Sains Malaysiana 51(9)(2022): 2757-2762
http://doi.org/10.17576/jsm-2022-5109-02

Bioaccumulation of Hexavalent Chromium in Commercially Edible Fish Grass Carp, *Ctenopharyngodon idella*

(Biopengumpulan Kromium Heksavalen dalam Ikan Rumput Komersial Boleh Dimakan, *Ctenopharyngodon idella*)

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Received: 19 August 2021/Accepted: 31 March 2022

ABSTRACT

The current research was conducted with the aim to investigate the rate of chromium accumulation in various tissues of commercially valuable freshwater fish, Grass Carp, *Ctenopharyngodon idella.* Investigation included various tissues of skin, muscles, liver, swim bladder, intestine, and gills in fish. For this purpose, the fish were exposed to sub lethal concentration of hexavalent chromium in the form of potassium dichromate with a dose of 120 mg/L for 40, 20 days and 10 mg/L for 7, 25, 40 and 60 days. Forty fish of the same weight and length (70.45±2.91 g and 7.32±0.16 inches) were used in the present research. Chromium was estimated after acid digestion of the sample tissues and further analyzed through atomic absorption spectrophotometer (Spectra AA6300 China). The results showed that in all the experiments intestine remained the highly accumulative tissue and the accumulation of chromium in the intestine increased with the increase in exposure time i.e., 0.63±0.21 after 7 days, 0.83±0.35 after 25 and 1.63±0.44 after 60 days. Finding of this research work supports that the route of metal uptake was mainly oral instead of absorption by gills or skin.

Keywords: Bioaccumulation; chromium; grass carp; potassium dichromate

ABSTRAK

Penyelidikan ini dijalankan untuk mengkaji kadar pengumpulan kromium dalam pelbagai tisu ikan air tawar yang bernilai komersial, Grass Carp, *Ctenopharyngodon idella*. Kajian dilakukan terhadap pelbagai tisu kulit, otot, hati, pundi renang, usus dan insang ikan. Untuk tujuan ini, ikan didedahkan kepada kepekatan kromium heksavalen sub-maut dalam bentuk kalium dikromat dengan dos 120 mg/L selama 40, 20 hari dan 10 mg/L selama 7, 25, 40 dan 60 hari. Empat puluh ikan dengan berat dan panjang yang sama (70.45±2.91 g dan 7.32±0.16 inci) telah digunakan dalam penyelidikan ini. Kromium dianggarkan selepas penghadaman asid tisu sampel dan selanjutnya dianalisis melalui spektrofotometer serapan atom (Spectra AA6300 China). Keputusan menunjukkan bahawa dalam semua uji kaji, usus kekal sebagai tisu terkumpul tinggi dan pengumpulan kromium dalam usus meningkat dengan peningkatan masa pendedahan iaitu 0.63±0.21 selepas 7 hari, 0.83±0.35 selepas 25 hari dan 1.63±0.44 selepas 60 hari. Penemuan penyelidikan ini menyokong bahawa laluan pengambilan logam berlaku melalui mulut dan bukannya penyerapan oleh insang atau kulit.

Kata kunci: Biopengumpulan; kalium dikromat; karp rumput; kromium

INTRODUCTION

Currently water pollution, as a result of heavy metal contamination has become a hot topic of concern to the ecologists and is one of the global challenges that the society must address in the 21st century aiming to reduce ecosystem health impacts (Zamora-Ledezma et al. 2021). Extensive industrialization, climate change as well as rapid urbanization have measurably imposed negative impact on the water quality of fish ponds, lakes and rivers throughout the globe (Praveena et al. 2013). This issue has become more critical because the industries frequently discharge their wastes containing metallic contaminants into the environment which surpasses the permissible limits (Velma et al. 2009). Even with the progression in ecological waste management system, the complications arise due to heavy metal discharge are still presenting unfavorable impact on aquatic biolife. Particularly lithophilic or class- B metals are noted to be more hazardous to the ecological community (Ahmed et al. 2013). Heavy metals can show high toxicity even in less concentration exerting cumulative deleterious effects in an aquatic environment.

Chromium pollution in aquatic medium is a key issue of concern as it is posing negative influence on human wellness as well as on society either directly or indirectly. As an element, Chromium is being very stable but does not exist pure in nature. The principle ore of Chromium is Chromite, from which ferro-chrome alloys and chromium metals are acquired. It may be present in divalent (Cr^{+2}), trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) forms, but trivalent and hexavalent are the predominant and stable forms (Velma et al. 2009). In biological system, Chromium typically exists in trivalent form, and is reported as a crucial element in mammalians because of its effective function in metabolic process of glucose, lipids and proteins. As a result of poor membrane permeability, non-corrosiveness and also very less potential to biomagnify in the food chain and food web, the toxic effect of trivalent chromium is extremely reduced (Ahmed et al. 2013; Bakshi 2016).

After entering the cell, hexavalent chromium changes to its trivalent form where it forms complexes with intracellular macromolecules and genetic materials. Easy permeability as well as biotransformation property is accountable for its toxicity and mutagenic activities (Rahman et al. 2012). Besides these properties, it is also considered to be a potential teratogen and carcinogen. In accordance with World Health Organization (WHO), the permissible limit of chromium in drinking water is 0.1 and 0.05 mg/L (Kumar & Puri 2012). According to WHO (1985) and FEPA or Federal Environmental Protection Agency (2003) reports, the maximum allowable limit of chromium in fish food is 0.05-0.15 mg/kg body weight.

Too much uptake of both essential and non-essential metals ends in accumulation in different tissues of fish. Metals in greater concentration alter the biological and physiological activities of the fish (Canli & Atli 2003). Therefore, various experiments pertaining to toxicity of heavy metals have already been carried out (Ali et al 2021) which depicted those heavy metals directly influences fish health and can cause damages to its population. Usage of such metal contaminated fishes by human beings can trigger serious health issues. Metals disturb and deteriorate the natural balance of the aquatic ecosystem as fish are at the end of the aquatic trophic level and have a greater tendency to accumulate metals in their body in comparison to land vertebrates (Kousar & Javed 2014; Mastan 2014).

Heavy metals accumulate in kidneys, skin, gills, liver, intestine and various other body organs of fish and creates steady damages in fish body. *C. idella* commonly called Grass carp is an edible commercially important freshwater fish of Asia. This design was intended to quantify the influence of chromium bioaccumulation in *C. idella* various tissues such as gills, intestine, liver, muscles, skin, and swim bladder.

Materials and Methods

SAMPLING AND ACCLIMATIZATION

Forty fish (*C. idella*) were transported in oxygenated bags from Sherabad hatchery Peshawar, Khyber Pakhtunkhwa Province, Pakistan to the Islamia College Laboratory (latitude and longitude coordinates are 34.0003, 71.4767). Ethical approval of the study was given by 16th Advance Study Research Board (ASRB) meeting under section 16 (iii) of Islamia College Peshawar on 10th November 2020. Fish were treated with 0.2% $KMnO_4$ solution for two minutes to remove any type of impurities and external infection. All the fish were then acclimatized for ten days in four different aquaria (4×2 feet) with a density of 10 fish each having 100 L normal, chlorine free tap water. Anti-chlorine was not used because tap water did not contain chlorine. After acclimatization, no mortality was observed, and the fish were found fresh and healthy.

EXPERIMENTAL DESIGN

For experimentation, a sample size of 5 fish from stock population was shifted to both control and test aquaria, having 50 L water. In all aquaria, fish were fed with commercial food pellet diet of Oryza Organics, Pakistan, composed of protein = 20.0%, phosphorus = 0.7%, fats = 3.0%, moisture = 10.0%, calcium = 0.7%, and fiber = 7.0%. Physiochemical parameters of water such as temperature 18.1 (degree Celsius), pH (7.6), total hardness (91.8 mg/L), dissolved oxygen (7.36 mg/L) and total alkalinity (163.6 mg/L) was also recorded and were found in permissible limits as per the recommended values of American Public Health Association (APHA). Fish were divided into four groups, each group consisted of 5 fish to which potassium dichromate was added with a concentration of 120, 40, 20, and 10 mg/L for 7, 25, 40, and 60 days. Control group was run simultaneously for each experimental group having 5 fish.

TISSUE DIGESTION AND ESTIMATION OF CHROMIUM

Fish were taken out from both the treated and control aquaria after the given exposure period and were dissected. A 0.5 g portion of skin, liver, intestine, swim bladder, muscles and gills were separated and washed with distilled water from both the treated and control fish in order to detect the amount of Cr.

Tissue digestion was carried out according to the methods described by Van Loon (1980), Du Preez and Steryn (1992), followed by Yousafzai and Shakoori (2006). Weighed tissues were defrosted, rinsed in distilled water, and blotted with blotting paper. The samples were transferred to 250 mL volumetric flasks and were put in 10 mL nitric acid (55%) and 5 mL perchloric acid (70%) and were kept overnight. Next day the flasks were then placed on a hot plate and allowed to digest at 200-250 °C until a transparent and clear solution was obtained. Dense white fumes from the flasks after brown fumes were an indication of completion of the process of digestion. By this method, the digestion process is completed in about 20 min instead of 3 to 4 h as stated by Van Loon (1980).

Samples after digestion were cooled and diluted to 10 mL with distilled water. Samples were stored in properly washed glass bottles until the metal concentration was taken out. Determination of chromium was carried out through atomic absorption spectrophotometer (Spectra AA 6300).

STATISTICAL ANALYSIS

Data was expressed in the form of mean and standard error of mean. Student's 't' test was applied for comparison of the data of control with the test samples. Values of P less than 0.05 were considered significant.

RESULTS

The accumulation of chromium in various tissues of *C. idella* is presented in Table 1. After 7 days of exposure with a dose of 120 mg/L potassium dichromate, intestine accumulated Cr (0.63±0.21) at a significantly ($p<0.05$) higher rate as compared to control. Gills (0.51±0.13) were second in metal bioaccumulation skin and muscles

TABLE 1. Chromium bioaccumulation in various tissues of *C. idella* after 7, 25, 40 and 60 days' exposure to different doses

Tissues	Exposure period	Control (Mean± SE) n=5	Treated (Mean± SE) n=5
Skin	7 days	0.00±0.00	0.11±0.06
	25 days	0.02±0.02	0.12±0.03
	40 days	0.01±0.01	0.10±0.01*
	60 days	0.02±0.02	0.07±0.03
Swim bladder	7 days	0.01±0.01	0.05±0.02
	25 days	0.09±0.08	0.37±0.10
	40 days	0.001±0.001	0.004±0.003
	60 days	0.003±0.003	0.15±0.08
Intestine	7 days	0.03±0.01	0.63±0.21*
	25 days	0.14±0.13	0.83±0.35
	40 days	0.05±0.03	0.72±0.39
	60 days	0.03±0.02	1.63±0.44*
Liver	7 days	0.01±0.01	0.21±0.13
	25 days	0.09±0.09	0.41±0.11
	40 days	0.001±0.001	0.44±0.09*
	60 days	0.01±0.01	0.16±0.06
Gills	7 days	0.02±0.02	0.51±0.13*
	25 days	0.01±0.01	0.58±0.33
	40 days	0.03±0.02	0.33±0.26
	60 days	0.007±0.005	0.47±0.0008
Muscles	7 days	0.002±0.002	0.10±0.03
	25 days	0.03±0.03	0.25±0.14
	40 days	0.008±0.006	0.02±0.005
	60 days	0.03±0.03	0.06±0.02

n=Number of samples, Student's t- test; *Values are Significant at $p<0.05$

have low concentration (0.11±0.06 and 0.10±0.03) compared to liver (0.21±0.13). While least accumulation was detected in the swim bladder (0.05±0.21). The accumulation of chromium in various tissues after seven days' exposure displayed the following order; intestine> gills> liver> skin> muscles> swim bladder.

Similar trends of accumulation were observed after 25 days (dose 40 mg/L) exposure. Intestine accumulated higher concentration (0.83±0.35) of Cr as compared to control. Gills (0.58±0.33) and liver (0.41±0.11) followed the same trend of accumulation as 7 days of exposure. After twenty-five days, the pattern of accumulation was; intestine> gills > liver> swim bladder > muscles> skin.

While exposing *C. idella* up to 40 days (dose 20 mg/L), the overall accumulation was high in the intestine (0.72±0.39) followed by liver (0.44±0.09) which indicated significant increase in values as compared to control. Likewise, skin also exhibited significant ($p<0.05$) increase in concentration (0.10±0.01). Muscles (0.02±0.005) and swim bladder (0.004±0.003) have less accumulation. After forty days, the overall accumulation sequence was intestine> liver> gills> skin> muscles> swim bladder.

Moreover, during 60 days of exposure with a dose of 10 mg/L significantly high accumulation of Cr was recorded in intestine (1.63±0.44) as compared to other tissues (Table 1). The pattern of accumulation after 60 days was in sequence like intestine> gills> liver> swim bladder> skin> muscles.

DISCUSSION

Heavy metals are amassed in living organisms when they are taken up and stored faster than they are metabolized or excreted. They accumulate in fishes via water, sediments and food such as algae upon which both herbivorous and omnivorous fishes feed (Joshi et al. 2002). Difference in the absorption of heavy metals depends on the fish species, chemical features of the test solution, tolerance limit of the organism, sensitivity as well as on the physical and chemical characteristics of water (Rauf et al. 2009; Yousafzai et al. 2010). In this study, all the fishes belonged to the same family cyprinidae and species idella. They were of the same size and age (size=7.32±0.16 inches, age 7 months) and were prone to Cr. Exposure of *C. idella* to different concentrations of potassium dichromate resulted in high bioaccumulation of chromium in the intestine.

Mean values of chromium in the intestine of treated group at day 7, 25, 40, and 60 are as follows: 0.63±0.21, 0.83±0.35, 0.72±0.39, and 1.63±0.44. Highest accumulation of chromium in intestine occurred at day 60 which suggests that accumulation pattern depends upon the duration of exposure. In the present study, the bioaccumulation of chromium in the fish intestine was acknowledged to be maximum followed by the gills and liver while minimal in the skin, muscle tissues, and swim bladder. Fish that accumulate heavy metals from food, exhibit high level of metals in the digestive tract in contrast to gills. Our findings are in accordance with Yousafzai et al. (2012) who also accounted highest possible accumulation of heavy metals in intestine of *Cyprinus carpio* followed by skin, liver, gills and least in muscles indicating that the foremost target body organ of heavy metals here is the intestine. Similarly Bury et al. (2003) also reported in gold fish that the intestine worked as the bulk pathway for the uptake of heavy metals like zinc. The outcome of this research is that the main route of absorption of chromium was food.

After intestine highest accumulation of chromium occurred in gills. In test group, the mean values of chromium in gills at day 7 is 0.51±0.13, 0.58±0.33, 0.33±0.26, and 0.47±0.0008 (mg/g) at day 25, 40, and 60. Gills are the very first target of waterborne contaminants and heavily prone to accumulation of heavy metals as a result of consistent contact with the outside environment and the main place for the uptake of heavy metals. Accumulation in gill tissues might be due to absorption of chromium via the gill surface which is not easy to eliminate. The highly branched and complicated morphology of gill tissues and the movement of water through it, results in greatest accumulation of chromium (Ruiz-Picos & López-López 2012). Mastan (2014) has also described the same pattern of bioaccumulation in *Labeo rohita*. Solangi et al. (2012) studied that chromium accumulation is maximum in gills than in the skin or muscular tissues. Gills are not only the respiratory organ in fish but also plays an important role in osmoregulation. Consequently, gill tissues as near to outer aquatic environment are affected directly by various toxins, it also alters the osmoregulatory function.

In liver mean values and standard error of mean for treated group for 7 days are 0.21± 0.13, 25 days are as 0.41±0.11, for 40 days 0.44±0.09 and for 60 days 0.16±0.06, respectively. The substantial accumulation of Cr in the liver might be associated to its essential role in amassing as well as detoxification. Higher concentration of metals in liver may be due to the storage and detoxification of heavy metals in liver coming through food (Noreña et al. 2012). Batool et al. (2014) reported that among various fish organs of *Channa marulius* and

Wallago attu, liver appeared as a major target organ that accumulates a significantly higher chromium content followed by kidney, gills, fins, skin, muscle and bones. Giguère et al. (2004) described greater tendency of liver for the accumulation of chromium and minimum in muscles of fish.

Chromium accumulated in the skin of the treated group fishes are 0.11±0.06 (mg/g wet weight) at day 7, 0.12±0.03 at day 25, 0.10±0.01 at day 40 and 0.07±0.03 (mg/g) at day 60. There looks a general decreasing trend of Cr accumulation by increase in exposure period which might be due to excretion by skin during mucous secretion. Small amount of chromium was also present in the control groups because chromium is also an essential metal for various biological and physiological activities. Skin is a crucial excretory organ for that reason chromium accumulation is minimum in the skin as compared to intestine, gills and liver. Skin is mainly the region exposed in a fish's body. The low level of chromium in skin tissues demonstrates that it is a crucial excretory organ, presumably by means of mucous secretions (Heath 1995). Another reason might be due to the presence of scales on the fish body which minimize the direct contact of skin with Cr intoxicated water. Skin also has a mucous layer existing on their external surface, which signifies that it is the probable means of excretion. It involves the disposing of metal containing mucus from such surfaces thus metal accumulation is minimal in the skin. Metals are accumulated in the skin through adsorption followed by the absorption. Our results are in line with the previous findings of Avenant-Oldewage and Marx (2000) who reported less chromium accumulation in skin tissues and muscles.

During 7, 25, 40, and 60 days' experiment, Cr accumulation in swim bladder was 0.05±0.02, 0.37±0.10, 0.004±0.003, and 0.15±0.08 (mg/g), respectively. Swim bladder functions as a hydrostatic organ. Chromium accumulation in the swim bladder can cause buoyancy issues. Any form of imbalance in fish can be considered as a potential threat to its health. Swim bladder is found in the lower half of the body and the affected fish face difficulties when trying to maintain their floating balance. Jovičić et al. (2015) reported lowest metal accumulation in swim bladder in comparison to other tissues. Xu et al. (2018) described the bioaccumulation of mercury (Hg) in different organs following the order; muscles > heart > liver > swim bladder > gills. Witeska et al. (2014) studied body malformations as well as effect on size of swim bladder during exposure to Cu and Cd in *Leuciscus idus*.

In the current study, chromium bioaccumulation is less in muscles. In treated group mean value of chromium in muscles at day 7 are 0.10±0.03, at day 25 are 0.25±0.14, 0.02±0.005 during 40 days experimentation and 0.06±0.02 during 60 days treatment. This may be due to the growth factor since growth can dilute the concentration of toxic substances, if growth is more rapid than accumulation. Yousafzai et al. (2012) reported considerably less amount of metals in the muscle tissues of *Cyprinus carpio*. Similar investigations were also reported by Shukla et al. (2007) that concentration of zinc, cadmium, and copper was optimal in liver and minimal in muscle tissues of *Channa punctatus*. Karadede-Akin and Unlu (2007) also observed that heavy metals in muscle tissues were at reduced levels in contrast with other body organs.

Conclusions

In the present study, the fish stored higher concentration of Cr in intestine followed by gills and liver whereas less accumulation occurred in other tissues like swim bladder, muscles, and skin which suggests that the main route of chromium uptake was alimentary canal or food. Chromium accumulation might cause deterioration of energy needed for crucial activities in the fish body.

Acknowledgements

We are thankful to the laboratory assistants of Department of Zoology, Islamia College Peshawar for support during the experimental work.

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