RESTORATION OF HEAVY METALS ACCUMULATION AND HEMATO-BIOCHEMICAL MARKERS IN *PUNTINS TICTO* FISH WITH ADMINISTRATION OF *ZIZIPHUS OXYPHYLLA* EDGEW EXTRACT

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ABSTRACT

The present study was aimed to investigate the curative effects of *Ziziphus oxyphylla* (Z.O) leaves aqueous extract on bioaccumulation of heavy metals along with blood and biochemical variables in *Puntius ticto* . The heavey metals such as Zn, Ni, Cd and Cr were analysed in muscle, liver, gills, skin and intestine while, complete hematology , lipid profile and some liver and kidney related biochemical parametes were analyzed. The fish were first exposed to $CaCl_2$ and then treted with extract of (Z.O) 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 revealed that extract alone (group 2) has no effect while the co administration 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).

Key words: *Puntius tict,* Bioaccumulation, Heavy metals, Skin, Gills and Muscles.

INTRODUCTION

The accumulation of heavy metals in aquatic life indicates the way of aqueous pollution and fishes are commonly used as bio-indicators of heavy metals contamination (Rasmussen and Anderson, 2000; Waqar 2006; Adami *et al.,* 2002; Rani, 2003). Heavy metals such as cobalt, Iron, copper, manganese, zinc and molybdenum are imprtant in trace amount for human being.(Lane and Morel, 2009). However, thses metals are lethal at higher concentrations.(Chronopoulos *et al*.,1997). The natural aqueous systems is broadly contaminated with heavy metals come out in the form of industrial, domestic and agricultrl effeluents which increasing at an alarming rate and has become a universal health issue (Malik *et al.,* 2010). 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 biomagnification (Agah *et al.,* 2009). Metals are considered as main environmental contaminants and are non-biodegradable which causes cytotoxic, carcinogenic and mutagenic effects in animals (More *et al*., 2003). Fishes are considerably affected higher than other vertebrates because of their feeding habits and habitat (Sun *et al*., 2011). To inspect the physiological state of both human or animal heamatological indices are very important (Khalid, 2011; Maiti *et al*., 2012). 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 (Medugu *et al*., 2010: 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 (Aldrin *et al*., 1982).

Thus current study was planned to analyzed useful impact of *Zizyphus oxyphyla* leaves aqueous extract on accumulation of some heavy metals in gills, skin, muscle and liver of the fish *Puntius ticto* along with hematobiochemicl indices.

MATERIALS AND METHODS

Fish grouping, treatment and dissection

Total of more than 60 fish i.e *Puntius ticto* (palpate) common name of equal length and size were reared in a separate fish aquarium i.e. group 1, group 2 and group 3. These fishes of various groups were treated for twenty days regularly as follows:

Group 1 retained as normal control fish , group 2 received $CaCl_2$ at dose rate 5 mg/kg body weight and *Zizyphus oxyphylla* leaves aqueous extract (250 mg /kg) while, group 3, exposed to $CaCl_2$ (5 mg), (Z.O) extract (250 mg) and ascorbic acid (10 mg) respectively. On day 21 of the fishes of various experimental groups were rinsed with aqueous with 0.7% NaCl mixed and dissected for various tissues such as skin, muscles, gills and liver tissues and were stored at -21^0C in the freezer.

Digestion of fish tissues

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 flasks were washed with distilled aqueous and dried in oven at 60^0C. The weight

of respectively tissue was shifted to these volumetric bottles after the identification. According to the method of Due Freez and Stein (1992) and Van Loon (1989) samples were digested. A small change was made in the process (Yousafzai and Shakoori, 2006). Instead of putting 5 mL 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 250^{0}C. 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 10mL 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 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.

Assessment of serum biomarkers and lipid profile

For the serum, each tube was centrifuged at 4000 rpm for 10 minutes. Clear serum after centrifugation was used for serum markers such as alkaline phosphatase (ALP) and alanine aminotransferase (ALT) and alkaline serum transaminase phosphatase (AST) similarly lipid profile like HDL, LDL, Triglycerides and cholesterol along with total proteins, urea creatinine and glucose were also measured on UV visible spectrophotometer (Agilent 8453) via commercially available kits (AMP Diagnostics, Austria).

Statistical analysis

Mean of the data and Standard error of mean was calculated using one way ANOVA test.

RESULTS

Zinc levels were found highest in the gills, skin, intestine, liver and muscles tissues of *Puntius ticto* that were exposed to $CaCl_2$ at dose rate 5mg/kg body weight and aqueous extract at 250 mg/kg body weight (group=2) when compared to control fish (group=1). However, co-administration of extract (250mg/kg body weight) and ascorbic acid (10 mg/kg body weight), 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 aqueous extract dose rate 250 mg/kg body weight after 5 mg/kg body weight exposure of $MgCl_2$ respectively. However, this elevated level of (Ni) was signinificanly (P< 0.05) reduced in fish (group=3) when compared to control fish (group=1) (Table 1; table 5).

Similarly, cadmium (Cd) and chromium (Cr) concentration were also found significantly high in fish (group= 2), showed that aqueous extract of (Z.O) at 250 mg/kg body weight had no effect. While, the administration of extract (200 mg/kg body weight) and ascorbic acid (10 mg/kg body weight) after the exposure of $MgCl_2$ (5mg/kg body weight) significantly (P<0.05) decline the levels of (Cd) and (Cr) when compared to control fish (Table 1,2,3,4 and table 5). Fish that were treated with aqueous extract (250 mg/kg body weight), after exposure of $CaCl_2$ (5 mg/kg body weight) showed no curative effects on blood profile. Hence these animals have significantly (P<0.05) irregular hematological parameters like red blood cells, white

blood cells, , HCT, Hb, MCV, MCH, MCHC, lymphocytes, monocytes and neutrophil), revealed toxicity (group=2). Although the combine treatment of animals with aqueous extract 250 mg/kg body weight and ascorbic acid at 10 mg/kg body weight significantly (P<0.05) improves all the hematological parameters after the exposure of $CaCl_2$ (5 mg/kg body weight) when compare to control animals (Table, 6).

Table 7, signifies 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 (Z.O) aqueous extract showed no optimistic effects on above mention serum parameters hence (group= 2), showed $CaCl_2$ 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 aqueous extract (250 mg/kg body weight) after exposure to $CaCl_2$ (5 mg/kg body weight) respectively (Table 8). The increased levels of above mentioned serum parameters indicates that the provision of extract alone, had no recovering effect, therefore $CaCl_2$ revealed toxicity in fish group=2. In other hand co-administration of extract 250 mg/kg body weight and ascorbic acid (10 mg/kg body weight), 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 8)

Table 1. Shows metals accumulation level in muscle tissue of *Puntius ticto* fish of various experimental groups

Metals	Group=1	Group = 2	Group = 3
Zinc	0.070±0.01	0.510±0.032	0.0048±0.0030
Nickle	0.0023±0.001	0.042±0.04	0.0415±0.0051
Cadmium	0.0040 ±0.002	0.0028 ±0.002	0.00687±0.0017
Chromium	0.00650±0.00081	0.014±0.003	0.0053±0.00208

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + $CaCl_2$=05 mg +Vitamin C=10 mg/kg

Table 2. Represents metals accumulation level in skin tissue of *Puntius ticto* fish of various experimental groups

Metals	Group=1	Group = 2	Group = 3
Metals	Group=C	Group = ES	Group = ESV
Zinc	1.33±0.50	1.82 ±0.130	0.04275±0.004349
Nickle	0.040±0.01	0. 085 ±0.050	0.0550±0.005
Cadmium	0.0167±0.007	0.07 ±0.02	0.023±0.0033
Chromium	0.055±0.001893	1.43±0.067	0.02775±0.004717

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + $CaCl_2$=05 mg +Vitamin C=10 mg/kg

Table3. Displays metals accumulation level in gills of *Puntius ticto* fish of different experimental groups

Metals	Group=1	Group = 2	Group = 3
Metals	Group=C	Group = ES	Group = ESV
Zinc	0.074±0.0061	0.74±0.007	0.06667±0.005774
Nickle	0.050±0.004	0.073±0.02	0.0400±0.009
Cadmium	0.023±0. 001	0.09 ±0.0	0.02633±0.0015
Chromium	0.053±0.008505	0.54±0.053	0.0600±0.034

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + $CaCl_2$=05 mg +Vitamin C=10 mg/kg

Table 4. Shows metals accumulation level in liver of *Puntius ticto* fish of various experimental groups

Metals	Group=1	Group = 2	Group = 3
Zinc	0.081±0.001	0.615±0.524	0.006467±0.0005859
Nickle	0.0031±0.0020	0.068±0.003	0.0400±0.0100
Cadmium	0.00523±0.000	0.0074±0.4	0.0087±0.0011
Chromium	0.0041 ±0.0004	0.025± 0.027	0.0093±0.001136

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + $CaCl_2$=05 mg +Vitamin C=10 mg/kg

Table 5. Shows metals accumulation level in intestine of *Puntius ticto* fish of various experimental groups

Metals	Group=1	Group = 2	Group = 3
Zinc	0.53±0.01	0.816±0.055	0.0735±0.00695
Nickle	0.024±0.0056	0.053±0.001	0.047±0.01835
Cadmium	0.037±0.012	0.083 ±0.02	0.0570±0.02539
Chromium	0.045±0.01242	0.5893±0.066	0.0507±0.008

Treatment= Group 1, control fish, group 2, Extract =250 mg + Cacl2=05 mg/kg and group 3, Extract =250 mg + Cacl2=05 mg +Vitamin C=10 mg/kg

Table 6. Shows mean hematological values of *Puntius ticto* fish in various experimental groups

Blood Markers	Group=1	Group = 2	Group = 3
RBCs (×106 µl)	1.717 ± 0.24	2.850±0.2170	1.950±0.04583
WBS ×103µl	4.467 ±0.4244	7.367±0.4606	5.600±0.6502
HCT (%)	33.01 ±2.4	22.34±1.125	29.08±1.979
Hb (g/dl)	10.29 ± 1.24	7.963±0.24	10. 31±0.58
MCV (fl)	175.5±1.550	153.8±3.292	169.1 ±6.2
MCH (pg)	44.08±2.37	36.08±1.476	41.15±0.73
MCHC (gr/d)	22.59±1.73	17.59±0.7304	20.89±0.577

Lymphocyte (%)	50.21 ± 0.3900	45.00±1.845	46.96± 1.82
Monocyte (%)	3.120 ± 0.23	2.100±0.1015	2.933±0. 0510
Neutrophil (%)	35.78±2.48	43.78±2.20	36.44±2.36

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + $CaCl_2$=05 mg +Vitamin C=10 mg/kg

Table7. Shows mean values of liver related and other serum biomarkers of *Puntius ticto* fish in various experimental groups

Serum biomarkers	Group=1	Group = 2	Group = 3
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
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
Glucose (mg/dl)	157±2.12	192.5±3.052***	161.9 ± 2.23

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + CaCl2=05 mg +Vitamin C=10 mg/kg

Table 8. Shows mean values of serum lipid profile of *Puntius ticto* fish in various experimental groups

Lipid biomarkers	Group=1	Group = 2	Group = 3
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
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

Treatment= Group 1, control fish, group 2, Extract =250 mg + $CaCl_2$=05 mg/kg and group 3, Extract =250 mg + $CaCl_2$=05 mg +Vitamin C=10 mg/kg

DISCUSSION

Heavy metals like Cd, Cr, Ni, and Zn were analyzed for the bioaccumulation in the muscle, liver, gills and skin tissues of fresh aqueous 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 (Yallapragda and Chinni, 2000). In the current study of Zinc concentration was observed more 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 (Yousafzai, 2004). Beveridge *et al.*, (1985) have also reported the lowest level of heavy metals in the muscles.

Skin of the fish is in direct contact with aqueous 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 Mulley, Wallago *attu* have accumulated high concentration of Zn as compared to other fish tissues. Excessive Zn increase can be toxic and has been connected to the neurodegeneration (Qiu and Hogstrand, 2005). Reid (1990) 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 aqueous. Similarly, Ishaq *et al.,* (2011) has recorded highest Zn concentration in gills of *Clarias gariepinus* which is inline of our detected toxic fish (group=PE) values. Previously Crepso *et al.,* (1979) 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 the present study the concentration of nickel in gills>skin >intestine>liver and >muscle. According to Muiruri *et al.,* (2013) 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,* and *Cyprinus carpio* .

Chromium is a vital trace metal both for human and animals but in high level it is neurotoxic and carcinogen (Gulfaraz *et al*., 2001). 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 and gills revealed highest chromium concentration which is due large surface area for exposure to the surrounding aqueous. Previously, Yilmas, (2003) has 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, Ishaq *et a*l., (2011) 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 (K.Mia *et al* 2006). 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. (Raspor and Filipovic, 2003; Anderson and Rasmussen, 2000).

Ibrahim and Samir, (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 and 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

The present studies confirm the presence of some hazardous element in aquatic environment. The bio-concentration of trace metal like Zn, Ni, Cr, and Cd were determine in altered tissues of *Puntius ticto*. Thus highly accumulated metal was Zn followed by Ni while Cd was the least accumulated metal in various experimental fish

groups. Similarly, serum related parameters and hematological indices were also been evaluated in different groups treated with various chemicals and plant extract. it was concluded that the toxicity caused by $CaCl_2$ has been regulated by the con administration of extract and ascorbic acid thus indicating the antioxidant potential of the study plant.

During our studies it was also been identified and confirm by the comparing of determine heavy metal with that of FAO (1989), WHO (1989) and U.S suggested daily dietary Allowance (RDA, 1989) showed that our detected values did not exceed the usual values. However inducing toxicity in laboratory can harm the fish health and immune system lead to high bioaccumulation of various metals in different tissues. Therefore can be concluded that progressive increase in the environmental pollutants from anthropogenic activates like industrialization, urbanization and miningingcan extremely pollute the natural aquatic ecosystem and aquatic life. therefore it is necessary to develop new and valuable drugs for incoming expected infections and diseases caused by environmental pollutants which are very toxic to human especially metals that are traveled through food chain to the human body. For the development of new drugs mankind need to explore the natural herbs. Since in the present study *Ziziphus oxyphylla* leaves aqueous extract was analyzed and showed best result in combine therapy either than alone. It is recommended that further exploration and analysis of the mention plant is needed.

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Author's Contributions

Conflicts of Intersts

The authors declare that they have no conflicts of interest.

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