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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1542-1550. **OPEN ACCESS**

Assessment of Some Heavy Metals and Hematobiochemical Alterations In *Cyprinus Carpio* Exposed To $MgCl_2$ and Treated With *Typha elephantina* Leaves Extract

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The present study was aimed to investigate the curative effects of *Typha elephantina* (T.E) leaves methanolic extract on bioaccumulation of heavy metals along with blood and biochemical variables in *Cyprinus carpio* (common carp).The heavy metals such as Zn, Ni, Cd and Cr were examined in Muscle, liver, gills, skin and intestine while complete hematology, lipid profile and some liver and kidney related biochemical parameters were analyzed The fish were first exposed to MgCl2 and then treated with extract of T.E alone and along with ascorbic acid. The results showed that all the tissues accumulate the substitutional amount of heavy metals. The accumulation of ZN was highest and Ni come second followed by Cr and in the last and least was Cd respectively i.e Zn > Ni > Cd >Cr. This accumulation was differential in each organ studied such as in the skin the accumulation was highest while in intestine it was high and followed by gills then muscles tissues. However the accumulation was least in the liver tissues. Analysis of blood and serum indices showed that extract alone (group 2) has no effect while the co admiration of extract and ascorbic acid (group 3) significantly ameliorates the altered hematological and biochemical parameters toward normal levels when compared to normal control fish (group 1).

Keywords: bioaccumulation, heavy metals, skin, gills, hematology, transaminases.

INTRODUCTION

The accumulation of heavy metals in aquatic life indicates the way of Water pollution and fishes are commonly used as bio-indicators of heavy metals contamination (Keke et al. 2020). Heavy metals such as cobalt ,Iron, copper, manganese, zinc and molybdenum are important in trace amount for human being (Mehrandish et al. 2019). However these are metals are lethal at higher concentrations (Castaldo et al. 2020). The natural water systems is broadly contaminated with heavy metals come out in the form of industrial, domestic and agricultural effluents which increasing at an alarming speed and has become a universal health issue(Deng et al. 2020). Therefore, substantial metals can be accumuated

in the living body (bioaccumulation) and pass through diet chain (trophic level) to the next organism or population resulting bio-magnification (Dar et al. 2019). Metals are considered as main environmental contaminants and are non-biodegradable which cytotoxic, carcinogenic and mutagenic effects in animals (Kanwar et al. 2020). Fishes are considerably affected higher than other vertebrates because of their feeding habits and habitat (water) (Fazio 2019). To inspect the physiological state of both human or animal heamatological indices are very important (Ohaeri and Eluwa 2011). Blood in animal's body serves as a medium of transporting nutrients absorbed from the digestive system or released from storage in adipose tissues or in liver. The blood picture changes with advancement of animal with age and with certain conditions such as nutrition. The heamatological parameters which are of significant diagnostic values include the packed cell volume (PCV), hemoglobin (Hb), total protein (TP) and Serum globulin (SG) are known to affect health, production and adaptability to environmental conditions in livestock(H. Karasov and Douglas 2013) ; (Adenkola et al. 2011)

Moreover, hematological tests and analysis of serum constituents have showed useful information in detection and diagnosis of metabolic disturbances and disease in fishes (Chagas et al. 2017).

Thus current study was planned to analyzed useful impact of *Typha elephantina* leaves methanolic extract on accumulation of some heavy metal f in gills, skin, muscle and liver of the fish (*Cyprinus carpio)* along with hematobiochemicl markers.

MATERIALS AND METHODS

Fish and aquaria

A total of "75" *Cyprinus carpio* fish of the same length and size were brought from the university fish pond to the laboratory and were kept in aquaria with regular supply of fresh air and food. All the fish were categorized into "3" groups, comprising of "25" animals in each group and the experiment was carried out for two weeks.

Group 1: served as control fish

Group 2: administered with MgCl2 at concentration of 50mg/L once a day and *Typha elephantina* methanolic extract at dose rate 100 mg/L.

Group C3: Received 50 mg/L vitamin C (Ascorbic acid), and 100 mg/L of *Typha elephantina* methanolic extract after exposure to 50mg/L of MgCl2

Digestion of fish tissue

Constant weighed tissues were digestion in perchloric acid (70%) and nitric acid (55%) respectively. The tissue digestion was passed out in the Chemistry lab Islamia College Peshawar For the assessment of heavy metals. In distilled water tissue samples were washed and marked with blotting papers and then shifted to 100ml volumetric bottles. The flask were washed with distilled water and dried in oven at 60 c^0. The weight of respectively tissue was shifted to these volumetric bottles after that the identification. According to the method of (Shah et al. 2020), samples were digested. A small change was made in the process (Yousafzai et al. 2012).Instead of putting 5ml per chloric acid (70%) and 10ml nitric acid (55%) at the time of digestion and kept for all night. Next day a second dose of 4ml (70%) per chloric acid (70%) and 5ml nitric acid (55%) was added to all flasks. Until a clear and transparent solution was prepared the flasks were kept on warm plates and permitted to absorb at 200 to 250c^0. The thick white fume from the flask after brown fumes was a sign of digestion process ending. As stated by Van Loon (1980) digestion was completed by this method in approximately 20 minutes instead of 3 hours to 4 hours. Samples were cooled after absorption and were dilute to 10 ml with purified water by good washing of the consumption flasks. Sample was stowed in well washed glass bottles awaiting the metals absorption possibly will be resolute.

Assessment of heavy metals

Heavy metals Zn^{+2} , Ni^{+2}, Cr^{3+}, Cd^{2+} in tissue sample of each fish was determined through the atomic absorption spectrophotometer (Spectra AA-700) in the CRL (Centralized Resource Laboratory) University of Peshawar. To identify the concentration of heavy metals present the ODs (Optical Densities) found were adjusted against the standard curvatures and Standard curves were organized(Cheng et al. 2018).

Table A: showing animal grouping and treatment schedule

Group	Group category	Treatment	medium
Group 1	Control fish	Served as control fish	Water
Group 2	MgCl2+ Extract	MgCl2 at concentration of 50mg/L once a day and Typha elephantina methanolic extract at dose rate 100 mg/L.	Water
Group 3	MgCl2+ Extract +Vitamin C	Received 50 mg/L vitamin C (Ascorbic acid), and 100 mg/L of Typha elephantina methanolic extract after exposure to 50mg/L of MgCl2.	Water

Analysis of hematology and serum biomarkers

Blood samples were collected for the assessment of hematology and serum biochemical parameters such as red blood cell, hemoglobine, mean corpuscular hemoglobine (MCH), mean corpuscular hemoglobine concentration (MCHC), mean corpuscular volume (MCV), white blood cell, monocytes and lymphocytes(Sheikh and Ahmed 2019). The serum biomarkers were include aspartate transaminase AST, alanine transaminase (ALT), alanine phosphatase (ALP) (Lala et al. 2020). Lipid profile like Cholesterol high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) along with glucose, total protein, urea and creatinine(Karimi-Nazari et al. 2019).

Statistical Analysis:

Mean of the data and Standard error of mean was reserved.

RESULTS

The concentration of Zinc in the gills, skin, intestine, and liver and muscles tissues was found highest in fish that were exposed to MgCl2 at dose rate 50 mg/L and methanolic extract at 200 mg/L (group= 2) when compared to control fish (group=1). However co-administration of extract (200mg/L) and ascorbic acid (50mg/L), significantly ($P < 0.05$) decreased the raised value of Zn (group=3) respectively. A significant raised level of nickel (Ni) deposition was observed in gills, skin, intestine, liver and in muscles of fish (group=2). These animals (group=2) were treated with methanolic extract at dose rate 200 mg/L after 50 mg/L exposure of MgCl2 respectively. However this elevated level of (Ni) was signinificanly ($P < 0.05$) reduced in fish of (group= 3) when compared to control fish (group= 1).

Similarly cadmium (Cd) and chromium (Cr) were concentration were also found significantly high in fish (group= PE), showed that methanolic extract of T.Eat dose rate 200 mg/L had no effect. While the administration of extract (200mg/L) and ascorbic acid (50mg/Lt) after the exposure of MgCl2 (50 mg/L) significantly ($P < 0.05$) decline the levels of (Cd) and (Cr) when compared to control fish (Table 1,2,3,4 and 5). Fish that were treated with methanolic extract ((200 mg after exposure to MgCl2 (50 mg) showed no curative effects on blood profile. Hence these animals have significantly ($P<0.05$) irregular hematological parameters like (RBCs, WBCs, HCT, Hb, MCV, MCH, MCHC, Lymphocytes, monocytes and neutrophil), revealed toxicity (group= 2).Although the combine treatment of animals with methanolic extract at 200 mg/L and ascorbic acid at 50 mg/L significantly ($P<0.05$) ameliorates all the hematological indices, after the contact of MgCl2 (50 mg/L) when compare to control animals (Table 6).

The significant elevation in the serum ALT, AST and ALP were observed in (group= 2). Likewise some other serum parameters such as glucose, total protein, urea and creatinine were also found significantly ($P<0.05$) higher levels in fish (group=2), when compared to control animals (group=1) respectively. As the administration of T.E methanolic extract showed no optimistic effects on above mention serum parameters hence (group= 2), showed MgCl2 toxicity. Animals of group=2 showed significant ($P<0.05$) elevated levels of serum cholesterol, low density lipo-proteins (LDL) and triglycerides (TG) while decreased in level of high density lipo-proteins (HDL) was observed when compared to control animals. In the same way level of serum urea and serum creatinine were found statistically significant ($P<0.05$) in fish that were administered with methanolic extract (200 mg/L) after exposure to MgCl2 (50 mg/L) respectively. The increased levels of above mentioned serum parameters indicates that the provision of extract alone, had no recovering effect, therefore MgCl2 revealed

toxicity in fish (group 2).

Table 1: Showing Mean ± SD values of some heavy metals bioaccumulation in the muscles tissues of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Heavy metals	Group1 Control Fish (N=25)	Group 2 MgCl2+Extract (N=25)	Group 3 MgCl2+Extract+Vit C (N=25)
Zn	0.069±0.01	0.500±0.06	0.048±0.030
Ni	0.0043 ±0.02	0.052±0.01	0.041±0.05
Cd	0.00350±0.081	0.0021 ±0.07	0.00687±0.0017
Cr	0.0041±0.001	0.013±0.003	0.0053±0.00208

Group 1: served as control fish. Group 2: 50mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50mg/L of MgCl2.

Table 2: Showing Mean ± SD values of some heavy metals bioaccumulation in the skin tissues of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Heavy metals	Group1 Control Fish (N=25)	Group 2 MgCl2+Extract (N=25)	Group 3 MgCl2+Extract+Vit C (N=25)
Zn	1.36±0.50	1.81 ±0.131	0.04275±0.004349
Ni	0.045±0.01	0. 0895 ±0.050	0.0550±0.005
Cd	0.0187±0.007	0.070 ±0.02	0.023±0.0033
Cr	0.05025±0.001893	1.43±0.067	0.02775±0.004717

Group 1: served as control fish. Group 2: 50mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50mg/L of MgCl2.

Table 3: Showing Mean ± SD values of some heavy metals bioaccumulation in the gills tissues of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Heavy metals	Group1 Control Fish (N=25)	Group 2 MgCl2+Extract (N=25)	Group 3 MgCl2+Extract+ Vit C (N=25)
Zn	0.071±0.0061	0.70±0.0017	0.06667±0.005774
Ni	0.051±0.004	0.071±0.01	0.0400± 0.009
Cd	0.0203±0. 001	0.03 ±0.51	0.02633±0.0015
Cr	0.0557±0.008505	0.54±0.053	0.0600±0.034

Group 1: served as control fish. Group 2: 50mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50mg/L of MgCl2.

Table 4: Showing Mean ± SD values of some heavy metals bioaccumulation in the liver tissues of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Heavy metals	Group1 Control Fish (N=25)	Group 2 MgCl2+Extract (N=25)	Group 3 MgCl2+Extract+Vit C (N=25)
Zn	0.0825±0.001	0.625±0.544	0.006467±0.0005859
Ni	0.0032±0.0020	0.069±0.002	0.0400±0.0100
Cd	0.00518±0.000	0.0098±0.1	0.0087±0.0011
Cr	0.0040 ±0.0004	0.025± 0.027	0.0093±0.001136

Group 1: served as control fish. Group 2: 50 mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50 mg/L of MgCl2.

Table 5: Showing Mean ± SD values of some heavy metals bioaccumulation in the intestine tissues of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Heavy metals	Group1 Control Fish (N=25)	Group 2 MgCl2+Extract (N=25)	Group 3 MgCl2+Extract+Vit C (N=25)
Zn	0.826±0.055	0.056±0.007	0.047±0.01835
Ni	0.026±0.0056	0.08 ±0.01	0.0570±0.02539
Cd	0.03775±0.012	0.5893±0.066	0.0501± 0.008
Cr	0.04825±0.01242	0.0735±0.00695	0.0491±0.01645

Group 1: served as control fish. Group 2: 50mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50mg/L of MgCl2.

Table 6: Showing blood hematology (Mean ± SD) values of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Variables	Group1 Control Fish (N=25)	Group 2 MgCl2+ Extract (N=25)	Group 3 MgCl2+Extract+ Vit C (N=25)
RBCs (×106 µL)	1.717 ± 0.24	2.850±0.217**+**	1.950±0.045
WBS ×103µL	4.467 ±0.42	7.367±0.460**+**	5.600±0.65*
HCT (%)	33.01 ±2.4	22.34±1.125**+**	29.08±1.97*
Hb (g/dL	10.29 ± 1.24	7.963±0.24**+**	10. 31±0.58
MCV (fl)	175.5±1.55	153.8±3.292**+**	169.1 ±6.2
MCH (pg)	44.08±2.37	36.08±1.476**+**	41.15±0.73
MCHC (g/dL	22.59±1.73	17.59±0.730**+**	20.89±0.57*
Lymphocyte (%)	50.21 ± 0.39	45.00±1.845**+**	46.96± 1.82 *
Monocyte (%)	3.120 ± 0.23	2.100±0.101**+**	2.933±0. 05*
Neutrophil (%)	35.78±2.48	43.78±2.201**+**	36.44±2.36

Note: (**+)** = significant difference from group 1animals and (***)** = significant difference from group 2 animals according to T test and one way Anova at (P<0.05) . Group 1: served as control fish. Group 2: 50mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50mg/L of MgCl2.

Table 7: Showing serum biochemical (Mean ± SD) values of *Cyprinus carpio* exposed to MgCl2, methanolic extract of *Typha elephantina* and ascorbic acid (Vitamin C).

Variables	Group1 Control Fish (N=25)	Group 2 MgCl2+Extract (N=25)	Group 3 MgCl2+Extract+ Vit C (N=25)
AST (U/dL)	179.9±1.84	199.3±0.5774**+**	182.0± 1.905
ALT (U/dL)	79.64±1.434	93.12±2.459**+**	83.64± 1.640
ALP (U/dL)	68.07±1.995	76.74±1.403**+**	69.30± 1.11*
HDL (mg/ dL)	39.08±1.517	32.37±1.009**+**	41.14±1.050
LDL (mg/dL)	106.1±1.010	116.3 ±3.530**+**	109.0± 1.000
Glucose (mg/dl)	157±2.12	192.5±3.052**+**	161.9 ± 2.23 *
Cholesterol mg/dl	165±0.57	196.3±0.5774**+**	170.1±3.74*
Triglycerides mg/dl)	137±1.76	164.3 ±1.058**+**	139.6± 0.800
Total Protein (mg/dl	10.1±0.254	8.747±0.5590*	9.747± 0.55
Urea (mg/dl)	17.67±0.87	21.96±1.004**	18.0± 0.78
Creatinine (mg/dl)	0.45±0.02	0.7133±0.01528***	0.493 ±0.005

Note: (**+)** = significant difference from group 1animals and (***)** = significant difference from group 2 animals according to T test and one way Anova at (P<0.05) . Group 1: served as control fish. Group 2: 50mg/L MgCl2 + 100 mg/L extract. Group C3: 50 mg/L vitamin C (Ascorbic acid) + 100 mg/L extract + 50mg/L of MgCl2.

In other hand co-administration of extract 200 mg/L and ascorbic acid (50 mg/L), to animals of group=3, significantly reduced ($P<0.05$) and recovered the levels of serum lipid profile, urea and creatinine toward normal range when compared with control animals shown in (Table 7)

DISCUSSION

Heavy metals like Cd, Cr, Ni, and Zn were analyzed for the bioaccumulation in the muscle, liver, gills and skin tissues of fresh water fish Mully. Combustion emission, Domestic manure, mining operations, industrial effluents and metallurgical activities are the sources of heavy metals such as Pb, Cd, Zn and Cr in the hydrosphere (Hembrom et al. 2020). In the current study of Zinc More concentration observed in gills, skin and intestine flowed by liver while muscles has shown the lowest accumulation. The reason is that the muscle is less active tissue metabolically that's why accumulated the least level of zinc (Ren et al. 2020) ; (Kwaansa–Ansah et al. 2019). (Asksonthong et al. 2018) have also reported the lowest level of heavy metals in the muscles.

Skin of the fish is in direct contact with water so the heavy metals accumulation in skin occurs due to the adsorption which is followed by the absorption through several mechanisms. In present study skin of *Cyprinus carpio* had accumulate high concentration of Zn as compared to other fish tissues. Excessive Zn increase can be toxic and has been connected to the neurodegeneration (Song et al. 2020). (Reid and McDonald 1991), has reported the gill surface is negatively charged and thus provides the potential site for positively charged metals, causing gill-metal communication.

According to (Muiruri et al. 2013) Zinc levels ranged between 28.00-49.50 (mg/kg DW) and 48.79 to 76.33 (mg/kg DW) in the dry and wet seasons respectively.

In the current study the concentration of Zn is high in gills due to the close contact of blood and water. Similarly (Mbuthia 2015) has recorded highest Zn concentration in gills of *Clarias gariepinus* which is inline of our detected toxic fish(group=2) values. Previously (Onuwa et al. 2012) has noted high concentration of Zinc in the dog fish gills.

Nickel is produces severe damage to respiratory system in fish and thus caused fish death (Palanaippan et al., 2003). In present study the concentration of nickel in gills>skin > intestine > liver and >muscle. According to (Kiema et al. 2019), from attribute of Athi-Galana-Sabaki river in Kenya the concentrations of Ni ranged from 0.29-1.75 mg/kg DW and 0.12-0.87 mg/kg DW in the wet and dry seasons respectively. Parallel study was conducted by (Abida et al. 2009) who's noticed the maximum Ni absorptions in the gills of, *Hypophthalmichthys molitrix, Catla catla fossilis, Heteropneustus, ,Cyprinus carpio* .

Chromium is a vital trace metal both for human and animals but in high level it is neurotoxic and carcinogen (Islam et al. 2015). In the current study Cr was detected in the different tissue in the order of Gills Skin >Liver> Muscle, more concentration in the fish tissues skin an gills revealed highest chromium concentration which is due large surface area for exposure to the surrounding water. Previously, (Yousafzai et al. 2014) have recorded of Chromium in the muscles of *Mugil cephalus* and *Trachur mediterraneus* was 1.48 and 1.46 ppm (wet weight) respectively. In gills tissue the accumulation is frequently related with physical damage to the gill epithelium and osmoregulatory function. Likewise, (Has-Schön et al. 2008) have recorded high level of Cr in the intestine of *Clarias gariepinus*.

Cadmium is anthropogenic metal pollutant extremely toxic to aquatic animals with a long biological half- life and produce renal and hepatic injuries in land animals and fish. (Mishra et al. 2019). In the present study the mean value of cadmium concentration in the tissue in order Cd= Gills > Skin> Liver>Muscle. Cd is a non-essential, and element non-biodegradable which is reflected to be a main contaminant that sources antagonistic special effects on the marine environment. (Shivakumar et al. 2014). (Saeed and Shaker 2008), have noted Cadmium concentration 0.19 ppm (dry.wt) in the muscles of fish, *Oreochromis niloticus* collected from Egypt, Northern Delta lakes which exceeds the values detected during this research. In the previous studies of Tiimub & Dzifa Afua 2013 the Concentrations of heavy metals in muscle of the fish samples analyzed in descending order of Fe > Mn > Cd were detected, but, the rest (Pb, Hg and As) were not detected.

CONCLUSION

In the present study *Typha elephantina* methanolic extract was analyzed and showed best result in combine therapy either than alone As the bio-concentration of trace metal like Zn, Ni, Cr, and Cd were determined in various tissues of *Cyprinus carpio.* And it was concluded that lesser concentration was observed when the fish were

treated with *Typha elephantina* extract alone and the concentration was least when treated with combination with vitamin C. For the development of new drugs mankind need to explore the natural herbs. It is recommended that further exploration and analysis of the mention plant is needed.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Current research work was facilitated by Islamia College Peshawar, Khyber Pakhtunkhwa Pakistan

AUTHOR CONTRIBUTIONS

B. Ahmad present the idea, A.M Yousafzai designed the experiment T.W Haq, F.Naz, D.Naz, S.Ahmad and A.Aziz performeed the experiments A.Khan, D.Naz, A.Ali and B. Ahmad wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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