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Original Article

Cadmium (Cd) influences calcium (Ca) levels in the skeleton of a freshwater fish *Channa gachua*

Influência do cádmio (Cd) nos níveis de Ca no esqueleto de peixes de água doce *Channa gachua*

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Abstract

Environmental contamination with heavy metals is a threat to the organisms due to their toxicity, persistence and bioaccumulation in food chains. The study was aimed to assess cadmium (Cd) effect on calcium (Ca) level in bones of a freshwater fish *Channa gachua*. 42 fish individuals were kept into six (6) aquaria; labelled aq.0, 1, 2, 3,4 and 5 in the laboratory for treatment. Aq.0 was control group and aq.1, 2,3,4,5 were experimental with treatment solution of Cd 0, 0.1ppm, 0.5ppm, 1ppm, 2.5ppm and 5ppm respectively for three months. After exposure, bones tissue were examined for Cd accumulation and Ca concentration. Highest accumulation of Cd were recorded in aq.5 mean 46.86 ± 0.46 $mgkg^{-1}$ and lowest in the control group with mean 0.61 ± 0.06 $mgkg^{-1}$. The order of Cd bioaccumulation in bones were aq.5 > aq.4 >aq.3 > aq.2 > aq.1 > aq.0. Highest concentration of Ca were noted in aq.0 (Control group) mean 7888.06 ± 4827.22 $mgkg^{-1}$ and lowest were 1132.36 ± 203.73 $mgkg^{-1}$ in aq.5 (at 5.0 ppm). Generally a pattern of decreasing Ca level were observed with each rise of Cd bioaccumulation aq.0 > aq.1 > aq.2 > aq.3 > aq.4 > aq.5. Current study indicated that Cd accumulation have substantial effect on Ca level in bones and hence on skeleton system. Strict rules must be implemented by government to control metals pollution and exploitations of biota.

Keywords: aquatic pollution, heavy metal, Cd toxicity, Ca level, Pakistan.

Resumo

A contaminação ambiental com metais pesados é uma ameaça aos organismos devido à sua toxicidade, persistência e bioacumulação nas cadeias alimentares. O estudo teve como objetivo avaliar o efeito do Cd sobre o nível de Ca em ossos de peixes de água doce *Channa gachua*. Quarenta e dois indivíduos de peixes foram mantidos em seis aquários; marcado aq. 0, 1, 2, 3, 4 e 5 no laboratório para tratamento. Aq. 0 foi o grupo controle e aq. 1, 2, 3, 4, 5 foram experimentais com solução de tratamento de Cd 0, 0,1 ppm, 0,5 ppm, 1 ppm, 2,5 ppm e 5 ppm respectivamente por três meses. Após a exposição, o tecido ósseo foi examinado quanto ao acúmulo de Cd e concentração de Ca. O maior acúmulo de Cd foi registrado em aq. 5 com média de 46,86 ± 0,46 mgkg-1 e o menor no grupo controle com média de 0,61 ± 0,06 $mgkg^{-1}$. A ordem de bioacumulação de Cd nos ossos foi aq. 5 > aq. 4 > aq. 3 > aq. 2 > aq. 1 > aq. 0. A concentração mais alta de Ca foi observada em aq. 0 (grupo controle) média de 7.888,06 ± 4.827,22 $mgkg^{-1}$ e a mais baixa foi de 1.132,36 ± 203,73 $mgkg^{-1}$ em aq. 5 (a 5,0 ppm). Geralmente um padrão de diminuição do nível de Ca foi observado com cada aumento da bioacumulação de Cd aq. 0 > aq. 1 > aq. 2 > aq. 3 > aq. 4 > aq. 5. O estudo atual indicou que o acúmulo de Cd tem efeito substancial no nível de Ca nos ossos e, portanto, no sistema esquelético. Regras estritas devem ser implementadas pelo governo para controlar a poluição por metais e a exploração da biota.

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Palavras-chave: poluição aquática, metal pesado, toxicidade do Cd, nível de Ca, Paquistão.

1. Introduction

Heavy metals are the most hazardous, toxic, bioavailable and persistent elements in the aquatic ecosystem (Jia et al., 2020). These toxic metals are released into the environment from various sources which may be natural such as bedrocks weathering (Wei and Yang, 2010; Muhammad et al., 2011) or anthropogenic including direct industrial discharge into rivers, smoke emissions from chimneys and vehicles, agricultural activities e.g fertilizers (Shao et al., 2018; Jin et al., 2019; Siddique and Aktar, 2012). When organisms are exposed to these metals various destructive effects have been reported to occur and the ultimate fate is no doubt humans in which lung cancer, abnormalities of kidneys, fertility, liver functions and bone fractures have been reported to chronic exposure of heavy metals (Rai et al., 2019; Hu et al., 2020). The ugly face of heavy metals in aquatic ecosystems has been widely recognized since the mid-1950s. There have been cases of fatal exposure to mercury (Hg) and Cd as a result of pollution of coastal, riverine and irrigation systems in Japan with industrial and mining wastewater (Lindqvist et al., 1991). Metal pollution in aquatic ecosystems is heterogeneous and depends on many factors; it is small in the open ocean and increases rapidly as it approaches coastal and estuarine bodies of water (Bryan and Langston, 1992; Siddique et al., 2021). These heavy metals precipitate bio-accumulate and reach significant concentrations in sediment and biota (Jeong et al., 2021; Bilal et al., 2022). Bioaccumulation of non-essential metals in tissues leads to toxicity, decreased fertility, tissue damage and dysfunction of various organs (Ribeiro et al., 2000). It was reported to be absorbed by various fish organs and cause morphological, histological and biochemical changes in tissues that can have a decisive effect on fish quality (Fadel and Gaber, 2007). Heavy metals are toxic to fish causing abnormalities including behavioural (eg, swimming, feeding, avoiding prey-predator interactions), physiological (eg, growth, reproduction, and development), biochemical (eg, blood levels of enzymes and ions), and histological changes (Sheehan et al., 1984). Toxic metals can mimic base metals by attaching to physiological positions normally reserved for the base element. Due to their abundance of chemical composition, they are involved in the main control of metals or various important metabolic and signalling functions (Kasprzak, 2002). Human exposure to Cd is possible from a variety of sources, including primary metal production, consumption of contaminated food, smoking and work in cadmium-contaminated workplaces, with smoking being the main cause, other sources of Cd are emissions from mining and industrial activities, including the manufacture of batteries, pigments, stabilizers and alloys (Hayat et al., 2019; Kim et al., 2019; Muntau and Baudo, 1992; Adams et al., 2011; Thornton, 1992; Hutton, 1983). Exposure to cadmium can cause various disorders due to bone destruction, such as calcium metabolism, and osteoporosis, chronic exposure to low Cd levels can also increase urinary calcium loss. And can increase skeletal demineralization, which can increase bone breakability and increase the risk of fracture in exposed people (Järup et al., 1998). Cd-induced toxicity in bones is rare and very few reports have been available in this regard. This is an urgent need to assess the metal's pollution and their toxicity in biota and to advise and develop ways and methods to control heavy metal pollution to keep ecosystems in their natural balance position. The objective of this study was to evaluate the impact of waterborne Cd on Ca level in bones of freshwater fish *Channa gachua* in laboratory conditions to reflect the impact of metal pollution on biota.

2. Material and Methods

2.1. Designing of experiment

The freshwater fish Snakehead (*Channa gachua*) were collected (n=42) with an average initial body weight of 10.2 ± 1.4 g and average body length of 5.1 ± 0.4 cm in River Barandu district Bunair, KP, Pakistan by using a fishing net. The Snakehead were acclimatized for 2 weeks in the rearing aquarium and aeration system was provided using air pumps were fixed in all aquarium to maintain dissolved oxygen with no stress and treatment so they acclimatized under controlled environment in the "Ecotoxicology and Ecology Laboratory" Department of Zoology, University of Malakand, KPK, Pakistan.The forty two fish randomly distributed in 6 aquariums, and everyone was filled with 40 L of water. One was the control group (labelled as aq.0) and 5 were the experimental groups (labelled as aq.1, aq.2, aq.3, aq.4, and aq.5) with one replicate per treatment.

2.2. Sample collection

Forty-two (42) samples of fish were collected and weighed in the laboratory by the digital balance and sized by the length measurement instrument. Fish were divided into 6 groups. The final total weight is 41.1 ± 0.2 g and final total length is 15.2 ± 0.1 cm and three individuals were randomly collected from each treatment.

2.3. Chemical treatment

Cd solutions of different concentrations were added to all experimental aquaria aq.1 (0.1 ppm), aq.2 (0.5 ppm), aq.3 (1.0 ppm), aq.4 (2.5 ppm) and aq.5 (5.0 ppm) with a frequency of two times in a week for three months to expose the fish individuals to Cd metal. While no Cd treatment was given to aq.0 (Control group). For balancing oxygen and other parameters, water in aquaria was continuously renewed twice in a week.

2.4. Sample preparation and chemical analysis

After fish dissecting, bone tissues were digested by concentrated HNO3 (75%) and HClO4 (25%) on a wet weight basis. After digestion, the sample were heated on a hot plate at 80 °C for 30 minutes until a clear solution formed and evaporation stopped. After cooling, the sample was filtered through Whatmann filter paper. Then the sample was diluted with distilled water to 50 ml. The digested samples were then transferred to separate plastic bottles, labelled and kept until analysis. Atomic Absorption Spectrometry was used for Cd and Ca analysis due to its high specificity and selectivity. AAS provide High degree of accuracy. Results typically have an accuracy of between 0.5 and 5%, though this could increase further depending on the testing and analytical standards used.It is a very delicate analytical technique.It can determine parts per billion of a gram in given material (García and Báez, 2012).

2.5. Statistical analysis

Descriptive statistics, ANOVA and Pearson Correlation were calculated by SPSS 18 and graphs were created through Origin Pro 2016.

3. Results and Discussion

3.1. Bioaccumulation of Cd and Ca level in the bones

Highest accumulation of Cd in bones were noticed in aq.5 having concentrated treatment solution (5.0 ppm) where the mean concentration of Cd in bone were detected 46.8667 ± 0.4618 mgkg^{-1} while lowest concentration were measured in the control group 0.6133 ± 0.06807 mgkg^{-1}. The concentration order of Cd in bones were 46.8667 ± 0.4618 > 43.50 ± 5.74 > 9.36 ± 5.42 > 2.56 ± 0.602 > 0.663 ± 0.104 > 0.61 ± 0.06 mgkg^{-1} in aq.5 (5.0ppm), aq.4 (2.5 ppm), aq.3 (1.0 ppm), aq.2 (0.5 ppm), aq.1(0.1 ppm) and aq.0 (0 ppm), respectively. The bioaccumulation of Cd in the bone tissues were comparatively higher in concentrated Cd treatment solution i.e aquarium 5 and 4, where the the concentration of treatment solutions were 5.0 ppm and 2.5 ppm respectively. Which simply reflect that the bioaccumulation of heavy metals directly related to the concentration of metals present in the medium (water) in which fishes swim (Figure 1).On the flip side the level of Ca in bones were highest in control group, where there were no exposure of Cd with mean 7888.06 ± 4827.22 mgkg^{-1} and lowest level of Ca were detected in aq.5 having concentrated treatment solution (5.0 ppm), with mean value of 1132.36 ± 203.73 mgkg^{-1}.Comparitively lower Ca level were detected in the bone tissues of fishes treated in concentrated Cd solutions (aq.5 and 4) than other aquaria. Highest Ca level in control group (with no Cd stress) and lowest Ca level in aq.5 (with 5.0 ppm Cd solution) reflect that bioaccumulation of Cd in fish have influences on Ca level in the bones tissues Furthermore, a pattern was observed in which the Ca concentration in bones decreased in accordance with the rise of Cd bioaccumulation, mean value of each group were 7888.06 ± 4827.22 > 4180.76 ± 6984 > 4209 ± 685.4 > 3915.63 ± 597.7 > 3521.1 ± 9107 > 1132.36 ± 203.73 mgkg^{-1} in aq.0 (control group) ( ), aq.1 (0.1 ppm), aq.2 (0.5 ppm), aq.3 (1.0 ppm), aq.4 (2.5 ppm) and aq.5 (5.0 ppm) Cd treatment solution. (Table 1 and Figures 2-3)

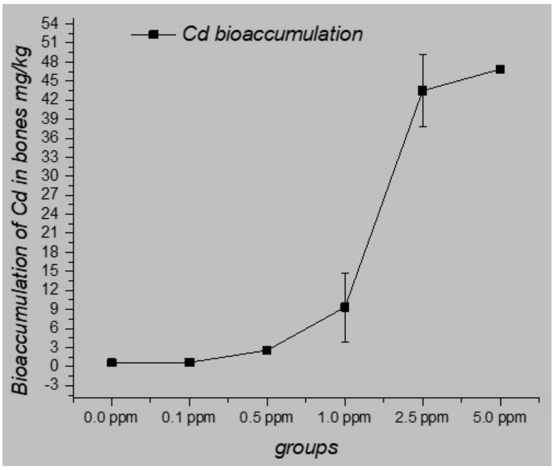

Figure 1. Bioaccumulation of Cd in bones exposed to different concentration of Cd solution.

In addition the concentration of Ca at each group were statistically significant at 95% confidence level *$p < 0.05$ ($p = 0.035$)* and the result of Pearson correlation showed moderate negative association between the accumulation of Cd with level of Ca in bones tissues of the fish *($r = - 0.611$, $p < 0.01$)* (Table 2)

Results of the current study fall in the range of various studies in this regard to Cd-induced toxicity in skeletons across the globe. Cadmium is a highly toxic nonessential element with no biological function. It is carcinogenic to aquatic biota and humans and can cause abnormalities such as reduction in development and growth rates as well as skeletal ossification even at the lowest concentration (Wright and Welbourn, 2002). Cadmium accumulates mainly in the kidneys and liver, but can also reach high concentrations in the gills, alimentary canal and spleen (Jezierska and Witeska, 2006). Bone mineral density in the femur and tibia bones of rats has been reported to be decreased by 6.5-11% when treated with Cd (Brzóska et al., 2010). And influence Ca metabolism and

Table 1. Comparison of Cd bioaccumulation and Ca level.

Groups	N	Mean Cd bioaccumulation (mgkg^{-1})	Std. Deviation	Mean Ca level (mgkg^{-1})	Std. Deviation
Aq.0 (Control)	3	0.61	0.068	7888.06	4827.62
Aq.1 (0.1 ppm)	3	0.66	0.104	4180.76	69.84
Aq.2 (0.5 ppm)	3	2.56	0.602	4209.00	685.42
Aq.3 (1.0 ppm)	3	9.36	5.426	3915.63	597.74
Aq.4 (2.5 ppm)	3	43.50	5.741	3521.10	91.07
Aq.5 (5.0 ppm)	3	46.86	0.461	1132.36	203.73
Total		17.26	20.741	4141.15	2645.49

Table 2. Showing moderate negative association between Cd bioaccumulation and Ca level.

Correlations			
		Bioaccumulation of Cd	**Ca level**
Bioaccumulation of Cd	Pearson Correlation	1	-.611**
	Sig. (2-tailed)		.007
	N	18	18
Ca level	Pearson Correlation	-.611**	1
	Sig. (2-tailed)	.007	
	N	18	18

**Correlation is significant at the 0.01 level (2-tailed).

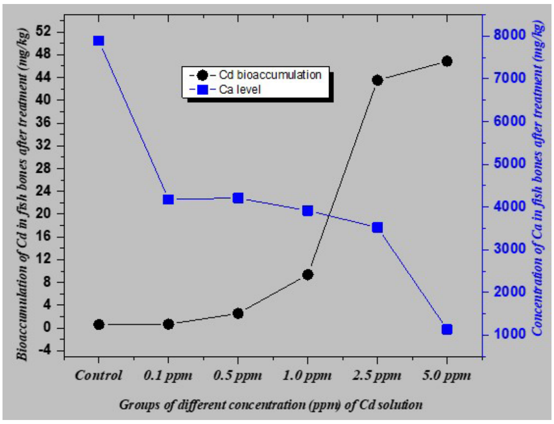

Figure 3. Comparison of Cd bioaccumulation and declined Ca level.

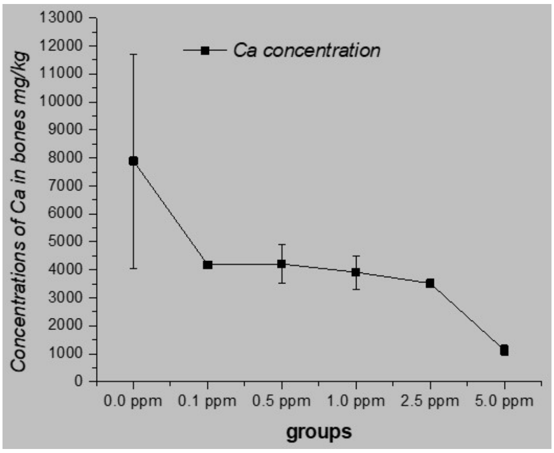

Figure 2. Ca level in bones of fish after exposed to Cd.

hence bone development and maturity (Brzóska and Moniuszko-Jakoniuk, 2005). When mice were exposed to Cd experimentally, within 24 h of oral dose cadmium caused demineralization. While long-term exposure leads to bone fractures in skeletons in rats (Bhattacharyya, 2009). Sassi et al. (2010) have further investigated that this Cd-induced toxicity in skeleton accelerates with temperature in juvenile Mosquitofish, *Gambusia affinis*. Exposure to water containing Cd leads to an increase in the Cd content in fish and a decrease in the Ca and P content in fish and other vertebrates (Muramoto, 1981).

4. Conclusion

Various reports have been published on heavy metal pollution across the globe in fishes and other aquatic animals, on this regard, the current report has shown that Cd bioaccumulation also caused toxicity in the skeleton system of fish. Cd was bioaccumulated in fish bones according to the treatment solution of Cd. Fish that were exposed to a concentrated solution (5.0 ppm), have accumulated the highest Cd content, while the lowest level of accumulation of Cd was recorded in that of the control group. Furthermore, the accumulation of Cd has shown a significant effect on Ca level in the bones of fish. As Ca levels were recorded highest in Control group with no Cd exposure while the lowest Ca levels were recorded in the group of higher Cd treatment solution (5.0 ppm). Moderate negative associations were observed between the accumulations of Cd and a level of Ca in the bones and tissues of the fish. Harmony of the present study with many published reports has exhibited that Cd has a substantial consequence on Ca which is the major component of the bone tissue. The current study aimed to highlight Cd toxicity in bones and would be valued support in this regard. Further investigations are needed to evaluate and fully understand the phenomena.

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