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Human Health Risk Assessment and Histopathological Alterations in *Puntius sophore* of Damsal Nala, Sukinda Chromite Valley, Odisha, India

Mondal Niladri Sekhar[1,2], Mandal Arghya[1], Kole Debraj[1], Patra Atanu[1], Das Subhas[1], Ghosh Apurba Ratan[1*]

[1]Ecotoxicology Lab, Department of Environmental Science, The University of Burdwan, Purba Bardhaman, West Bengal, India
[2]Department of Environmental Science, School of Sciences, Netaji Subhas Open University, DD-26, Sector-I, Salt Lake City, Kolkata 700 064, West Bengal, India

**Corresponding Author*

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Abstract: The aim of the present study was to assess the concentration of potentially toxic metals (PTMs) like As, Cd, Cr, Cu, Fe and Pb in the Damsal Nala of Sukinda Chromite Valley, Jajpur of Odisha (India) and its subsequent histopathological lesions in the fish *Puntius sophore*. Energy Dispersive X-Ray Fluorescence (EDXRF) and Flame Atomic Absorption Spectroscopy (FAAS) methods were used to evaluate the concentrations of PTMs in the muscles of fish, *Puntius sophore* collected from different zones namely upstream discharge zone (UDZ) and upstream zone (UZ) of the Damsal Nala and control water bodies (CW). In the UDZ of Nala, the concentration of metals viz., Cd, Pb and Fe were 2 to 7 times, and Cr was 77 times higher than the maximum permissible limits recommended by WHO and FAO in the fish muscles. The values of Target Hazard Quotients (THQ) of all these 6 metals were <1 in CW and UZ, but in UDZ the THQ values of Cr and Fe were >1. Hazard Index (HI) for UDZ was 3.47, indicated the alarming concentration of metal which can pose serious risk to human health. Histopathological observations in liver and kidney of *Puntius sophore* collected from UDZ demonstrated the serious lesions, but mild damage in fish collected from UZ. These histological alterations and the health risk indices disclosed the effects of effluents of chromite mining and the impact on the ecological balance which may result into health hazards to the local human population who are maintaining their livelihood by consuming these fish as protein source.

Keywords: Potentially toxic metals, Health risk assessment, Histopathology, Chromite mine, *Puntius sophore*, Hazard index

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Introduction

Increasing production and extraction of metals from mines are meant to cater the rising demands of human well-being. Huge amount of metals from these mining industries are liberating large amount of metal ions in different ecosystems leading to long term bioaccumulation, which cause the major environmental hazards. Several authors (Banerjee *et al*., 2016; Naz *et al*., 2016a) studied the health hazards to the exposed human population. Chromite mines produce large amount of chromium (Cr) which is mostly used in various manufacturing and processing industries like glass manufacture and paints industries, steel alloy, tannery, dyes, rust proof chrome-plating etc. (Godgul and Sahu, 1995; Das and Singh, 2011; Dutta, 2015; Saha *et al*., 2017). As a result to meet up the increasing need day-by-day the production of Cr is also increased by 0.81 million tons yearly in 2016 (3.73 million tons) from 2015 (2.92 million tons) (IBM, 2019). A contribution of 98% of chromite from chromite ore ($FeO.Cr_2O_3$ or $FeCr_2O_4$) is coming from Sukinda Chromite Valley, in Jajpur district of Odisha (India) with regard to total Indian deposits (Das and Mishra, 2010; Naz *et al*., 2016b); as a consequence it produces huge amount of overburden which subsequently drain off the mining residues consisting of different ions of potentially toxic metals viz., Fe, Cr, Ni, Cu, Zn, Pb, As and Cd that are contaminating our different ecosystems (Iyer and Mastorakis, 2010; Rashed, 2010; Mohapatra and Kirpalani, 2017; Saha *et al*., 2017). The ionic forms of these metals are posing damage to the natural environment because of their properties of bioaccumulation and biomagnifications (Das and Singh 2011; P. Madejón *et al*., 2002; Paula Madejón *et al*., 2006). Damsal Nala is passing through the Sukinda Chromite Valley and is carrying the residues from the adjacent overburden and mining activities and causing contamination to the surrounding areas, including aquatic bodies (Iyer and Mastorakis, 2010; Dutta, 2015). Kumar and Maiti (2015), Banerjee *et al*. (2016) and Zhang *et al*. (2016) studied the effects of these leachates and drainage containing a huge amount of heavy metals that undergoing accumulation in the habitants, resulting into acute or chronic abnormalities in the target organs, which may finally cause potential human health risk. Various researchers explained the haematological, histological, cytotoxic and genotoxic effects of Cr and other PTMs like Cd, Pb and Fe on fish (Marchese *et al*., 2008; Poleksic *et al*., 2010; Velma and Tchounwou, 2010; Fatima and Usmani, 2013; Kole *et al*., 2016; Nawab *et al*., 2016, 2017). Fish is an excellent model to assess the magnitude of the toxicity of an aquatic ecosystem (Javed *et al*., 2016; Samanta *et al*., 2018; Mandal *et al*., 2020). It can also be used to calculate the long term and cumulative, as well as magnified toxicosis of these metals to the higher tropic levels, even in human beings who use fish as a common protein source.

Present study intended to assess the extent of toxicity of these PTMs and their accumulation concentration in the fish muscle of Damsal Nala (wild catch) causing consequent histopathological alterations; and finally, to evaluate the human health risk assessment of metal contamination through various indices like THQ, Hazard Index (HI) etc.

Materials and Methods

Study area:

Present experimental site known as Sukinda Chromite Valley is located in the Jajpur district of Odisha (India). The sampling zones of Damsal Nala is geographically situated within latitudes $21^0 25'$ and $21^0 45'$ and longitudes $85^0 46'2''$ and $85^0 5'8''$ (Fig. 1). The normal water bodies (as control) named as CW1 and CW2 are situated geographically $21^0 56'45.89''$N- $86^0 7'40.18''$E and $21^0 56'30.28''$N- $86^0 8'34.51''$E, respectively. Actually, this Damsal Nala is the source of water for agriculture and surrounding residents, which is also carrying the total drain off from the overburden of adjacent mining areas. So, it was chosen to estimate the effects and ecological impact of Cr and other associated PTMs in the different zones of Damsal Nala.

Sample collection:

A total number of twenty-four fish (*Puntius sophore*) samples were procured from eight sites, i.e. UZ (S1 to S3), UDZ (S4 to S6) and two normal

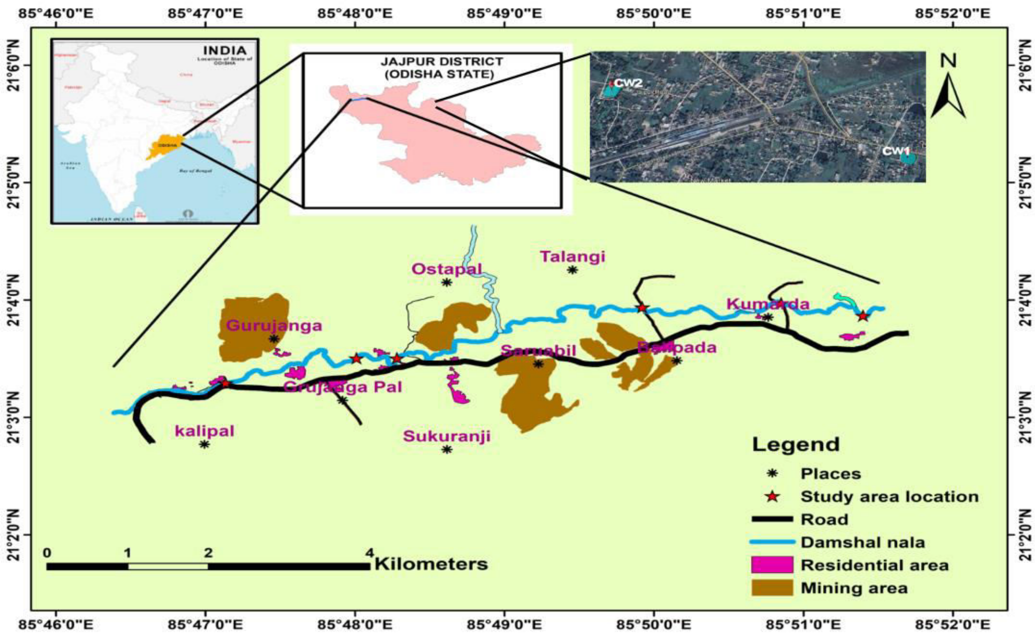

Fig. 1: Location map of the study area

water body (CW) as CW1 and CW2 to compare with contaminated areas (Fig. 1). The collected fish specimens from respective zones and the desired tissues like liver and kidney were collected and fixed into Bouin's solution for histopathological study. The desired amount of fish muscles were taken for estimation of metals' concentration using Energy Dispersive X-Ray Fluorescence (EDXRF) and Flame Atomic Absorption Spectroscopy (FAAS). The present study was conducted during the year 2018-2019.

Sample preparation:

Freeze-dried fish muscles were prepared into pellets of 13 mm diameter using a tabletop palletizer (100-130 kg/cm^2) for elemental analysis by EDXRF. For elemental assessment via FAAS, the fish muscle was digested through microwave digestion at 450W for 7 min with 10 ml of concentrated HCl, 5 ml of concentrated HNO_3 and 2 ml of $HClO_4$ followed by H_2O_2 treatment.

Human health risk assessment:

The health risk assessment includes the identification of the pathway through which a toxicant comes to an organism (EPA, 1989). It can be done through ingestion, inhalation, or by dermal contact. In the present study, we used fish muscles for evaluating the potential human health risk because fish muscle is mainly used for consumption as a protein source by human population. We have assessed the risk by Estimation of Daily Intake (EDI) of the metals, Target Hazard Quotients (THQ) and Hazard Index (HI). The estimated daily intake of PTMs depends on both the concentration of the PTMs in fish and the amount of fish ingested. EDI is calculated by following equation:

$$\text{EDI } (\mu g/kg/day) = \frac{C_{metal} \times \text{FIR}}{B_W}$$

Where, C_{metal} is the concentration of individual metal in fish (µg/g dry weight), a conversion factor of 4.8 is used to get dry to fresh weight of fish (Rahman *et al*., 2012). Food Information Regulation (FIR) represents the daily consumption of fish per person (g/day/capita). Average FIR is 21 g/person/day and B_W is the average body weight of an adult in kilogram, which is 52 kg in context of India (Dang *et al*., 1996; Giri and Singh, 2015).

THQ is the most competent and reasonable index to assess the potential risk from the intake of toxic metals on human health as recommended by the USA Environmental Protection (EPA, 1989; Maurya *et al*., 2019). Accordingly, if the value of THQ is more than '1', then the exposed population is under the deleterious effects of that contaminated food. It can be calculated by following equations:

$$THQ = \frac{Ffr \times ED \times FIR \times C_{metal} \times 10^{-3}}{R_f D \times B_W \times AT_n}$$

$$THQ = \frac{EDI \times Ffr \times ED \times 10^{-3}}{R_f D \times AT_n}$$

Where, *Ffr* is exposure frequency (365 d/yr), *ED* is exposure duration (70 yr, for this study), R_fD is the reference dose that is the non-deleterious tolerable daily intake of a specific metal (µg/kg/day), and AT_n is the average time of exposure for the toxic metal (365 d x no. of exposure years). For multiple metal risk assessment present in fish, HI is introduced by summing the THQ values of all the metals through equation:

$$HI = \sum_{i=1}^{n} THQ_i$$

Where, THQ_i refers to target hazard quotient against a single metal, and in the present study, HI indicates the total thrust of all metals considered here.

Histopathological observations:

Histopathological effects due to exposure of potential toxic metals were investigated in the tissues like liver and kidney from the fish samples collected from control water body (CW), UZ and UDZ. The Bouin's fixed tissues were routinely processed for staining with Hematoxylin-Eosin (HE) and observed under a Light Microscope (Leica DM 2000 with an attached camera, EZ).

Statistical analysis:

SPSS program (v.16.0) was used for calculating mean and standard deviation. ANOVA-one way and Tukey test were also performed.

Results and Discussion

Distribution of metal concentration in fish:

The concentration of heavy metals in fish muscle is illustrated in Table 1. In the control water body (CW), the average concentration of the metals like As, Cd, Cr, Cu, Fe and Pb was 0.22±0.08, 0.22±0.04, 0.88±0.02, 2.57±0.19, 86.64±3.70 and 0.26±0.01 mg/kg of fresh weight of fish, respectively. All the concentrations of metals seem to be low, or very near to the permissible limits (as per the recommendation of WHO and FAO). In UZ, the concentrations of Cu, Cd, and Fe were 30, 0.5, and 43 mg/kg of fresh weight of fish, respectively, which were below and near to the permissible limit as recommended by WHO and FAO (Nauen, 1983). The concentrations of Cu, Cd and Fe were 5.08±1.49, 0.71±0.12 and 57.34±27.85 mg/kg of fresh weight of fish, respectively. The concentrations of Cr and Pb (4.84±2.26 and 5.45±1.20 mg/kg of fresh weight of fish, respectively) were much higher (about 30 and 10 times higher, the permissible limit of Cr and Pb is 0.15 and 0.5 mg/kg of fresh weight, respectively) than the recommended limit by FAO (Nauen, 1983) and the Indian Standard (Giri and Singh 2015). In the UDZ, the concentration of all other metals except Cu was much higher such as cadmium (1.17±0.08 mg/kg) two times, chromium (11.60±3.68 mg/kg) seventy-seven times, iron (326.90±29.22 mg/kg) seven times and lead (1.11±0.46 mg/kg) two times higher than the maximum permissible limit.

The bioavailability of metals in the fish muscles was maximum in the zone, which is very near to the mines and at the point of discharge area of UDZ. The maximum concentration of these metals in fish has also been estimated by various researchers throughout the world, but this concentration varies according to their study fields, however, in most of the cases, the mining areas are suffering from associated metal contaminations (Mohamed and Mohamed, 2005; Rashed, 2010; Shah *et al*., 2012). The impacts of enrichment of metal in water were best evaluated by the analysis of aquatic organisms especially fish, as it is a good bioindicator. In the present study, the concentration of metals measured in the edible part (muscle) in UDZ was in the order of Fe>Cr>Cu>Cd>Pb>As and in UZ was Fe>Pb>Cu>Cr>Cd>As. The concentration of Fe, which was found to be higher, was also recorded by other researchers (Maurya *et al*., 2019), and the concentrations of Cr, Cd and Pb exceeded the permissible limits due to the discharged effluents from chromite mining and their trailing also by leaches (Godgul and Sahu, 1995; Dutta, 2015; Naz *et al*., 2016a; Saha *et al*., 2017). The process of bioaccumulation of these metals for a period of time in fish fauna may also affect the local community who are used to consume these intoxicated fish regularly.

Pollution level and Evaluation of EDI, THQ, and HI of metals:

Average fish consumption by human being is 21 g of fresh weight per day (Rahman *et al*., 2012); accordingly, in the present study, the EDI values of the metal concentrations in fish can be calculated from Table 1 and are shown in Table 2. The Referred dose (R_fD) is also listed in Table 2, as established by the United States Environmental Protection Agency (Smith, 1995; USEPA, 2000). Daily dietary intake (EDI) of Fe and Cu was highest in control pond, in UZ and UDZ the concentrations of metals like Fe, Cu and Cr were higher than the other metals. THQ value is an integrated risk index used to assess the potential risk from the intake of toxic metals on human health, as recommended by the USA Environmental Protection Agency (USEPA, 1998; Storelli, 2008; Rehman *et al*., 2018). In the control pond, THQ values of all the metals were less than '1', so, it is without any potential risk. In UZ, the THQ values of all other metals were lower than 1, but for Cr and Pb, they were very high in comparison to the others, and in UDZ, the THQ values of Cr and Fe were more than '1', i.e., 1.56 and 1.20, respectively. These results indicated that fish consumption from UDZ would pose a deleterious impact on human health. Hazard Index (HI) is the sum of the impacts of all metals, and its value in the control water body was less than '1', which means it is safe for human intake. But, in UDZ, it was very high, i.e., 3.47, and in UZ it was 1.89. So, both the values are of serious concern, especially, consumption of fish from UDZ is deleterious and it can affect the human population badly due to long term consumption because of the presence of Cr and Pb in the edible part (muscle) of the fish species from Damsal Nala (Guang *et al*., 2014; Islam *et al*., 2016; Maurya *et al*., 2019; Nargis *et al*., 2020). This also indicated the potential contamination of the aquatic environment and its fish inhabitants by metals due to chromite mines and their leachates.

Histological alterations:

Liver:

The sections of liver tissue of fish collected from the CW showed a regular arrangement of normal parenchymatous hepatocytes as lattice network. It is made up of polygonal hepatocytes with central spherical and densely stained nucleus and nucleolus (Fig. 2a). Under light microscopy, the liver is composed of irregular lobules separated by the hepatopancreas and bile duct. Central veins were observed randomly throughout the hepatic parenchyma between the cords of hepatocytes endorsing a three-dimensional network of cylindrical blood sinusoids.

The light microscopic changes in fish liver exposed to the area before the zone of discharge (UZ) showed damage in parenchymatous tissuesin some regions, and the hepatopancreas was degenerated and scattered. Vacuolations occurred in the cytoplasm and the hepatocytes with the appearance of slightly distorted blood capillaries in the central veins (Fig. 2b). Vacuolization of hepatocytes is a significant symptom under the exposure of metals (Hermenean *et al*., 2015). Comparable histopathological variations, viz., vacuolization, necrosis, inflammation etc. in liver were also reported by few authors (Radhakrishnan and Hemalatha, 2010; Subburaj *et al*., 2015). The histopathological variations are frequently linked to fish living in the aquatic body

Table 1: Estimation of concentration of metals in fish muscle (mg/kg of fresh weight) of Damsal Nala

Elements	CW		UZ			UDZ		
	CW1	CW2	S1	S2	S3	S4	S5	S6
As	0.16±0.02^{a}	0.27±0.02^{ab}	0.45±0.00^{c}	0.43±0.01^{c}	0.46±0.01^{c}	0.51±0.00^{c}	0.45±0.02^{c}	0.37±0.01^{b}
Cd	0.24±0.02 ^{a}	0.19±0.01 ^{a}	0.58±0.02^{ab}	0.71±0.08^{b}	0.83±0.01^{bc}	1.10±0.0^{c}	1.15±0.00^{cd}	1.25±0.01^{d}
Cr	0.87±0.07^{a}	0.89±0.01^{a}	2.75±0.13^{b}	4.54±0.04^{bc}	7.23±0.14^{c}	14.90±1.41^{d}	12.27±0.62^{d}	7.64±0.74^{c}
Cu	2.43±0.16^{a}	2.71±0.13^{a}	6.60±0.14^{c}	5.01±0.23^{bc}	3.63±0.23^{b}	3.79±0.35^{b}	5.54±0.02^{bc}	4.76±0.38^{bc}
Fe	84.03±2.09^{b}	89.26±5.62^{b}	28.61±0.27^{a}	59.20±0.29^{ab}	84.22±1.19^{ab}	321.11±7.43^{c}	358.58±9.31^{c}	301.00±3.80^{c}
Pb	0.26±0.01^{a}	0.26±0.05 ^{a}	6.80±0.50 ^{d}	5.08±1.21^{cd}	4.48±0.31^{c}	0.71±0.16^{a}	1.01±0.17^{ab}	1.62±0.53^{b}

Different letters indicated significant differences ($p < 0.05$) of the parameters between each site based on Tukey's HSD test.

Table 2: Analysis of Estimation of Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI) of metals through fish consumption from different zones of Damsal Nala

Elements	*CW*			*UZ*			*UDZ*			*RfD (mg/kg/D)*
	Concentration	*EDI*	*THQ*	*Concentration*	*EDI*	*THQ*	*Concentration*	*EDI*	*THQ*	
As	*0.22*	*0.088*	*0.029*	*0.45*	*0.181*	*0.060*	*0.44*	*0.179*	*0.060*	*0.003*
Cd	*0.22*	*0.087*	*0.087*	*0.71*	*0.286*	*0.286*	*1.17*	*0.472*	*0.472*	*0.001*
Cr	*0.88*	*0.355*	*0.118*	*4.84*	*1.956*	*0.652*	*11.60*	*4.686*	*1.562*	*0.003*
Cu	*2.57*	*1.037*	*0.026*	*5.08*	*2.051*	*0.051*	*4.70*	*1.898*	*0.047*	*0.040*
Fe	*86.64*	*34.990*	*0.318*	*57.34*	*23.158*	*0.211*	*326.90*	*132.015*	*1.200*	*0.110*
Pb	*0.26*	*0.105*	*0.030*	*5.45*	*2.202*	*0.629*	*1.11*	*0.449*	*0.128*	*0.004*
HI	*0.609*			*1.890*			*3.470*			

RfD = recommended doses of heavy metals as established by the United States Environmental Protection Agency (Smith, 1995). The concentration of Metals is mean concentration from the respective zone in mg/kg.

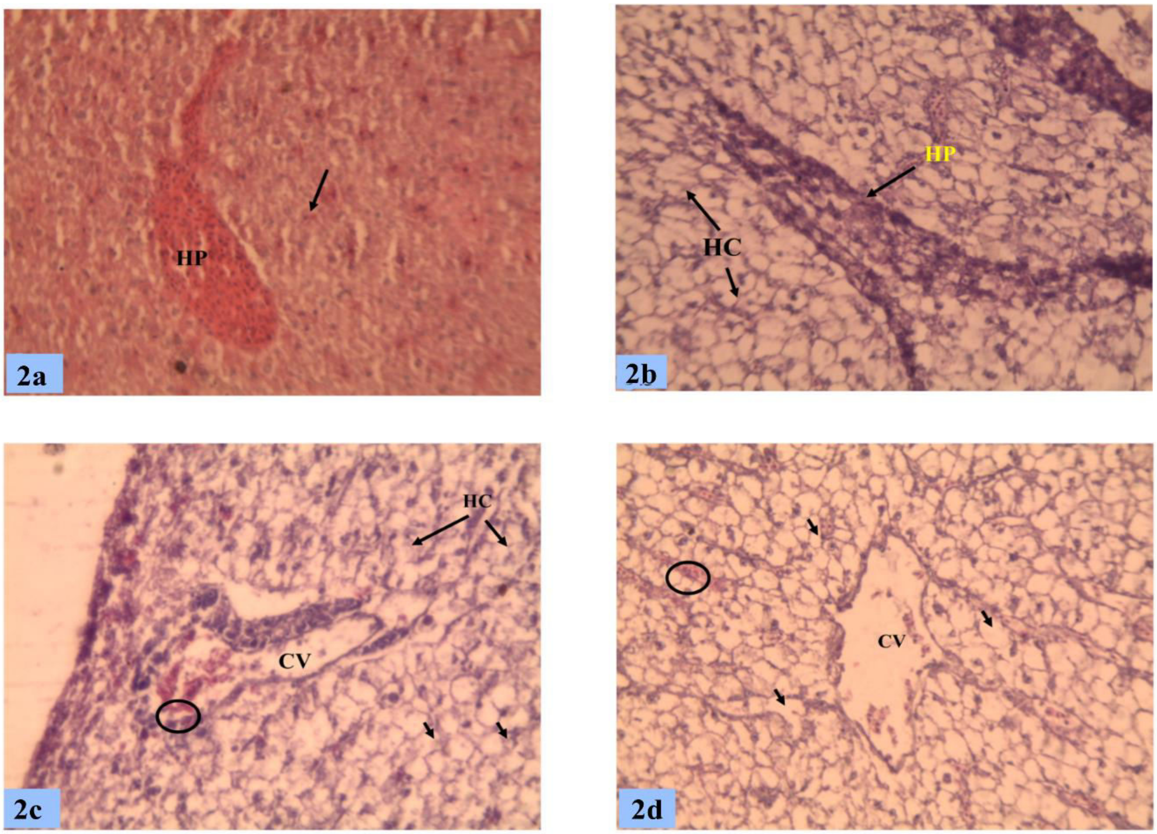

Fig. 2: Photomicrographs of T.S. of Liver of *Puntius sophore.* (2a)- Control fish showing hepatopancreas (HP), hepatocytes (arrow), and sinusoids (bold arrow) (400x); (2b)- Fish liver from UZ showing HP, hepatic cells (HC), and large nucleus (N) (400x); (2c)- Fish liver from UDZ showing sinusoids (bold arrow), HP, Hepatocytes (arrow), and damaged hepatic lobule (oval) (400x); (2d)- Fish liver from UDZ showing central vein (CV) and damaged hepatic lobule (oval) (400x).

where mining activities are caused (Carrola *et al*., 2009; Jordanova *et al*., 2016). The present study also displayed the similar results.

Severe histopathological lesions in the liver tissue were observed in the UDZ, showing profound damage to parenchymatous tissues and degeneration of hepatocytes (Figs. 2c, 2d). Fibrosis of parenchyma as the intoxication-related modification was comparatively infrequent in fish, differing to mammals, but cholangiofibrosis (Wolf and Wolfe, 2005) and fibrosis of periportal and portal areas (Rašković *et al*., 2015) were common symptoms. Proliferation of bile ducts was also a predominant histopathological change in the mine draining and tailings areas (Carrola *et al*., 2009; Jordanova *et al*., 2016), but this damage was not observed in the present study. This modification is likely to be distinguished for certain species, as observed by Lukin *et al*. (2011). Widespread histopathological abrasions in the liver tissue were detected because of the oxidative stress caused by metal accumulation in the organs. Hepatic lobules showed serious distortion and damaged capillary networks. The fibrosis was detected as a result of hepatic cellular injury and also liver necrosis was noticed due to enzymatic inhibition and may be due to instabilities in the protein and carbohydrate metabolism. In the liver, lesions were noticed because of excessive fatty infiltration and irregular lipid accretion in the liver cells. The cytoplasm of the hepatocytes was highly degenerated, vacuolated and the cell contour of the hepatocytes became altered. The nuclei were reduced in size. The hepatocytes in some areas showed a syncytial mass in appearance (Fig. 2d). Since, the liver is the

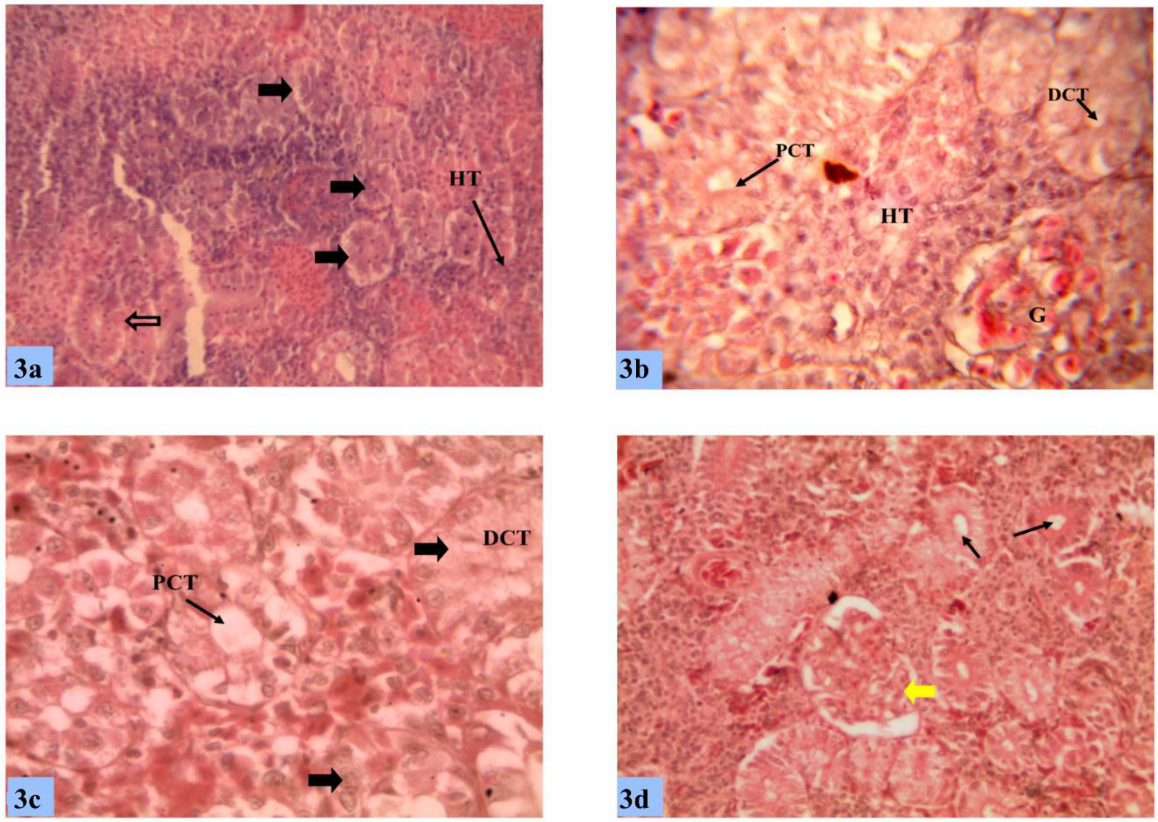

Fig. 3: Photomicrographs of T.S. of Kidney of *Puntius sophore*. (3a)- Control fish showing proximal convoluted tubules (bold arrow), distal convoluted tubules (arrow) and hematopoietic tissue (HT) (400x); (3b)- Fish kidney from UZ showing PCT, DCT, glomeruli (G), and HT (1000x); (3c)- Fish kidney from UDZ showing PCT, DCT (bold arrow), and HT (1000x); (3d)- Fish kidney from UDZ showing shrinkage of glomeruli (yellow arrow) and PCT (arrow) (400x).

foremost organ for detoxification, any modifications in it can be a beneficial marker that can designate the preceding exposure to environmental stressors (Patil and David, 2013; Dey *et al*., 2019).

Kidney:

Histological architecture of kidney of fish from control water body displayed the normal arrangement of haematopoietic tissue (HT) comprising renal corpuscles or the Malpighian tubules and renal tubules. The renal corpuscles are numerous, containing vascularized glomerulus. The renal tubules are made up of columnar epithelial cells and differentiated into proximal convoluted tubules (PCT) that are rounded in shape and the distal convoluted tubules (DCT) which are oval with elongated collecting ducts (Fig. 3a).

In the upstream zone, the kidney showed regular arrangement of glomerulus, PCT and DCT, but there were also few damaged glomeruli at some places. Haematopoietic tissues, PCT and DCT revealed slight damage (Fig. 3b). Javed *et al*. (2016) and Velma and Tchounwou (2010) also reported similar histopathological alterations in fishes.

In the discharge zone, the histopathological analysis revealed the shrinkage of glomeruli and hypertrophied PCT, and disorganization of haematopoietic tissues in kidney (Figs. 3c, 3d). Gupta and Srivastava (2006) and Loganathan *et al*. (2006) also reported the hepatic and renal histological changes in various fish models intoxicated with zinc. Brush border in the inner lining of PCT and DCT were markedly damaged and blood vessels showed rupture (Figs. 3c, 3d).

Histopathological lesions in liver and kidney of *Puntius sophore* from UZ and UDZ endorsed the impacts and distinct damage in tissues as well as subsequent effects of accumulation of chromium. Omar *et al*. (2013) also designated the gross histopathological changes as the end-point marker for estimation of toxic potential and risk assessment of heavy metals in gill, liver and kidney of fishes, *Oreochromis niloticus* and *Mugil cephalus*. Ratn *et al*. (2018) described the histopathological alterations like reduction in renal tubules and damage of tubules in fishes exposed to heavy metals.

Conclusion

Present study revealed that the upstream discharge zone of Damsal Nala is severely contaminated with excess concentrations of metals like Cr and Fe. In the upstream discharge zone (UDZ), the THQ values for Cr and Fe and the sum of THQ values, i.e., HI of all 6 metals were more than '1', which indicated potential high health risk to the exposed consumers. Liver and kidney of the fish *Puntius sophore* from UDZ showed serious damage and degeneration that strongly support the ecological impact of PTMs coming from chromite mining activity on aquatic habitat. Therefore, some strategic management plans like judicial pre-treatment before discharge, phytoremediation through locally available macrophytes/weeds etc. to minimize the excess metallic thrust from the mining zones may be developed and practiced to combat the possible health risks to humans and different life forms.

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