration of Zn in liver, gills and muscle

After treatment with Cd solution the level of Zn in different tissue were evaluated and the highest and lowest mean concentration of Zn were recorded in liver 51.56 ± 23.04 mg kg^{-1} and 14.56 ± 15.07 mg kg^{-1} in Control group (0 ppm) and group V (5.0 ppm) respectively. Followed by gills having highest Zn level 26.20 ± 2.81 mg kg^{-1} in Control group while lowest level of Zn in gills were recorded 16.76 ± 1.35 mg kg^{-1} in group V (5.0 ppm). The same pattern were followed by muscles tissue in which the highest level of Zn were 15.10 ± 1.17 and lowest level were 4.60 ± 0.72 in control and group V (5.0 ppm). The order of Zn level in liver was 51.56 mg kg^{-1} > 24.43 mg kg^{-1} > 23.03 mg kg^{-1} > 21.06 mg kg^{-1} > 16.80 mg kg^{-1} > 14.46 mg kg^{-1} in control group (0.0 ppm), I (0.1 ppm), II (0.5 ppm), III (1.0 ppm) , IV (2.5 ppm) and V (5.0 ppm) respectively. Same pattern were recorded in gills 26.20 ± 2.81 mg kg^{-1} >22.0 ± 4.67 mg kg^{-1} > 19.80 ± 2.85 mg kg^{-1} > 19.70 ± 2.76 mg kg^{-1} > 19.36 ± 2.23 mg kg^{-1} > 16.76 ± 1.35mg kg^{-1} in control group (0.0 ppm), I (0.1 ppm), II (0.5 ppm), III (1.0 ppm) , IV (2.5 ppm) and V (5.0 ppm) respectively. While in muscle the Zn level were 15.10 ± 1.17 mg kg^{-1} > 8.33 ± 1.41 mg kg^{-1} > 5.80 ± 0.43 mg kg^{-1} 5.13 ± 0.57 mg kg^{-1} > 4.80 ± 0.72 mg kg^{-1} > 4.60 ± 0.72 mg kg^{-1} in control group (0.0 ppm), I (0.1 ppm), II (0.5 ppm), III (1.0 ppm) , IV (2.5 ppm) and V (5.0 ppm) respectively (Figure 2).

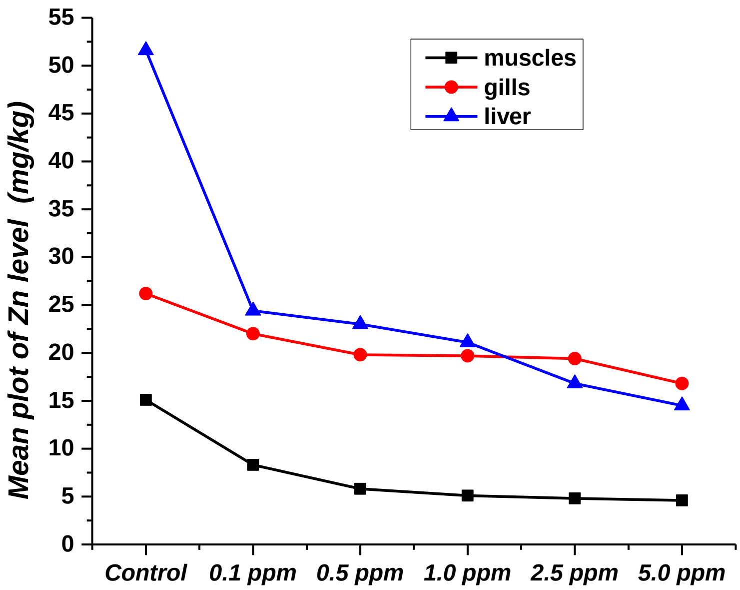

Figure 2 mean concentrations of Zn in different tissues of fish after treatment

The result of two way Anova showed that there is highly significant difference in Zn level among all groups of treatment solutions *$p < 0.05$ ($p=0.000$)* as well highly significant difference among liver , gills and muscles tissues of fish *$p < 0.05$ ($p=0.000$).* Pearson correlation showed that there is significant negative effect of Cd bioaccumulation on Zn level in fish liver, gills and muscle with correlation coefficient *$r = -0.45$, - 0.54 and - 0.10* respectively and were statistically significant $p < 0.05$ .The correlation between Cd bioaccumulation and Zn level in liver and gills were negative moderate while negative weak correlation were exist between Cd bioaccumulation and Zn level in muscle. In simple language which mean that the bioaccumulation of Cd in tissue of fish decrease the Zn level according to tissue type as our statistical analysis shows that the effect were high in liver than gills and muscle. The statistical correlation in present study indicated when the concentrations of Cd in fish organs increased the level of Zn decreased accordingly. Results of the current study do match with few studies reported by Brzo´ ska *et al*., (2000, 2001) and Ogoshi *et al*., (1992) according to their published report Zn level (concentration) found to be lowered due to Cd bioaccumulation in bones. Similar findings also have been reported by (Mahaffey *et al*., 1981; Chmielnicka *et al*., 1985) where the Cd interaction with Zn resulted into declined of Zn concentration in organisms. Further the bioaccumulation of Cd in biota suppress the uptake of Zn (Coppen-Jaeger and Wilhelm, 1989). This phenomenon of Zn suppression by Cd, would be big threat as many important enzymes need Zn for their proper functioning and in absence of Zn these enzymes will not work properly for instance an enzyme involved in synthesis of matrix of bone are dependent of Zn (Okano, 1996; Tanaka *et al*., 1995). (Figure 3, 4 and 5)

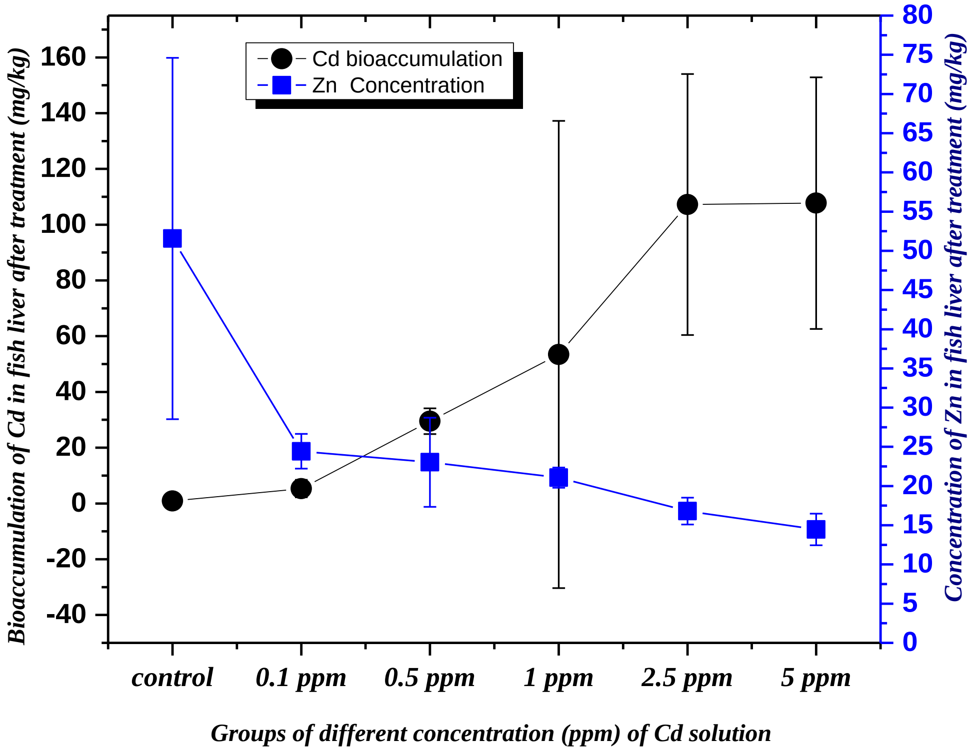

Figure 3 Comparison of Cd and Zn level in fish liver of different groups

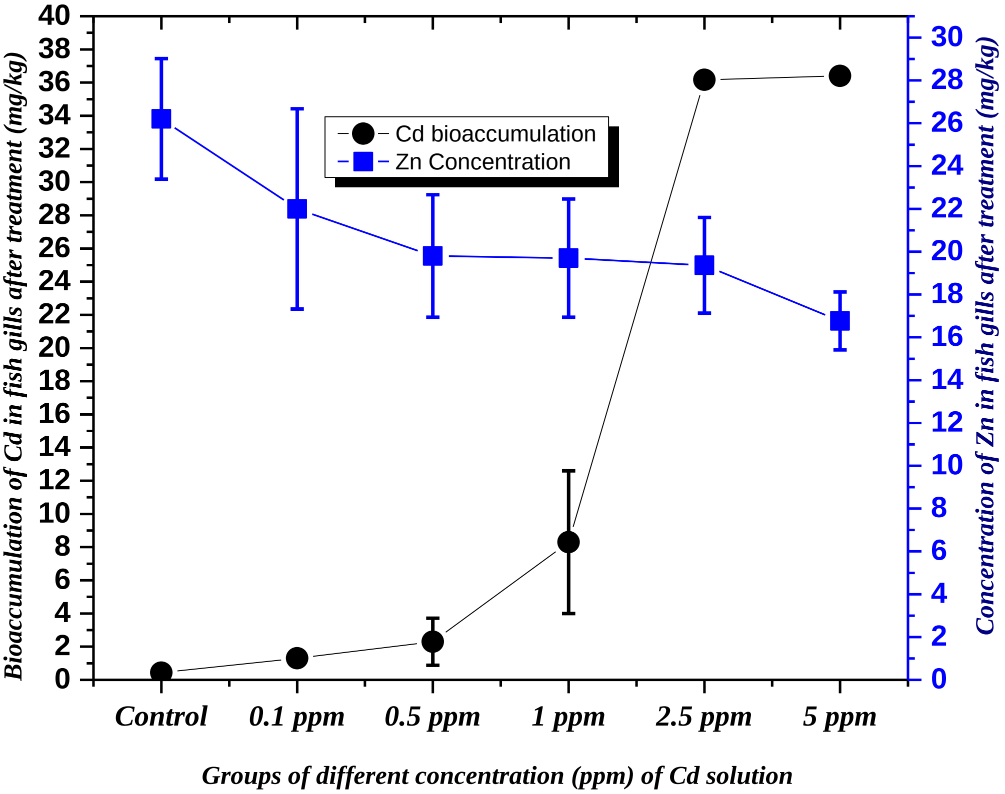

Figure 4 Comparison of Cd and Zn level in fish gills of different groups

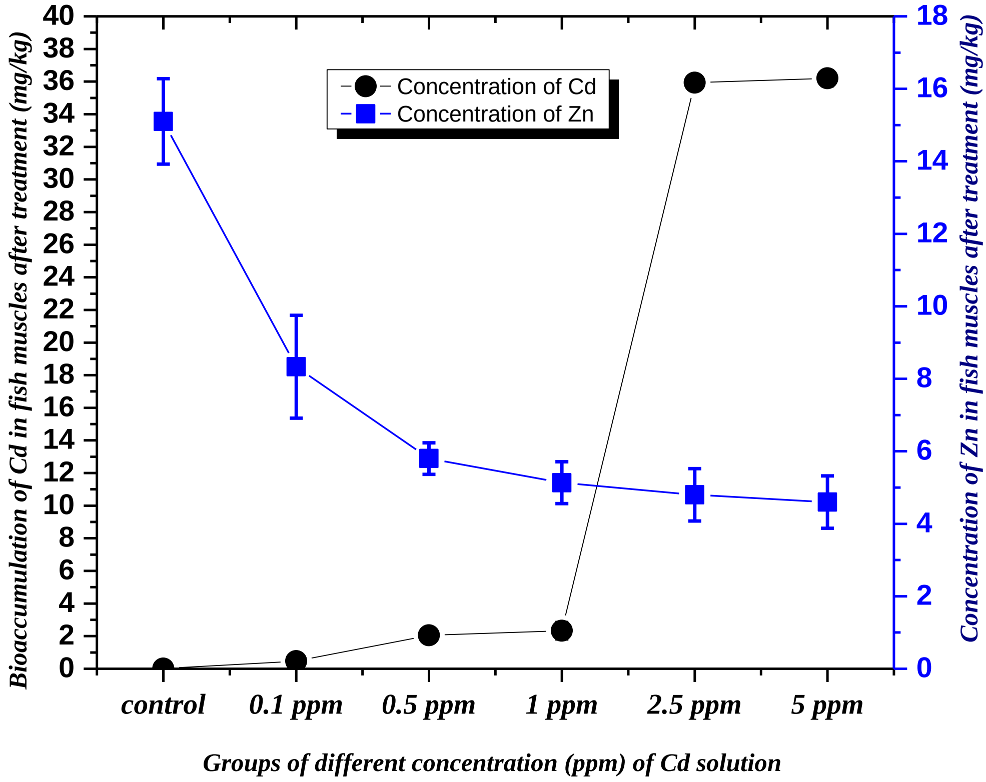

Figure 5 Comparison of Cd and Zn level in fish muscles of different groups

4. CONCLUSION

From different studies it is becoming evident that heavy metal pollution is predominant in freshwater bodies, the reported investigation showed that the bioaccumulation was highest in fish treated with higher Cd concentration solution (5.0 ppm) and were lowest in fish whom are in control group having. This order of bioaccumulation clearly reflect that higher the concentration in medium higher will be bioaccumulation in fish. Furthermore it was observed that the Cd accumulation were more dominant in liver followed by gills and then muscles of fish. While the Zn concentration were highest in fish of control group and lowest in fishes whom are treated with highest Cd concentration solution (5.0 ppm).This showed that Cd have significant effect on Zn level. The current study was aimed to highlight the heavy metal pollution and their toxicity in aquatic ecosystems. Further investigations are recommended to fully highlight the effect of Cd on Zn level.

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