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Advances in Bioresearch

ORIGINAL ARTICLE

Bioaccumulation of selected metals and histopathological alterations in tissues of *Adenoscolex oreini* from Dal Lake, Kashmir valley

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ABSTRACT

The concentration of some heavy metals (Cu, Zn, Co, Ni, Mn, Cr, Al, Fe, Ca, Cd, Pb and Hg) in water samples, parasites and tissues of Schizothorax niger obtained from four sites (Dalgate, Saidakadal,Hazratbal and Telbal) of Dal Lake was investigated (atomic absorption spectrophotometry) with emphasis on the histological alterations in these organs. Histopathologically, the parasite induced various intensities of enteritis coupled with hyperplastic goblet cells with increased acid mucopolysaccharide concentrations. In Dal lake the Adenoscolex oreini; S. niger liver and muscle and the ranking of the 12 metals were Ca> Zn> Fe> Mn> Al> Cu> Co> Ni> Cd> Cr;Fe> Ca> Mn> Al> Cu> Zn> Cd> Ni> Co> Cr and Fe> Ca> Al> Mn> Zn> Cu> Ni> Cd> Cr> Co respectively. In river Jhelum it were Ca> Fe> Cr> Mn> Al> Zn>Cu> Cd> Ni> Co; Ca> Fe> Al> Zn> Cu> Mn> Ni> Co> Cd> Cr and Ca> Fe> Al> Zn> Cu> Cr> Ni> Mn> Co> Cd respectively. In Dal Lake the concentrations of copper (1.25µg/g), zinc (5.69µg/g) and iron (29.3µg/g) were maximum in the muscles of infected fishes which were still lower than the Maximum Limit recommended by WHO/ FEPA/IAEA-407 whereas on the other the concentration of nickel (0.80µg/g), manganese (1.69µg/g), cadmium (0.78µg/g) and chromium (2.86µg/g), aluminum (2.99µg/g) were higher than the permissible limits. Cu concentration in Pomphorhynchus kashmirensis and Adenoscolex oreini showed positive correlation with water temperature. Cd concentration in Adenoscolex showed positive correlation with pH of water. However, the Adenoscolex oreini have direct relation with water. Adenoscolex oreini was positively correlated with Cd concentration in water during autumn and winter. Therefore, the parasites seem to act as bio-indicators for the corresponding metals.

***Keywords**: Dal Lake; River Jhelum; Hyperplastic; Goblet cell; Schizothorax Spp; Helminth; physicochemical parameters.*

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INTRODUCTION

Intestinal helminthes of fish are of increasing interest as potential bioindicators for heavy metal contamination in aquatic habitats. Among these parasites cestodes and acanthocephalans in particular have an enormous heavy metal accumulation capacity exceeding that of established free living sentinels. Metal concentrations several thousand times higher in acanthocephalans than in host tissues has been described from field and laboratory studies. Whereas larval stages inside their intermediate hosts are not able to take up high quantities of metals, young worms begin to take up metals immediately after infection of the final host. After 4-5 weeks of exposure, the parasites reach a steady-state concentration and order of magnitude higher than the ambient water level. Thus, acanthocephalans are not only very effective in taking up metals, but they can also respond very rapidly to changes in environmental exposure. The mechanism which enables acanthocephalans to take up metals from the intestinal lumen of the host appears to be based on the presence of bile acids, which form organo-metallic complexes that are easily absorbed by the worms due to their lipophilicity [1]. The permanent contamination of aquatic habitats caused by human activities has become one of the major problems in the era of global industrialization and urbanization. Mostly, chemical pollution and in particular contamination with heavy metals is considered to have an anthropogenic source. A wide range of contaminants are continuously introduced into the aquatic environment mainly due to increased industrialization, technological development, growing human population, oil exploration and exploitation, agricultural and domestic waste run-off [2]. Among these contaminants, heavy metals constitute one of the most dangerous groups because of their persistent nature, toxicity, tendency to accumulate in organisms and undergoing food chain amplifications and being non-degradable [3]. Therefore, this study was conducted to determine the levels of some metals (Cu, Zn, Co, Ni, Mn, Cr, Al, Fe, Ca, Cd, Pb and Hg) in water and tissues.

MATERIAL AND METHODS

Study site

The valley of Kashmir is situated in the mid-Himalayas in the North West and South East direction within the coordinates 33◦01-35◦00 N latitude and 73◦48-75◦30 E longitude at an altitude of ≥1500 m above sea level. The study was carried out in the Dal Lake (34◦07 N- 74◦52 E). Four sites that were selected from Dal Lake viz, Hazratbal Basin, Telbal Nallah, Dalgate ghat and Saidakadal. The field collection was conducted on seasonal basis. The four seasons include spring (March-May, summer (June- August), autumn (September November), spring (March- May) and winter (December-February).

Sample collection

The water samples were acidified immediately after collection by adding 5 ml nitric acid to minimize adsorption of heavy metals onto the walls of the bottles [4, 5]. Fish species (*Schizothorax niger*) along with their parasites were collected randomly from water body with the aid of local fishermen, quickly killed and stored on ice.

Metal estimation

Preparation of standard solutions: Standard solutions were prepared from the stock solution (1000 μg/ml) of each metal. The calibration standards for all of the metals had a concentration working range of 0.1 to 2.0 ppm.

Instrumentation: The absorbance of the calibration standards and samples were measured by Atomic Absorption Spectrophotometer (model, A Analyst 800; Make, Perkin Elmer Ltd).

Water sample preparation and treatment: Processing of water samples for metal analysis was carried out as per the standard methodology of American Public Health Association [6].

Fish tissue and parasites: Fish tissues and parasite were processed for metal analysis [7, 8]. Samples were digested by wet digestion method. The tissue sub-samples were oven dried at 105^{o}C until they reached a constant weight [9].

Gross pathology Fishes were systematically subjected to detailed macroscopic examination with special emphasis on liver, intestine and the lesions were recorded.

Histopathology Representative tissue samples from the liver, intestine affected by parasites were collected in 10% formalin. The tissue samples were processed for routine paraffin embedding technique and 5u thin section were stained with Haris haematoxylin and Eosin [10]. **Histochemistry** Parallel tissue section selected on the basis of histopathological examination was stained for following histochemical observation.

1. Determination of acid and neutral mucin by combined alcian blue Periodic-acid Schiff (PAS) stain [11].
2. Determination of mast cells by toluidine blue staining protocol [12].

RESULTS AND DISCUSSION

Concentration of heavy metals in water: Concentration of heavy metals in water: We performed repeated measure test, ANOVA technique and post hoc test for seasonal comparison. The mean concentrations of the metals in the water of the four selected sites (Dalgate, Saidakadal, Hazratbal and Telbal) of Dal Lake were tabulated in Table 1 to 4. The ranking of mean Concentrations of the twelve metals at different sites of Dal Lake was as Dalgate site Fe > Ca > Mn > Al > Zn > Cd > Cu > Ni > Co > Cr; Saidakadal site as Fe > Mn > Ca > Al > Zn > Cd > Ni > Co > Cr > Cu; Hazratbal site as Al > Fe > Ca > Mn > Zn > Cd > Ni > Co > Cr > Cu; Telbal site as Fe > Al > Ca > Mn > Cu > Ni > Co > Zn > Cd > Cr. Applying one way

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ANOVA, showed highly significant difference (p<0.01) between the four investigated sites for all heavy metals concentration.

Concentration of heavy metals in fish tissues:

The liver and muscle tissue pieces along with the parasites recovered from the same *Schizothorax niger* were analyzed for the heavy metals. A parasite species was recovered and identified as *Adenoscolex oreini* from *Schizothorax niger* from Dal Lake. The concentrations of the metals in the tissues and the corresponding parasite are presented in the (Tables 5 to 7).

The mean concentrations of the twelve heavy metals in the tissues of *Schizothorax niger* and their helminthes parasites in the investigated water body are ranked in both infected and uninfected fishes. The mean concentrations of heavy metals for fish muscle, liver and *Adenoscolex* parasite of Dal Lake are ranked as Fe > Ca > Al > Mn > Zn > Cu > Ni > Cd > Cr > Co, Fe > Ca > Mn > Al > Cu > Zn > Cd > Ni > Co > Cr and Ca > Zn > Fe > Mn > Al > Cu > Co > Ni > Cd > Cr. Compared to the metal concentrations of muscle and liver tissues of infected fishes and the tissues concentrations of uninfected fishes from Dal Lake were ranked as Fe > Ca > Zn > Cu > Ni > Cd > Al > Mn > Co > Cr and Fe > Cd > Cu > Ca > Al > Mn > Zn > Ni > Co > Cr .

Highly significant (P<0.01) effects of the type of metal and the site of location were seen on their concentration in the tissues and parasites. Two factors interaction revealed high significance (P<0.01) in the case of (heavy metal*site), significance (P<0.05) in the case of (Fish*heavy metal) and non significance in the case of (Fish*site) while, three factors interaction (fish*site*heavy metal) showed high significance (P<0.01) on the concentration of heavy metals in the infected fishes at the two sites.

Correlation studies: The correlation tables are presented in Tables 8 and 9.

During summer Al and Cu concentrations in livers of the fishes, infected with *Adenoscolex* revealed negative correlation (p<0.05, r= -0.922 and p<0.05, r= -0.930) respectively, with water.). Mn concentration in liver of fishes infected with *Adenoscolex* showed negative correlation (p<0.05, r= -0.890) with water levels. Fe concentration of *Adenoscolex* was found negatively correlated with water level (p<0.05, r= -0.929). Cu concentration in Liver of fishes infected with *Adenoscolex* was found to be positively correlated with temperature (p<0.05, r = 0.916). Mn concentration in Liver of fish infected with *Adenoscolex* was found positively correlated with pH (p<0.01, r=0.885) of water. Muscle tissue of uninfected fish had positive correlation with free carbon dioxide (p<0.05, r=0.892). During autumn Zn concentration in liver of fishes infected with *Adenoscolex* revealed significant negative correlation (p<0.05, r = -0.947. Cu concentration in *Adenoscolex* parasite revealed significant positive correlation (p<0.05, r = 0.909) with water temperature. Ca concentration in *Adenoscolex* was positively correlated with pH (p<0.05, r= -0.939).

Histopathology of *Adenoscolex orenii* infection of *Schizothorax niger:* The fishes infected with *Adenoscolex oreini* were anemic and the abdomen appeared slightly pot bellied (Fig 1). Viscera appeared red on opening the abdomen and the abdominal fluid was tinged red (Fig 2). On opening the intestine necrotic debris was present on the surface and numerous parasites were pesent (Fig 3 & 4). During spring Enteritis characterized by inflammatory cells in the lamina propria and lamina epithelialis was seen (Fig 5). Eosinophils granule cells are seen in lamina propria. Goblet cells hyperplasia was seen with positivity for acid mucopolysaccharide (Fig 6).

Adenoscolex oreini* infection in *Schizothorax niger

Fig1. The fishes infected with *Adenoscolex oreini* were anemic and the abdomen appeared slightly pot bellied.

Fig2. Viscera appeared red on opening the abdomen and the abdominal fluid was tinged red.

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Fig3. Intestinal mucosa showed necrotic debris and numerous parasites were pesent.

Fig4.Numerous *Adenoscolex oreini* were recovered from the intestines

Liver: Cells were swollen showing vacuolar degeneration and Kupffer cell hyperplasia (Figs 7 & 8).

During summer severe chronic enteritis was seen in intestines with infiltration of lymphocytes and fibroblasts in the lamina propria (Fig 9). The necrotic villi were completely denuded of epithelial mucosa (Fig 10). Only the goblet cells in surviving epithelia revealed hyperplasia changes with evidence of acid mucopolysaccharide. Mast cells were occasionally seen.

Liver: Cells showed moderate degenerative changes with cellular swelling and distortion (Figs 11& 12).

During autumn season Enteritis was comparatively less severe. Lamina propria was infiltrated with mononuclear and eosinophilic granule cells (Fig 13). Necrosis of some villi was seen represented by fibrillar networks (Fig 14). Alcian blue PAS staining revealed goblet cells hyperplasia with presence of acid mucopolysaccharide (Fig 15). Mast cells were occasionally seen in lamina propria.

Liver: cells revealed degenerative changes; cellular swelling and pyknotic nuclei were occasionally seen (Fig 16).

During winter enteritis was characterized by infiltration of inflammatory cells in lamina propria and lamina epithelial along with necrosis of mucosal epithelial cells (Fig 17). Intestinal villi had become thickened and crypts were obliterated. Eosinophiles granules were seen in lamina propria (Fig 18). Goblet cell hyperplasia was clearly seen having acid mucopolysaccharide (Fig 19). Mast cells were evident.

Liver: cells revealed vascular degeneration and cellular disorganization (Fig 20).

Spring Season

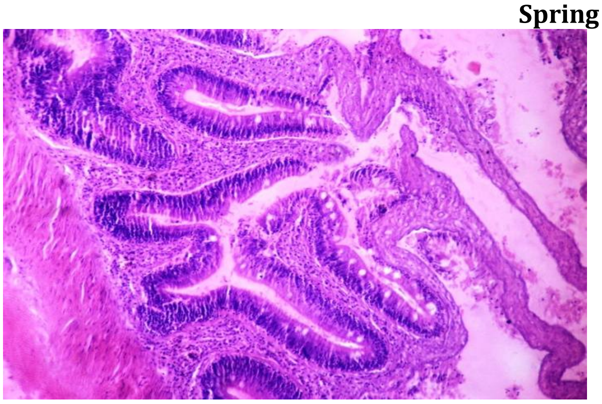

Fig 5. Intestine of fish infected with *Adenoscolex oreini* revealing enteritis. Infiltration of inflammatory cells in lamina propria and lamina epithelia were seen. **H & E X 25.**

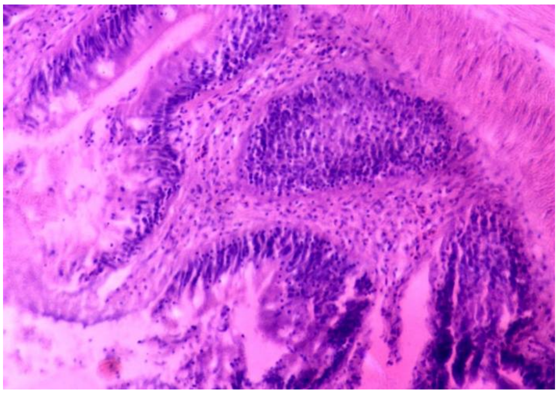

Fig 6. Epithelial desquamation was evident and hyperplasia of lymphoid nodules seen. **H & E X 65.**

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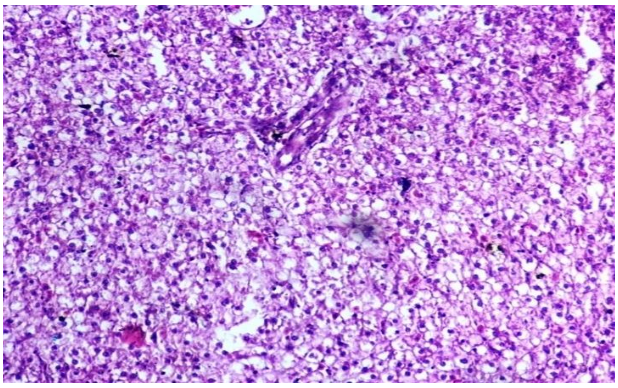

Fig 7. Cells were swollen showing vacuolar degeneration. **H & E X 57.**

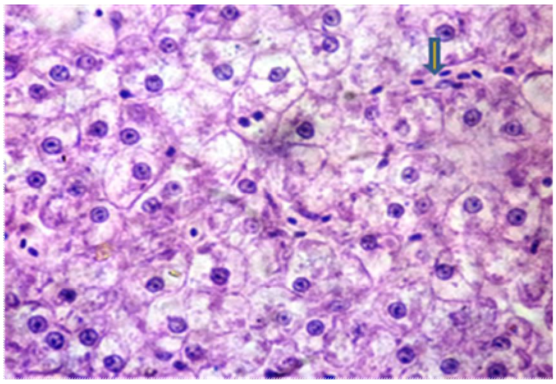

Fig 8. Higher magnification of the same showing Kupffer cell hyperplasia (arrow). **H & E X 160.**

Summer Season

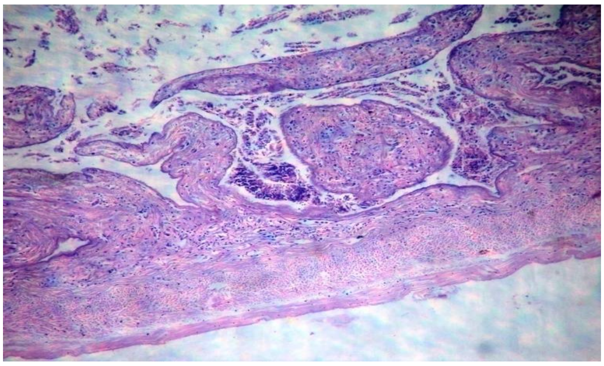

Fig 9. Severe chronic enteritis was seen with infiltration of lymphocytes and fibroblasts in the lamina propria. **H & E X 28.**

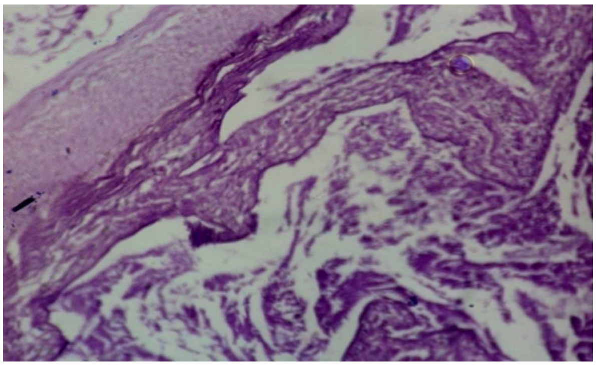

Fig10.The necrotic villi were completely denuded of epithelial cells. **Alcian blue PAS X 40.**

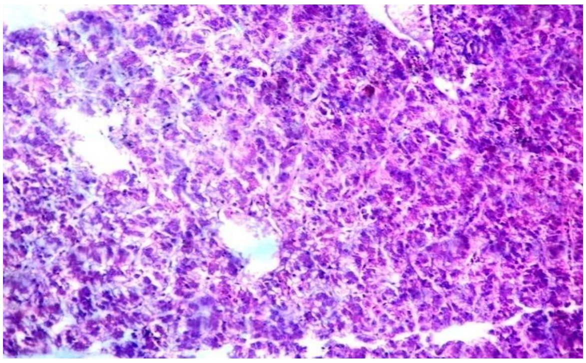

Fig 11. Cells showed moderate degenerative changes with cellular swelling and distortion**. H & E X 35.**

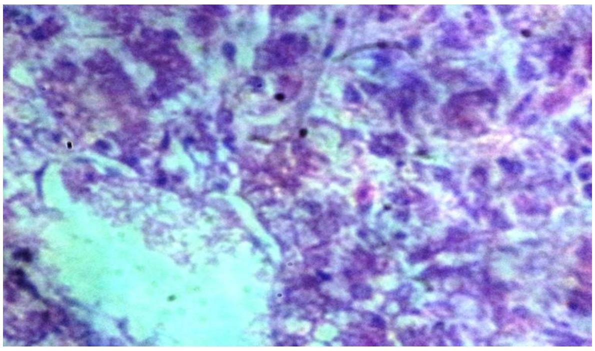

Fig 12. Higher magnification of the same. **H & E X 140.**

Autumn Season

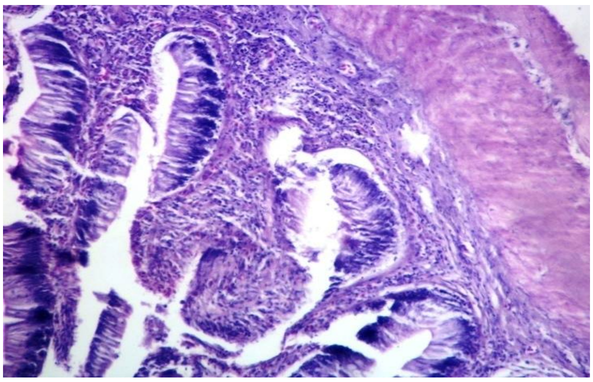

Fig 13. Enteritis was comparatively less severe. Lamina propria was infiltrated with mononuclear and eosinophilic granule cells. **H & E X 41.**

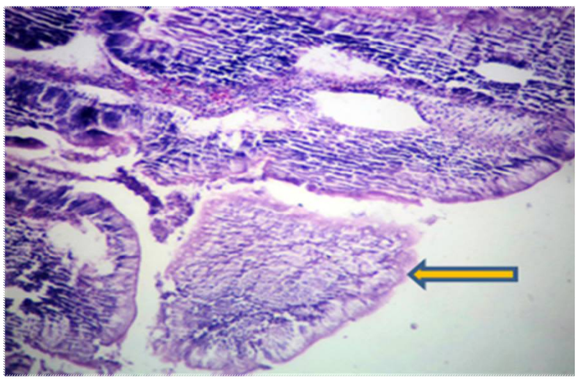

Fig 14. Necrosis of some villi was seen represented by fibrillar networks (arrow). **H & E X 30.**

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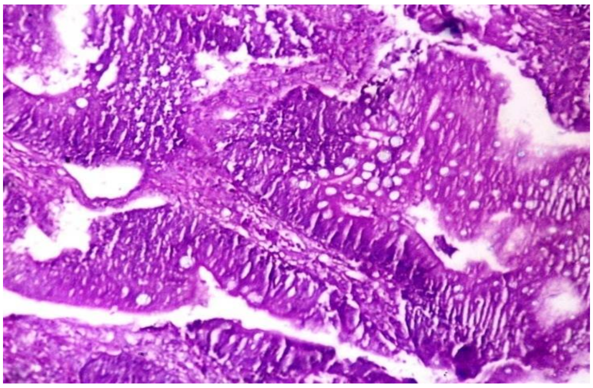

Fig 15. Goblet cell hyperplasia with presence of acid mucopolysaccharide. **Alcian blue PAS staining 35.**

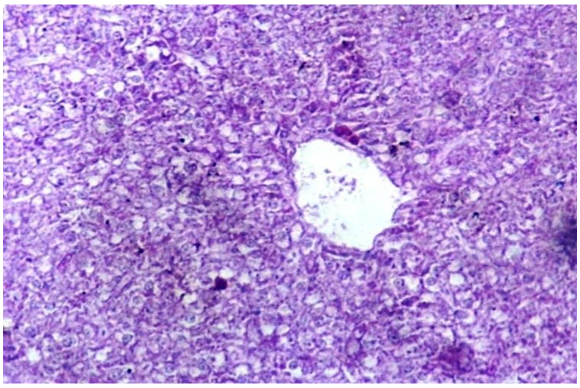

Fig 16. Hepatic cells revealed degenerative changes with vacuolar degeneration. **H & E X 80.**

Winter season

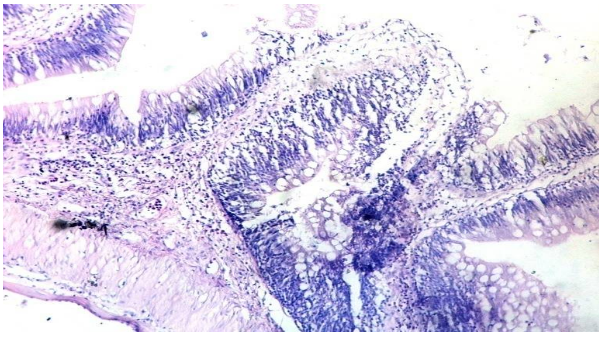

Fig 17. Enteritis characterized by infiltration of inflammatory cells in lamina propria and lamina epithelial along with necrosis of mucosal epithelial cells. **H & E X 33.**

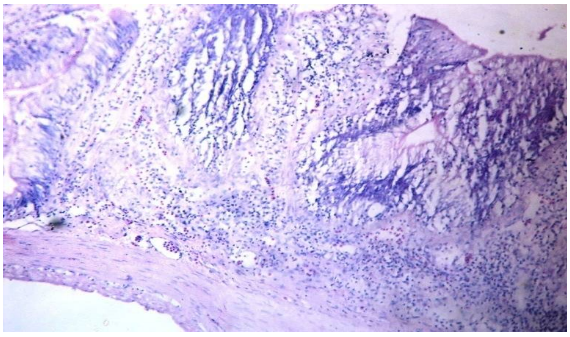

Fig 18. Intestinal villi had become thickened and crypts were obliterated. **H & E X 33.**

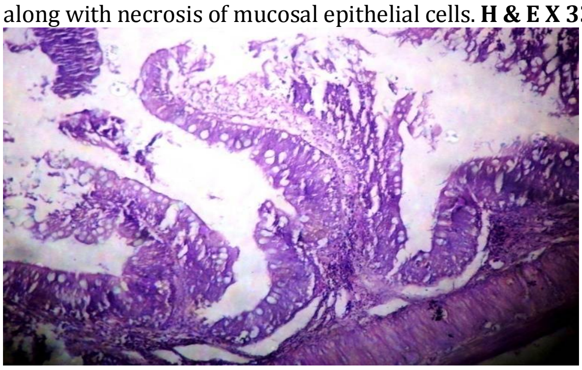

Fig 19. Goblet cell hyperplasia was clearly seen having acid mucopolysaccharide. **Alcian blue PAS staining X 28**

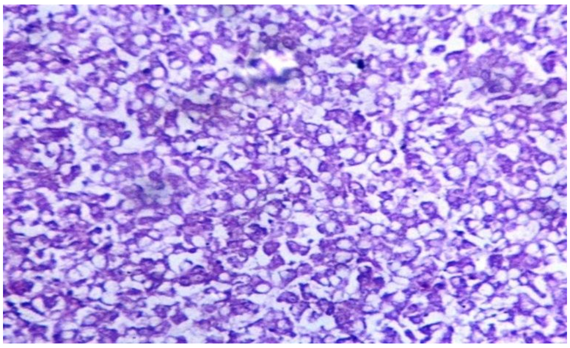

Fig 20. Liver: cells revealed vascular degeneration and cellular disorganization. **H & E X 80.**

Table 1: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Dalgate of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.047 ± 0.015	0.156 ± 0.022	0.288 ± 0.049	0.331 ± 0.036	0.20 ± 0.12	**0.100**
Zinc (Zn)	0.768 ± 0.056	0.039 ± 0.007	0.325 ± 0.033	1.437 ± 0.194	0.64 ± 0.60	**0.309**
Cobalt(Co)	0.537 ± 0.037	0.160 ± 0.051	0.003 ± 0.002	0.003 ± 0.002	0.17 ± 0.25	**0.095**
Nickel (Ni)	0.483 ± 0.056	0.316 ± 0.016	0.003 ± 0.002	0.003 ± 0.002	0.20 ± 0.23	**0.089**
Manganese (Mn)	1.455 ± 0.083	1.581 ± 0.085	5.705 ± 0.327	5.507 ± 0.521	3.56 ± 2.36	**0.946**
Chromium (Cr)	0.268 ± 0.049	0.363 ± 0.157	0.003 ± 0.002	0.001 ± 0.001	0.15 ± 0.18	**0.249**
Aluminium (Al)	1.676 ± 0.049	2.426 ± 0.156	1.413 ± 0.101	1.481 ± 0.085	1.74 ± 0.46	**0.317**
Iron(Fe)	6.501 ± 0.205	12.315 ± 1.026	12.530±0.626	11.978 ±0.534	10.8 ±2.89	**2.013**
Calcium (Ca)	1.855 ± 0.040	4.097 ± 0.213	5.584 ± 0.401	4.192 ± 0.435	3.93 ±1.54	**0.953**
Cadmium(Cd)	0.613 ± 0.054	0.050 ± 0.013	0.089 ± 0.009	0.342 ± 0.032	0.27 ±0.26	**0.098**
Lead(Pb)	BDL	BDL	BDL	BDL	BDL	**-**
Mercury(Hg)	BDL	BDL	BDL	BDL	BDL	**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

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Table 2: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Saidakadal of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.051 ± 0.013	0.025 ± 0.021	0.051 ± 0.010	0.730 ± 0.049	0.21±0.34	**0.084**
Zinc (Zn)	0.697 ± 0.012	0.396 ± 0.034	0.482 ± 0.033	1.016 ± 0.031	0.64±0.27	**0.088**
Cobalt(Co)	0.549 ± 0.038	0.378 ± 0.025	0.023 ± 0.011	0.014 ± 0.008	0.24±0.26	**0.072**
Nickel (Ni)	0.424 ± 0.074	0.053 ± 0.014	0.013 ± 0.008	0.004 ± 0.001	0.12±0.20	**0.114**
Manganese(Mn)	1.427 ± 0.075	4.379 ± 0.235	8.245 ± 0.194	2.857 ± 0.160	4.22±2.93	**0.532**
Chromium (Cr)	0.099 ± 0.030	0.291 ± 0.058	0.011 ± 0.006	0.005 ± 0.002	0.10±0.13	**0.099**
Aluminium (Al)	3.434 ± 0.061	1.769 ± 0.064	1.442 ± 0.036	2.639 ± 0.087	2.32±0.89	**0.195**
Iron(Fe)	6.698 ± 0.054	8.560 ± 0.544	8.945 ± 0.256	13.682 ±0.369	9.47±2.97	**1.070**
Calcium (Ca)	1.365 ± 0.038	4.804 ± 0.239	4.705 ± 0.119	3.789 ± 0.088	3.66±1.60	**0.429**
Cadmium(Cd)	0.588 ± 0.031	0.475 ± 0.054	0.077 ± 0.010	0.063 ± 0.009	0.30±0.27	**0.097**
Lead(Pb)	BDL	BDL	BDL	BDL		**-**
Mercury(Hg)	BDL	BDL	BDL	BDL		**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

Table 3: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Hazratbal Basin of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.022 ± 0.012	0.046 ± 0.020	0.038 ± 0.006	0.063 ± 0.010	0.04±0.01	**N.S.**
Zinc (Zn)	0.556 ± 0.078	1.536 ± 0.186	0.062 ± 0.011	0.011 ± 0.006	0.54±0.70	**0.305**
Cobalt(Co)	0.555 ± 0.054	0.482 ± 0.070	0.006 ± 0.002	0.004 ± 0.001	0.26±0.29	**0.133**
Nickel (Ni)	0.325 ± 0.052	0.759 ± 0.061	0.183 ± 0.047	0.024 ± 0.015	0.32±0.31	**0.142**
Manganese(Mn)	1.433 ± 0.065	6.492 ± 0.673	4.719 ± 0.118	0.011 ± 0.007	3.16±2.96	**1.037**
Chromium (Cr)	0.254 ± 0.089	0.005 ± 0.002	0.002 ± 0.001	0.004 ± 0.001	0.06±0.12	**0.135**
Aluminium (Al)	2.533 ± 0.127	2.006 ± 0.256	6.851 ± 0.350	13.212 ± 1.181	6.15±5.18	**1.912**
Iron(Fe)	5.061 ± 0.471	2.373 ± 0.110	4.031 ± 0.293	5.665 ± 0.287	4.28±1.44	**0.959**
Calcium (Ca)	1.967 ± 0.228	5.094 ± 0.486	5.249 ± 0.326	4.155 ± 0.342	4.11±1.51	**1.081**
Cadmium(Cd)	0.717 ± 0.096	0.058 ± 0.011	0.378 ± 0.058	0.568 ± 0.079	0.43±0.28	**0.207**
Lead(Pb)	BDL	BDL	BDL	BDL		**-**
Mercury(Hg)	BDL	BDL	BDL	BDL		**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

Table 4: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Telbal of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.036 ± 0.009	0.297 ± 0.067	0.439 ± 0.077	1.831 ± 0.234	**0.65±0.80**	**0.386**
Zinc (Zn)	0.517 ± 0.023	0.288 ± 0.078	0.271 ± 0.054	0.004 ± 0.002	**0.27±0.20**	**0.148**
Cobalt(Co)	0.494 ± 0.052	0.266 ± 0.048	0.379 ± 0.108	0.009 ± 0.003	**0.28±0.20**	**0.196**
Nickel (Ni)	0.683 ± 0.092	0.406 ± 0.018	0.987 ± 0.080	0.012 ± 0.009	**0.52±0.41**	**0.187**
Manganese(Mn)	1.657 ±0.304	2.211 ± 0.197	5.337 ± 1.370	0.003 ± 0.001	**2.30±2.23**	**2.142**
Chromium (Cr)	0.071± 0.011	0.003 ± 0.001	0.002 ± 0.002	0.745 ± 0.057	**0.20±0.36**	**0.088**
Aluminium (Al)	0.488 ± 0.060	2.675 ± 0.239	6.054 ± 0.338	4.967 ± 0.502	**3.54±2.47**	**0.988**
Iron(Fe)	7.764 ± 0.262	4.869 ± 0.338	12.827 ± 1.062	32.178 ±6.441	**14.4±12.29**	**9.892**
Calcium (Ca)	1.890 ± 0.126	3.427 ± 0.236	4.781 ± 0.159	3.868 ± 0.219	**3.49±1.20**	**0.575**
Cadmium(Cd)	0.441 ± 0.032	0.224 ± 0.033	0.067 ± 0.009	0.297 ± 0.018	**0.25±0.15**	**0.076**
Lead(Pb)	BDL	BDL	BDL	BDL		**-**
Mercury(Hg)	BDL	BDL	BDL	BDL		**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

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Table 5: Seasonal effect on the comparative concentrations (µg/g) of the heavy metals in tissues of the *Schizothorax niger* and ***Adenoscolex oreini*** collected from Dal Lake

Tissues				Copper (Cu)	Zinc (Zn)	Cobalt(Co)	Nickel (Ni)	Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron(Fe)
Muscle	C.D			0.243	0.142	0.035	0.157	0.256	0.147	0.333	0.768
	Overall Mean			1.25±1.26	1.30±0.84	0.38±0.39	0.80±0.90	1.69±2.20	0.74±1.20	2.92±2.80	29.3±28.6
	Summer (Mean ± SD)	**Mean ±SD**		1.871 ± 0.036	1.821 ± 0.054	0.005 ± 0.002	0.003 ± 0.002	5.277 ± 0.084	0.011 ± 0.006	7.194 ± 0.130	77.19 ± 0.142
	Spring (Mean ± SD)	**Mean ±SD**		2.957 ± 0.155	0.009 ± 0.004	1.031 ± 0.018	2.257 ± 0.089	0.009 ± 0.004	2.779 ± 0.094	3.424 ± 0.174	18.654 ±0.174
	Winter (Mean ± SD)	**Mean ±SD**		0.168 ± 0.023	2.141 ± 0.056	0.257 ± 0.013	0.699 ± 0.045	1.381 ± 0.145	0.008 ± 0.004	0.533 ± 0.019	14.628 ± 0.449
	Autumn (Mean ± SD)	**Mean ±SD**		0.014 ± 0.004	1.252 ± 0.053	0.263 ± 0.006	0.275 ± 0.029	0.099 ± 0.027	0.197 ± 0.024	0.566 ± 0.031	6.971 ± 0.075
Liver	C.D			0.154	0.109	0.044	0.092	0.313	0.056	2.454	27.928
	Overall Mean			1.74±0.009	1.16±0.14	0.09±0.04	0.55±0.01	2.55±0.01	0.06±0.081	2.21±0.21	33.7±2.21
	Summer (Mean ± SD)	**Mean ±SD**		4.246 ± 0.091	1.497 ± 0.024	0.196 ± 0.023	0.011 ± 0.005	3.538 ± 0.142	0.002 ± 0.001	5.477 ± 1.562	16.25 ± 2.028
	Spring (Mean ± SD)	**Mean ±SD**		2.726 ± 0.045	0.002 ± 0.002	0.003 ± 0.001	1.141 ± 0.059	1.305 ± 0.049	0.001 ± 0.000	2.566 ± 0.346	23.76 ± 1.703
	Winter (Mean ± SD)	**Mean ±SD**		0.007 ± 0.004	1.603 ± 0.022	0.003 ± 0.002	0.902 ± 0.009	5.329 ± 0.142	0.010 ± 0.006	1.023 ± 0.254	66.68 ± 10.77
	Autumn (Mean ± SD)	**Mean ±SD**		0.006 ± 0.004	1.550 ± 0.064	0.188 ± 0.018	0.155 ± 0.007	0.053 ± 0.005	0.228 ± 0.037	1.696 ± 0.095	27.55 ± 14.76
Adenoscolex oreini	C.D			0.587	9.250	0.261	0.238	1.681	N.S.	0.947	1.040
	Overall Mean			1.59±2.78	6.60±11.8	0.54±0.50	0.52±0.63	3.01±3.32	0.005±0.006	2.82±3.95	5.09±2.93
	Summer (Mean ± SD)	**Mean ±SD**		6.254 ± 0.387	23.623 ±6.103	1.286 ± 0.058	0.005 ± 0.002	6.865 ± 0.942	0.005 ± 0.003	9.109 ± 0.544	6.514 ± 0.535
	Spring (Mean ± SD)	**Mean ±SD**		0.079 ± 0.022	0.010 ± 0.007	0.006 ± 0.002	0.030 ± 0.017	5.138 ± 0.590	0.006 ± 0.003	0.399 ± 0.096	7.965 ± 0.385
	Winter (Mean ± SD)	**Mean ±SD**		0.005 ± 0.002	0.739 ± 0.087	0.412 ± 0.092	1.477 ± 0.095	0.005 ± 0.002	0.006 ± 0.004	0.003 ± 0.002	0.560 ± 0.069
	Autumn (Mean ± SD)	**Mean ±SD**		0.033 ± 0.018	2.062 ± 0.414	0.490 ± 0.135	0.574 ± 0.124	0.064± 0.011	0.004 ± 0.002	1.607 ± 0.295	5.326 ± 0.182
Metals				Copper (Cu)	Zinc (Zn)	Cobalt(Co)	Nickel (Ni)	Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron(Fe)

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Calcium (Ca)	10.329 ± 0.390	28.864 ± 2.029	34.336 ±1.785	0.007 ± 0.002	18.38±14.47	4.129	7.222 ± 1.158	2.490 ± 0.151	9.399 ± 7.273	19.856 ±5.560	10.15±0.19	N.S.	24.58 ± 0.193	6.034 ± 0.019	9.852 ± 0.221	74.92 ± 3.636	28.8±28.4	5.515
Cadmium(Cd)	0.010 ± 0.003	0.020 ± 0.012	0.044 ± 0.015	0.024 ± 0.012	0.02±0.026	N.S.	0.536 ± 0.467	1.773 ± 0.409	0.553 ± 0.387	2.524 ± 0.444	1.13±0.027	1.294	0.042 ± 0.007	0.005 ± 0.005	0.030 ± 0.010	3.059 ± 0.475	0.78±1.43	0.719
Lead (Pb)	BDL	BDL	BDL	BDL			BDL	BDL	BDL	BDL			BDL	BDL	BDL	BDL		
Mercury (Hg)	BDL	BDL	BDL	BDL			BDL	BDL	BDL	BDL			BDL	BDL	BDL	BDL		

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

Table 6: Seasonal effect on the comparative concentrations (µg/g) of the heavy metals in tissues of the *Schizothorax niger* without parasites collected from Dal Lake

Metals	**Tissues**											
	Liver						**Muscle**					
	Autumn	**Winter**	**Spring**	**Summer**	Overall Mean	C.D	**Autumn**	**Winter**	**Spring**	**Summer** (Mean± SD)	Overall Mean	C.D
	Mean ±SD	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**			**Mean ±SD**	**Mean ±SD**	**Mean ±SD**	**Mean ±SD**		
Copper (Cu)	0.900 ± 0.026	0.073 ± 0.009	5.156 ± 0.072	2.380 ± 0.083	2.12±1.98	0.171	0.005 ± 0.002	0.008 ± 0.005	4.394 ± 0.126	0.746 ± 0.030	1.28±1.87	0.196
Zinc (Zn)	0.898 ± 0.041	0.260 ± 0.027	2.152 ± 0.061	1.229 ± 0.079	1.13±0.70	0.168	1.137 ± 0.052	5.960 ± 0.028	1.680 ± 0.044	2.439 ± 0.145	2.80±1.93	0.246
Cobalt(Co)	0.603 ± 0.029	0.642 ± 0.013	0.006 ± 0.003	0.204 ± 0.014	0.36±0.27	0.053	0.505 ± 0.026	0.545 ± 0.033	0.055 ± 0.008	0.007 ± 0.004	0.27±0.25	0.064
Nickel (Ni)	0.004 ± 0.002	3.106 ± 0.071	0.812 ± 0.019	0.063 ± 0.059	0.99±1.29	0.143	0.325± 0.167	2.897 ± 0.110	1.556 ± 0.039	0.002 ± 0.001	1.19±1.18	0.308

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0.098	0.246	0.237	1.690	2.698 .	0.210		
0.53±0.93	0.09±0.23	0.80±0.61	19.60±12.08	19.56±12.26	1.01±0.97		
2.107 ± 0.064	0.004± 0.003	1.391 ±0.112	25.85± 0.810	29.46± 0.553	2.243± 0.077	BDL	BDL
0.009± 0.004	0.005± 0.002	0.026± 0.024	10.88± 0.608	31.55± 1.652	1.622± 0.114	BDL	BDL
0.002 ± 0.001	0.003± 0.001	0.478± 0.046	35.52± 0.465	2.400± 0.118	0.025± 0.011	BDL	BDL
0.022 ± 0.010	0.365± 0.163	1.334± 0.098	6.140± 0.085	14.84 ± 0.366	0.188± 0.017	BDL	BDL
0.226	0.024	N.S.	13.308	N.S.	3.085		
1.41±1.56	0.01±0.02	1.96±0.89	28.08±12.7	2.10±3.40	3.65±3.63		
1.211 ± 0.073	0.001 ± 0.001	1.344± 0.184	25.884± 5.775	0.240 ± 0.237	7.560± 1.762	BDL	BDL
3.937 ± 0.125	0.006± 0.004	1.143± 112.4	44.430± 5.315	6.388± 2.134	1.553± 0.756	BDL	BDL
0.009 ± 0.007	0.003± 0.002	2.283± 0.174	21.25 ± 0.708	1.423± 0.215	3.720± 0.696	BDL	BDL
0.484 ± 0.039	0.054± 0.015	3.082± 0.052	17.197± 3.922	4.245± 3.801	0.133 ± 0.034	BDL	BDL
Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron(Fe)	Calcium (Ca)	Cadmium(Cd)	Lead(Pb)	Mercury(Hg)

CD=Critical difference; BDL=below detection limit; N.S= Non significant

Table 7: International guidelines for heavy metals in water and fish

Heavy Metals	Maximum Limit WHO (1984)/ FEPA (mg/L) for water	Maximum Limit WHO (1984)/ FEPA/ IAEA-407(ug/g) for fish
Copper(Cu)	0.84	3.28
Zinc(Zn)	4.65	30 (FAO, 1983)
Cobalt(Co)	0.004	-
Nickel(Ni)	0.1-0.2	0.60
Manganese (Mn)	0.050	0.5
Chromium(Cr)	0.05	0.73
Aluminum(Al)	-	-
Iron(Fe)	0.300	146 (Wyse *et al.*,2003)
Calcium(Ca)	-	-
Cadmium (Cd)	0.003	0.18
Lead (Pb)	0.010	0.12
Mercury(Hg)	0.001	-

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Table 8: Correlation between the concentrations of heavy metals of Fish tissues and ***Adenoscolex oreini*** parasites during different seasons in Dal Lake

Seasons	water	Copper (Cu)	Zinc (Zn)	Cobalt (Co)	Nickel (Ni)	Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron (Fe)	Calcium (Ca)	Cadmium (Cd)	Lead (Pb)	Mercury (Hg)
Summer	Adenoscolex	-.235	-.286	-.263	-.599	-.774	-.578	.174	-.258	.874	-.409	-	-
	LAP	**-.930***	-.027	.147	.142	.175	-.229	**-.922***	-.378	-.193	-.657	-	-
	MAP	.322	-.439	.350	-.490	-.070	.402	-.534	.104	.551	.027	-	-
Spring	Adenoscolex	-.503	.204	-.141	.690	-.003	.633	-.370	**-.929***	.552	-.381	-	-
	LAP	.089	-.139	.784	.791	**-.890***	-.554	-.363	.053	-.123	-.060	-	-
	MAP	-.279	.201	.791	.886*	-.149	.183	.641	-.396	-.183	-.874	-	-
Autumn	Adenoscolex	-.595	.432	.313	.675	-.800	.784	-.207	-.513	-.253	**.984****	-	-
	LAP	-.522	**-.947***	.835	-.717	-.009	.583	-.679	-.557	-.591	-.644	-	-
	MAP	-.798	-.418	.670	.255	.634	.148	-.626	-.274	-.582	-.609	-	-
Winter	Adenoscolex	-.248	-.870	-.005	.190	-.377	-.462	-.316	.388	.192	**.929***	-	-
	LAP	-.253	.091	.096	.295	.373	.283	.285	-.597	.019	-.842	-	-
	MAP	.864	.686	**.909***	-.207	.648	-.164	**.881***	**-.882***	-.554	**.907***	-	-

LAP=liver of fishes infected with *Bothriocephalus*; MAP= muscle of fishes infected with *Bothriocephalus* *. Correlation is significant at the
0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

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Table 9: Correlation between physicochemical parameters and heavy metal concentrations of water during different seasons of Dal Lake

Seasons	Summer				Spring				Autumn				Winter			
water	Temp	DO	CO2	pH	Temp	DO	CO2	pH	Temp	DO	CO2	pH	Temp	DO	CO2	pH
Copper (Cu)	-0.706	0.55	-0.672	0.654	0.34	-0.558	0.414	**-.933***	-0.752	-0.258	0.541	-0.698	-0.136	-0.403	0.255	-0.618
Zinc (Zn)	-0.665	-0.402	**.885***	-0.57	0.619	0.218	0.073	0.194	**.982****	0.48	0.754	-0.68	-0.665	-0.402	**.885***	-0.57
Cobalt (Co)	-0.625	0.615	-0.621	0.405	-0.318	-0.704	-0.098	-0.158	0.638	0.175	-0.707	0.441	-0.661	-0.684	0.345	-0.59
Nickel (Ni)	0.056	0.286	-0.135	-0.054	0.69	0.239	0.128	0.11	0.254	-0.436	-0.477	0.275	-0.63	-0.404	0.435	-0.366
Manganese (Mn)	0.832	-0.419	0.471	-0.36	**.879***	-0.275	0.693	-0.625	-0.578	0.748	0.135	-0.306	**.972****	0.852	-0.866	0.832
Chromium (Cr)	-0.269	0.772	-0.726	0.607	-0.479	0.412	-0.046	0.806	**-.928***	0.472	0.856	-0.54	-0.108	-0.363	0.215	-0.607
Aluminium (Al)	.927*	-0.716	0.721	-0.569	-0.598	**.939***	-0.755	0.367	-0.815	0.416	0.869	-0.368	-0.363	-0.518	0.414	-0.356
Iron(Fe)	0.716	**-.953***	**.914***	**-.905***	0.743	-0.795	0.825	-0.666	**.952***	0.324	0.856	-0.65	-0.423	-0.398	0.484	-0.701
Calcium (Ca)	**.957***	-0.829	0.841	-0.723	0.434	0.473	0.304	0.62	**-.936***	0.571	0.878	-0.575	0.162	0.004	0.01	0.094
Cadmium (Cd)	0.211	0.229	-0.106	0.153	0.707	-0.846	**.918***	-0.434	**-.902***	0.478	**.911***	-0.658	0.287	0.037	-0.639	0.286
Lead (Pb)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mercury (Hg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

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CONCLUSION

In Dal Lake the concentrations of copper (1.25µg/g), zinc (5.69µg/g) and iron (29.3µg/g) were maximum in the muscles of infected fishes which were still lower than the Maximum Limit recommended by WHO/ FEPA/IAEA-407 whereas on the other the concentration of nickel (0.80µg/g), manganese (1.69µg/g), cadmium (0.78µg/g) and chromium (2.86µg/g), aluminum (2.99µg/g) were higher than the permissible limits. Cr, Mn, Cu, Zn, Al, Ca concentrations in water showed positive correlation with water temperature. Cu concentration in *Adenoscolex oreini* showed positive correlation with water temperature. Cd concentration in *Adenoscolex* showed positive correlation with pH of water. The parasite *Adenoscolex oreini* have direct relation with water. *Adenoscolex oreini* was positively correlated with Cd concentration in water during autumn and winter. Therefore, the parasite seem to act as bio-indicators for the corresponding metals.

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Advances in Bioresearch

ORIGINAL ARTICLE

Bioaccumulation of selected metals and histopathological alterations in tissues of *Adenoscolex oreini* from Dal Lake, Kashmir valley

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ABSTRACT

The concentration of some heavy metals (Cu, Zn, Co, Ni, Mn, Cr, Al, Fe, Ca, Cd, Pb and Hg) in water samples, parasites and tissues of Schizothorax niger obtained from four sites (Dalgate, Saidakadal,Hazratbal and Telbal) of Dal Lake was investigated (atomic absorption spectrophotometry) with emphasis on the histological alterations in these organs. Histopathologically, the parasite induced various intensities of enteritis coupled with hyperplastic goblet cells with increased acid mucopolysaccharide concentrations. In Dal lake the Adenoscolex oreini; S. niger liver and muscle and the ranking of the 12 metals were Ca> Zn> Fe> Mn> Al> Cu> Co> Ni> Cd> Cr;Fe> Ca> Mn> Al> Cu> Zn> Cd> Ni> Co> Cr and Fe> Ca> Al> Mn> Zn> Cu> Ni> Cd> Cr> Co respectively. In river Jhelum it were Ca> Fe> Cr> Mn> Al> Zn>Cu> Cd> Ni> Co; Ca> Fe> Al> Zn> Cu> Mn> Ni> Co> Cd> Cr and Ca> Fe> Al> Zn> Cu> Cr> Ni> Mn> Co> Cd respectively. In Dal Lake the concentrations of copper (1.25μg/g), zinc (5.69μg/g) and iron (29.3μg/g) were maximum in the muscles of infected fishes which were still lower than the Maximum Limit recommended by WHO/ FEPA/IAEA-407 whereas on the other the concentration of nickel (0.80μg/g), manganese (1.69μg/g), cadmium (0.78μg/g) and chromium (2.86μg/g), aluminum (2.99μg/g) were higher than the permissible limits. Cu concentration in Pomphorhynchus kashmirensis and Adenoscolex oreini showed positive correlation with water temperature. Cd concentration in Adenoscolex showed positive correlation with pH of water. However, the Adenoscolex oreini have direct relation with water. Adenoscolex oreini was positively correlated with Cd concentration in water during autumn and winter. Therefore, the parasites seem to act as bio-indicators for the corresponding metals.

***Keywords**: Dal Lake; River Jhelum; Hyperplastic; Goblet cell; Schizothorax Spp; Helminth; physicochemical parameters.*

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INTRODUCTION

Intestinal helminthes of fish are of increasing interest as potential bioindicators for heavy metal contamination in aquatic habitats. Among these parasites cestodes and acanthocephalans in particular have an enormous heavy metal accumulation capacity exceeding that of established free living sentinels. Metal concentrations several thousand times higher in acanthocephalans than in host tissues has been described from field and laboratory studies. Whereas larval stages inside their intermediate hosts are not able to take up high quantities of metals, young worms begin to take up metals immediately after infection of the final host. After 4-5 weeks of exposure, the parasites reach a steady-state concentration

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and order of magnitude higher than the ambient water level. Thus, acanthocephalans are not only very effective in taking up metals, but they can also respond very rapidly to changes in environmental exposure. The mechanism which enables acanthocephalans to take up metals from the intestinal lumen of the host appears to be based on the presence of bile acids, which form organo-metallic complexes that are easily absorbed by the worms due to their lipophilicity [1]. The permanent contamination of aquatic habitats caused by human activities has become one of the major problems in the era of global industrialization and urbanization. Mostly, chemical pollution and in particular contamination with heavy metals is considered to have an anthropogenic source. A wide range of contaminants are continuously introduced into the aquatic environment mainly due to increased industrialization, technological development, growing human population, oil exploration and exploitation, agricultural and domestic waste run-off [2]. Among these contaminants, heavy metals constitute one of the most dangerous groups because of their persistent nature, toxicity, tendency to accumulate in organisms and undergoing food chain amplifications and being non-degradable [3]. Therefore, this study was conducted to determine the levels of some metals (Cu, Zn, Co, Ni, Mn, Cr, Al, Fe, Ca, Cd, Pb and Hg) in water and tissues.

MATERIAL AND METHODS

Study site

The valley of Kashmir is situated in the mid-Himalayas in the North West and South East direction within the coordinates 33◦01-35◦00 N latitude and 73◦48-75◦30 E longitude at an altitude of ≥1500 m above sea level. The study was carried out in the Dal Lake (34◦07 N- 74◦52 E). Four sites that were selected from Dal Lake viz, Hazratbal Basin, Telbal Nallah, Dalgate ghat and Saidakadal. The field collection was conducted on seasonal basis. The four seasons include spring (March-May, summer (June- August), autumn (September November), spring (March- May) and winter (December-February).

Sample collection

The water samples were acidified immediately after collection by adding 5 ml nitric acid to minimize adsorption of heavy metals onto the walls of the bottles [4, 5]. Fish species (*Schizothorax niger*) along with their parasites were collected randomly from water body with the aid of local fishermen, quickly killed and stored on ice.

Metal estimation

Preparation of standard solutions: Standard solutions were prepared from the stock solution (1000 μg/ml) of each metal. The calibration standards for all of the metals had a concentration working range of 0.1 to 2.0 ppm.

Instrumentation: The absorbance of the calibration standards and samples were measured by Atomic Absorption Spectrophotometer (model, A Analyst 800; Make, Perkin Elmer Ltd).

Water sample preparation and treatment: Processing of water samples for metal analysis was carried out as per the standard methodology of American Public Health Association [6].

Fish tissue and parasites: Fish tissues and parasite were processed for metal analysis [7, 8]. Samples were digested by wet digestion method. The tissue sub-samples were oven dried at 105°C until they reached a constant weight [9].

Gross pathology Fishes were systematically subjected to detailed macroscopic examination with special emphasis on liver, intestine and the lesions were recorded.

Histopathology Representative tissue samples from the liver, intestine affected by parasites were collected in 10% formalin. The tissue samples were processed for routine paraffin embedding technique and 5u thin section were stained with Haris haematoxylin and Eosin [10]. **Histochemistry** Parallel tissue section selected on the basis of histopathological examination was stained for following histochemical observation.

1. Determination of acid and neutral mucin by combined alcian blue Periodic-acid Schiff (PAS) stain [11].
2. Determination of mast cells by toluidine blue staining protocol [12].

RESULTS AND DISCUSSION

Concentration of heavy metals in water: Concentration of heavy metals in water: We performed repeated measure test, ANOVA technique and post hoc test for seasonal comparison. The mean concentrations of the metals in the water of the four selected sites (Dalgate, Saidakadal, Hazratbal and Telbal) of Dal Lake were tabulated in Table 1 to 4. The ranking of mean Concentrations of the twelve metals at different sites of Dal Lake was as Dalgate site Fe > Ca > Mn > Al > Zn > Cd > Cu > Ni > Co > Cr; Saidakadal site as Fe > Mn > Ca > Al > Zn > Cd > Ni > Co > Cr > Cu; Hazratbal site as Al > Fe > Ca > Mn > Zn > Cd > Ni > Co > Cr > Cu; Telbal site as Fe > Al > Ca > Mn > Cu > Ni > Co > Zn > Cd > Cr. Applying one way

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ANOVA, showed highly significant difference ($p<0.01$) between the four investigated sites for all heavy metals concentration.

Concentration of heavy metals in fish tissues:

The liver and muscle tissue pieces along with the parasites recovered from the same *Schizothorax niger* were analyzed for the heavy metals. A parasite species was recovered and identified as *Adenoscolex oreini* from *Schizothorax niger* from Dal Lake. The concentrations of the metals in the tissues and the corresponding parasite are presented in the (Tables 5 to 7).

The mean concentrations of the twelve heavy metals in the tissues of *Schizothorax niger* and their helminthes parasites in the investigated water body are ranked in both infected and uninfected fishes. The mean concentrations of heavy metals for fish muscle, liver and *Adenoscolex* parasite of Dal Lake are ranked as Fe > Ca > Al > Mn > Zn > Cu > Ni > Cd > Cr > Co, Fe > Ca > Mn > Al > Cu > Zn > Cd > Ni > Co > Cr and Ca > Zn > Fe > Mn > Al > Cu > Co > Ni > Cd > Cr. Compared to the metal concentrations of muscle and liver tissues of infected fishes and the tissues concentrations of uninfected fishes from Dal Lake were ranked as Fe > Ca > Zn > Cu > Ni > Cd > Al > Mn > Co > Cr and Fe > Cd > Cu > Ca > Al > Mn > Zn > Ni > Co > Cr .

Highly significant ($P<0.01$) effects of the type of metal and the site of location were seen on their concentration in the tissues and parasites. Two factors interaction revealed high significance ($P<0.01$) in the case of (heavy metal*site), significance ($P<0.05$) in the case of (Fish*heavy metal) and non significance in the case of (Fish*site) while, three factors interaction (fish*site*heavy metal) showed high significance ($P<0.01$) on the concentration of heavy metals in the infected fishes at the two sites**.**

Correlation studies: The correlation tables are presented in Tables 8 and 9.

During summer Al and Cu concentrations in livers of the fishes, infected with *Adenoscolex* revealed negative correlation ($p<0.05$, $r= -0.922$ and $p<0.05$, $r= -0.930$) respectively, with water.). Mn concentration in liver of fishes infected with *Adenoscolex* showed negative correlation ($p<0.05$, $r= -0.890$) with water levels. Fe concentration of *Adenoscolex* was found negatively correlated with water level ($p<0.05$, $r= -0.929$). Cu concentration in Liver of fishes infected with *Adenoscolex* was found to be positively correlated with temperature ($p<0.05$, $r = 0.916$). Mn concentration in Liver of fish infected with *Adenoscolex* was found positively correlated with pH ($p<0.01$, $r=0.885$) of water. Muscle tissue of uninfected fish had positive correlation with free carbon dioxide ($p<0.05$, $r=0.892$). During autumn Zn concentration in liver of fishes infected with *Adenoscolex* revealed significant negative correlation ($p<0.05$, $r = -0.947$. Cu concentration in *Adenoscolex* parasite revealed significant positive correlation ($p<0.05$, $r = 0.909$) with water temperature. Ca concentration in *Adenoscolex* was positively correlated with pH ($p<0.05$, $r= -0.939$).

Histopathology of *Adenoscolex orenii* infection of *Schizothorax niger:* The fishes infected with *Adenoscolex oreini* were anemic and the abdomen appeared slightly pot bellied (Fig 1). Viscera appeared red on opening the abdomen and the abdominal fluid was tinged red (Fig 2). On opening the intestine necrotic debris was present on the surface and numerous parasites were pesent (Fig 3 & 4). During spring Enteritis characterized by inflammatory cells in the lamina propria and lamina epithelialis was seen (Fig 5). Eosinophils granule cells are seen in lamina propria. Goblet cells hyperplasia was seen with positivity for acid mucopolysaccharide (Fig 6).

Adenoscolex oreini* infection in *Schizothorax niger

Fig1. The fishes infected with *Adenoscolex oreini* were anemic and the abdomen appeared slightly pot bellied.

Fig2. Viscera appeared red on opening the abdomen and the abdominal fluid was tinged red.

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Fig3. Intestinal mucosa showed necrotic debris and numerous parasites were pesent.

Fig4.Numerous *Adenoscolex oreini* were recovered from the intestines

Liver: Cells were swollen showing vacuolar degeneration and Kupffer cell hyperplasia (Figs 7 & 8).

During summer severe chronic enteritis was seen in intestines with infiltration of lymphocytes and fibroblasts in the lamina propria (Fig 9). The necrotic villi were completely denuded of epithelial mucosa (Fig 10). Only the goblet cells in surviving epithelia revealed hyperplasia changes with evidence of acid mucopolysaccharide. Mast cells were occasionally seen.

Liver: Cells showed moderate degenerative changes with cellular swelling and distortion (Figs 11& 12).

During autumn season Enteritis was comparatively less severe. Lamina propria was infiltrated with mononuclear and eosinophilic granule cells (Fig 13). Necrosis of some villi was seen represented by fibrillar networks (Fig 14). Alcian blue PAS staining revealed goblet cells hyperplasia with presence of acid mucopolysaccharide (Fig 15). Mast cells were occasionally seen in lamina propria.

Liver: cells revealed degenerative changes; cellular swelling and pyknotic nuclei were occasionally seen (Fig 16).

During winter enteritis was characterized by infiltration of inflammatory cells in lamina propria and lamina epithelial along with necrosis of mucosal epithelial cells (Fig 17). Intestinal villi had become thickened and crypts were obliterated. Eosinophiles granules were seen in lamina propria (Fig 18). Goblet cell hyperplasia was clearly seen having acid mucopolysaccharide (Fig 19). Mast cells were evident.

Liver: cells revealed vascular degeneration and cellular disorganization (Fig 20).

Spring Season

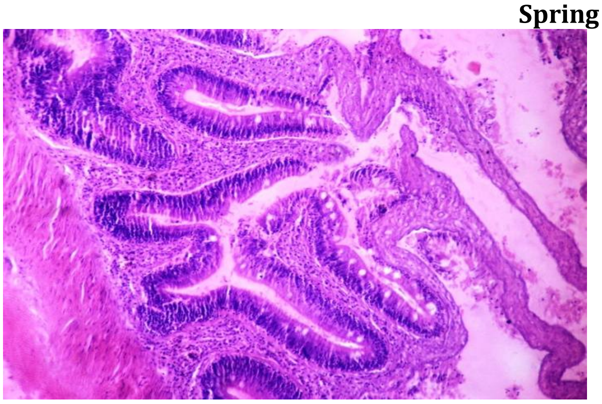

Fig 5. Intestine of fish infected with *Adenoscolex oreini* revealing enteritis. Infiltration of inflammatory cells in lamina propria and lamina epithelia were seen. **H & E X 25.**

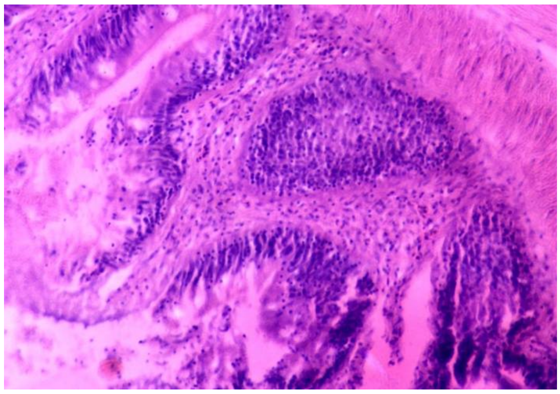

Fig 6. Epithelial desquamation was evident and hyperplasia of lymphoid nodules seen. **H & E X 65.**

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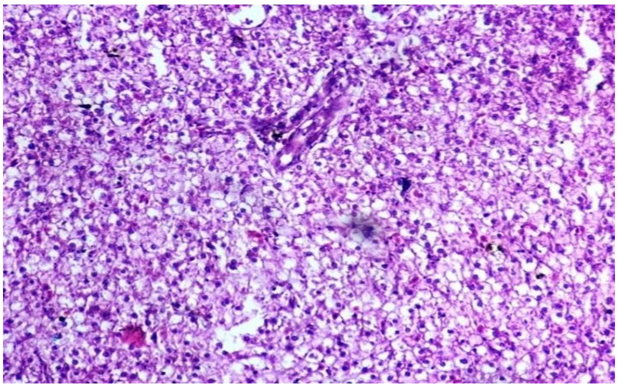

Fig 7. Cells were swollen showing vacuolar degeneration. **H & E X 57.**

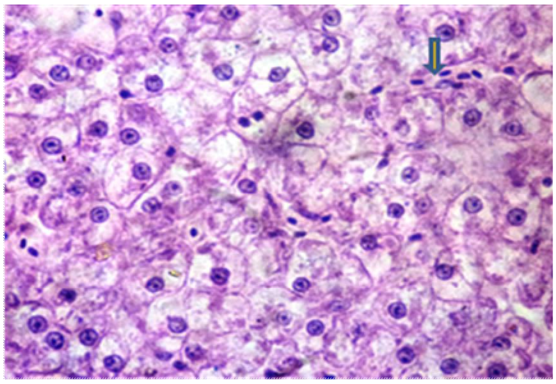

Fig 8. Higher magnification of the same showing Kupffer cell hyperplasia (arrow). **H & E X 160.**

Summer Season

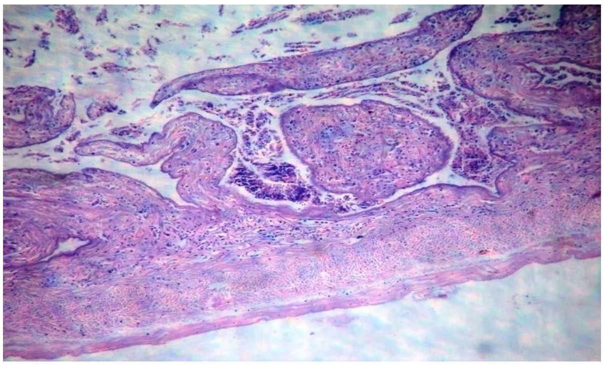

Fig 9. Severe chronic enteritis was seen with infiltration of lymphocytes and fibroblasts in the lamina propria. **H & E X 28.**

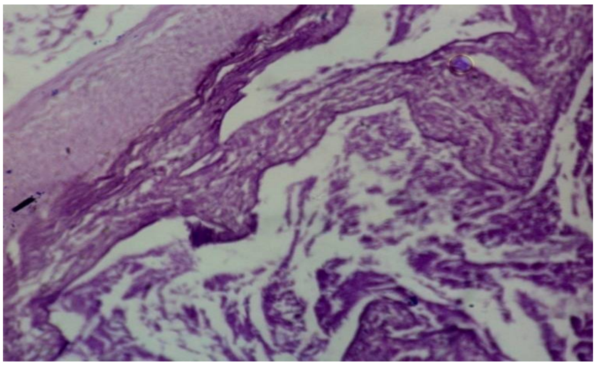

Fig10.The necrotic villi were completely denuded of epithelial cells. **Alcian blue PAS X 40.**

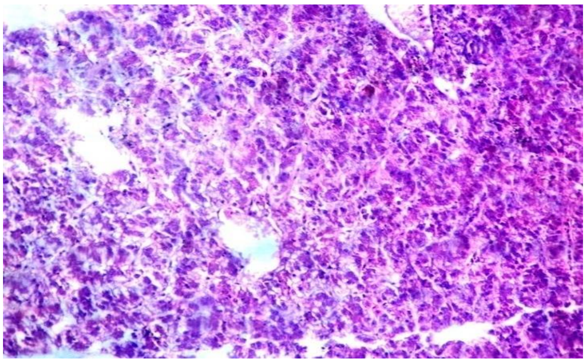

Fig 11. Cells showed moderate degenerative changes with cellular swelling and distortion**. H & E X 35.**

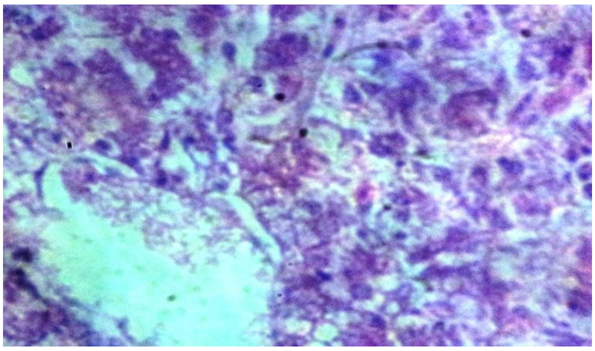

Fig 12. Higher magnification of the same. **H & E X 140.**

Autumn Season

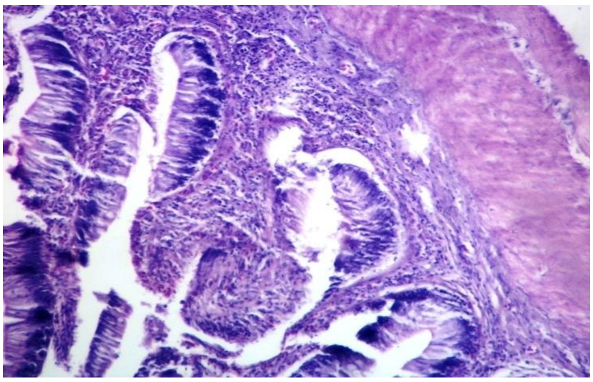

Fig 13. Enteritis was comparatively less severe. Lamina propria was infiltrated with mononuclear and eosinophilic granule cells. **H & E X 41.**

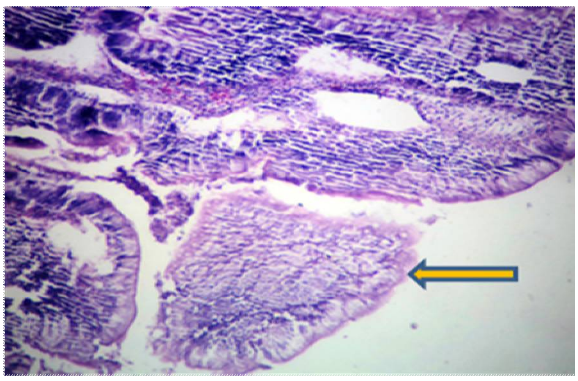

Fig 14. Necrosis of some villi was seen represented by fibrillar networks (arrow). **H & E X 30.**

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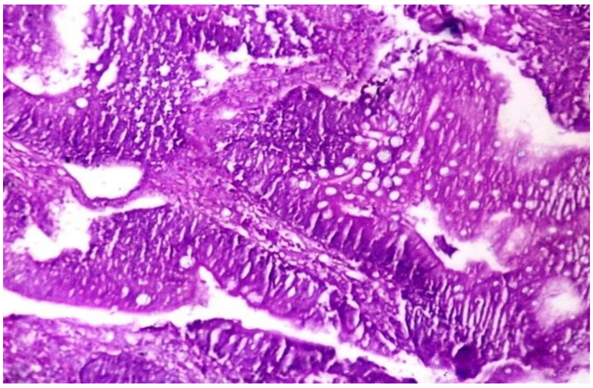

Fig 15. Goblet cell hyperplasia with presence of acid mucopolysaccharide. **Alcian blue PAS staining 35.**

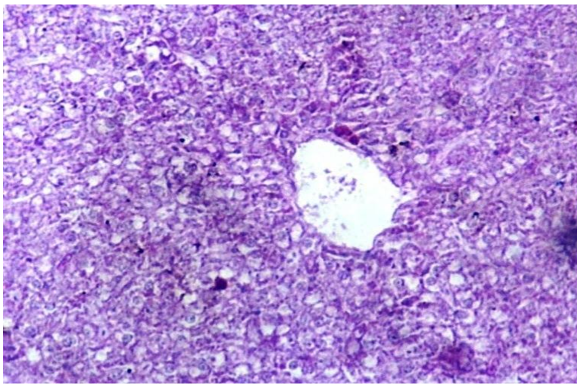

Fig 16. Hepatic cells revealed degenerative changes with vacuolar degeneration. **H & E X 80.**

Winter season

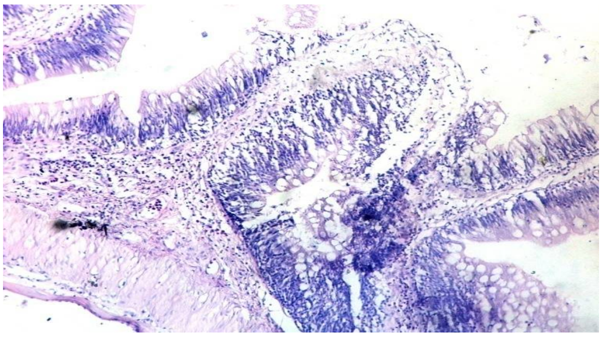

Fig 17. Enteritis characterized by infiltration of inflammatory cells in lamina propria and lamina epithelial along with necrosis of mucosal epithelial cells. **H & E X 33.**

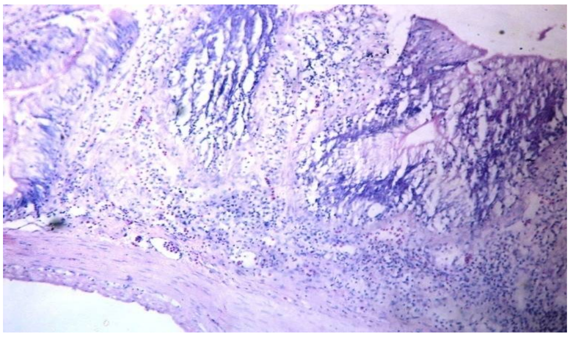

Fig 18. Intestinal villi had become thickened and crypts were obliterated. **H & E X 33.**

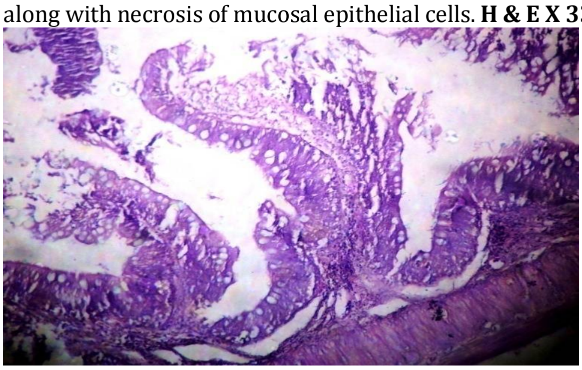

Fig 19. Goblet cell hyperplasia was clearly seen having acid mucopolysaccharide. **Alcian blue PAS staining X 28**

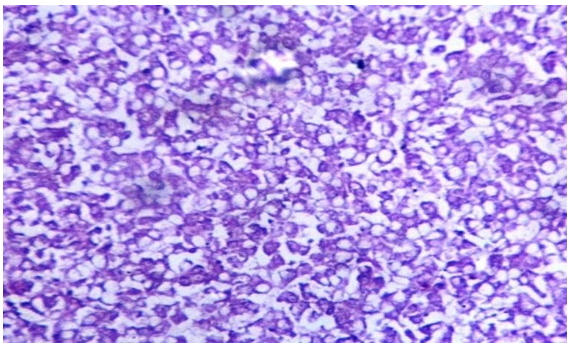

Fig 20. Liver: cells revealed vascular degeneration and cellular disorganization. **H & E X 80.**

Table 1: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Dalgate of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.047 ± 0.015	0.156 ± 0.022	0.288 ± 0.049	0.331 ± 0.036	0.20 ± 0.12	**0.100**
Zinc (Zn)	0.768 ± 0.056	0.039 ± 0.007	0.325 ± 0.033	1.437 ± 0.194	0.64 ± 0.60	**0.309**
Cobalt(Co)	0.537 ± 0.037	0.160 ± 0.051	0.003 ± 0.002	0.003 ± 0.002	0.17 ± 0.25	**0.095**
Nickel (Ni)	0.483 ± 0.056	0.316 ± 0.016	0.003 ± 0.002	0.003 ± 0.002	0.20 ± 0.23	**0.089**
Manganese (Mn)	1.455 ± 0.083	1.581 ± 0.085	5.705 ± 0.327	5.507 ± 0.521	3.56 ± 2.36	**0.946**
Chromium (Cr)	0.268 ± 0.049	0.363 ± 0.157	0.003 ± 0.002	0.001 ± 0.001	0.15 ± 0.18	**0.249**
Aluminium (Al)	1.676 ± 0.049	2.426 ± 0.156	1.413 ± 0.101	1.481 ± 0.085	1.74 ± 0.46	**0.317**
Iron(Fe)	6.501 ± 0.205	12.315 ± 1.026	12.530±0.626	11.978 ±0.534	10.8 ±2.89	**2.013**
Calcium (Ca)	1.855 ± 0.040	4.097 ± 0.213	5.584 ± 0.401	4.192 ± 0.435	3.93 ±1.54	**0.953**
Cadmium(Cd)	0.613 ± 0.054	0.050 ± 0.013	0.089 ± 0.009	0.342 ± 0.032	0.27 ±0.26	**0.098**
Lead(Pb)	BDL	BDL	BDL	BDL	BDL	**-**
Mercury(Hg)	BDL	BDL	BDL	BDL	BDL	**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

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Table 2: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Saidakadal of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.051 ± 0.013	0.025 ± 0.021	0.051 ± 0.010	0.730 ± 0.049	0.21±0.34	**0.084**
Zinc (Zn)	0.697 ± 0.012	0.396 ± 0.034	0.482 ± 0.033	1.016 ± 0.031	0.64±0.27	**0.088**
Cobalt(Co)	0.549 ± 0.038	0.378 ± 0.025	0.023 ± 0.011	0.014 ± 0.008	0.24±0.26	**0.072**
Nickel (Ni)	0.424 ± 0.074	0.053 ± 0.014	0.013 ± 0.008	0.004 ± 0.001	0.12±0.20	**0.114**
Manganese(Mn)	1.427 ± 0.075	4.379 ± 0.235	8.245 ± 0.194	2.857 ± 0.160	4.22±2.93	**0.532**
Chromium (Cr)	0.099 ± 0.030	0.291 ± 0.058	0.011 ± 0.006	0.005 ± 0.002	0.10±0.13	**0.099**
Aluminium (Al)	3.434 ± 0.061	1.769 ± 0.064	1.442 ± 0.036	2.639 ± 0.087	2.32±0.89	**0.195**
Iron(Fe)	6.698 ± 0.054	8.560 ± 0.544	8.945 ± 0.256	13.682 ±0.369	9.47±2.97	**1.070**
Calcium (Ca)	1.365 ± 0.038	4.804 ± 0.239	4.705 ± 0.119	3.789 ± 0.088	3.66±1.60	**0.429**
Cadmium(Cd)	0.588 ± 0.031	0.475 ± 0.054	0.077 ± 0.010	0.063 ± 0.009	0.30±0.27	**0.097**
Lead(Pb)	BDL	BDL	BDL	BDL		**-**
Mercury(Hg)	BDL	BDL	BDL	BDL		**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

Table 3: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Hazratbal Basin of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.022 ± 0.012	0.046 ± 0.020	0.038 ± 0.006	0.063 ± 0.010	0.04±0.01	**N.S.**
Zinc (Zn)	0.556 ± 0.078	1.536 ± 0.186	0.062 ± 0.011	0.011 ± 0.006	0.54±0.70	**0.305**
Cobalt(Co)	0.555 ± 0.054	0.482 ± 0.070	0.006 ± 0.002	0.004 ± 0.001	0.26±0.29	**0.133**
Nickel (Ni)	0.325 ± 0.052	0.759 ± 0.061	0.183 ± 0.047	0.024 ± 0.015	0.32±0.31	**0.142**
Manganese(Mn)	1.433 ± 0.065	6.492 ± 0.673	4.719 ± 0.118	0.011 ± 0.007	3.16±2.96	**1.037**
Chromium (Cr)	0.254 ± 0.089	0.005 ± 0.002	0.002 ± 0.001	0.004 ± 0.001	0.06±0.12	**0.135**
Aluminium (Al)	2.533 ± 0.127	2.006 ± 0.256	6.851 ± 0.350	13.212 ± 1.181	6.15±5.18	**1.912**
Iron(Fe)	5.061 ± 0.471	2.373 ± 0.110	4.031 ± 0.293	5.665 ± 0.287	4.28±1.44	**0.959**
Calcium (Ca)	1.967 ± 0.228	5.094 ± 0.486	5.249 ± 0.326	4.155 ± 0.342	4.11±1.51	**1.081**
Cadmium(Cd)	0.717 ± 0.096	0.058 ± 0.011	0.378 ± 0.058	0.568 ± 0.079	0.43±0.28	**0.207**
Lead(Pb)	BDL	BDL	BDL	BDL		**-**
Mercury(Hg)	BDL	BDL	BDL	BDL		**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

Table 4: Descriptive statistics obtained by ANOVA to compare concentration (mg/l) of the selected heavy metals of the water samples collected along Telbal of Dal Lake seasonally

Location	Seasons				Overall Mean	C.D
	Autumn (Mean ± SD)	**Winter** (Mean ± SD)	**Spring** (Mean ± SD)	**Summer** (Mean ± SD)		
Copper (Cu)	0.036 ± 0.009	0.297 ± 0.067	0.439 ± 0.077	1.831 ± 0.234	**0.65±0.80**	**0.386**
Zinc (Zn)	0.517 ± 0.023	0.288 ± 0.078	0.271 ± 0.054	0.004 ± 0.002	**0.27±0.20**	**0.148**
Cobalt(Co)	0.494 ± 0.052	0.266 ± 0.048	0.379 ± 0.108	0.009 ± 0.003	**0.28±0.20**	**0.196**
Nickel (Ni)	0.683 ± 0.092	0.406 ± 0.018	0.987 ± 0.080	0.012 ± 0.009	**0.52±0.41**	**0.187**
Manganese(Mn)	1.657 ±0.304	2.211 ± 0.197	5.337 ± 1.370	0.003 ± 0.001	**2.30±2.23**	**2.142**
Chromium (Cr)	0.071± 0.011	0.003 ± 0.001	0.002 ± 0.002	0.745 ± 0.057	**0.20±0.36**	**0.088**
Aluminium (Al)	0.488 ± 0.060	2.675 ± 0.239	6.054 ± 0.338	4.967 ± 0.502	**3.54±2.47**	**0.988**
Iron(Fe)	7.764 ± 0.262	4.869 ± 0.338	12.827 ± 1.062	32.178 ±6.441	**14.4±12.29**	**9.892**
Calcium (Ca)	1.890 ± 0.126	3.427 ± 0.236	4.781 ± 0.159	3.868 ± 0.219	**3.49±1.20**	**0.575**
Cadmium(Cd)	0.441 ± 0.032	0.224 ± 0.033	0.067 ± 0.009	0.297 ± 0.018	**0.25±0.15**	**0.076**
Lead(Pb)	BDL	BDL	BDL	BDL		**-**
Mercury(Hg)	BDL	BDL	BDL	BDL		**-**

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

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Table 5: Seasonal effect on the comparative concentrations (µg/g) of the heavy metals in tissues of the *Schizothorax niger* and ***Adenoscolex oreini*** collected from Dal Lake

Tissues			Copper (Cu)	Zinc (Zn)	Cobalt(Co)	Nickel (Ni)	Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron(Fe)
Muscle	C.D		0.243	0.142	0.035	0.157	0.256	0.147	0.333	0.768
	Overall Mean		1.25±1.26	1.30±0.84	0.38±0.39	0.80±0.90	1.69±2.20	0.74±1.20	2.92±2.80	29.3±28.6
	Summer (Mean ± SD)	**Mean ±SD**	1.871 ± 0.036	1.821 ± 0.054	0.005 ± 0.002	0.003 ± 0.002	5.277 ± 0.084	0.011 ± 0.006	7.194 ± 0.130	77.19 ± 0.142
	Spring (Mean ± SD)	**Mean ±SD**	2.957 ± 0.155	0.009 ± 0.004	1.031 ± 0.018	2.257 ± 0.089	0.009 ± 0.004	2.779 ± 0.094	3.424 ± 0.174	18.654 ±0.174
	Winter (Mean ± SD)	**Mean ±SD**	0.168 ± 0.023	2.141 ± 0.056	0.257 ± 0.013	0.699 ± 0.045	1.381 ± 0.145	0.008 ± 0.004	0.533 ± 0.019	14.628 ± 0.449
	Autumn (Mean ± SD)	**Mean ±SD**	0.014 ± 0.004	1.252 ± 0.053	0.263 ± 0.006	0.275 ± 0.029	0.099 ± 0.027	0.197 ± 0.024	0.566 ± 0.031	6.971 ± 0.075
Liver	C.D		0.154	0.109	0.044	0.092	0.313	0.056	2.454	27.928
	Overall Mean		1.74±0.009	1.16±0.14	0.09±0.04	0.55±0.01	2.55±0.01	0.06±0.081	2.21±0.21	33.7±2.21
	Summer (Mean ± SD)	**Mean ±SD**	4.246 ± 0.091	1.497 ± 0.024	0.196 ± 0.023	0.011 ± 0.005	3.538 ± 0.142	0.002 ± 0.001	5.477 ± 1.562	16.25 ± 2.028
	Spring (Mean ± SD)	**Mean ±SD**	2.726 ± 0.045	0.002 ± 0.002	0.003 ± 0.001	1.141 ± 0.059	1.305 ± 0.049	0.001 ± 0.000	2.566 ± 0.346	23.76 ± 1.703
	Winter (Mean ± SD)	**Mean ±SD**	0.007 ± 0.004	1.603 ± 0.022	0.003 ± 0.002	0.902 ± 0.009	5.329 ± 0.142	0.010 ± 0.006	1.023 ± 0.254	66.68 ± 10.77
	Autumn (Mean ± SD)	**Mean ±SD**	0.006 ± 0.004	1.550 ± 0.064	0.188 ± 0.018	0.155 ± 0.007	0.053 ± 0.005	0.228 ± 0.037	1.696 ± 0.095	27.55 ± 14.76
Adenoscolex oreini	C.D		0.587	9.250	0.261	0.238	1.681	N.S.	0.947	1.040
	Overall Mean		1.59±2.78	6.60±11.8	0.54±0.50	0.52±0.63	3.01±3.32	0.005±0.006	2.82±3.95	5.09±2.93
	Summer (Mean ± SD)	**Mean ±SD**	6.254 ± 0.387	23.623 ±6.103	1.286 ± 0.058	0.005 ± 0.002	6.865 ± 0.942	0.005 ± 0.003	9.109 ± 0.544	6.514 ± 0.535
	Spring (Mean ± SD)	**Mean ±SD**	0.079 ± 0.022	0.010 ± 0.007	0.006 ± 0.002	0.030 ± 0.017	5.138 ± 0.590	0.006 ± 0.003	0.399 ± 0.096	7.965 ± 0.385
	Winter (Mean ± SD)	**Mean ±SD**	0.005 ± 0.002	0.739 ± 0.087	0.412 ± 0.092	1.477 ± 0.095	0.005 ± 0.002	0.006 ± 0.004	0.003 ± 0.002	0.560 ± 0.069
	Autumn (Mean ± SD)	**Mean ±SD**	0.033 ± 0.018	2.062 ± 0.414	0.490 ± 0.135	0.574 ± 0.124	0.064± 0.011	0.004 ± 0.002	1.607 ± 0.295	5.326 ± 0.182
Metals			Copper (Cu)	Zinc (Zn)	Cobalt(Co)	Nickel (Ni)	Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron(Fe)

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Calcium (Ca)	Cadmium(Cd)	Lead (Pb)	Mercury (Hg)
10.329 ± 0.390	0.010 ± 0.003	BDL	BDL
28.864 ± 2.029	0.020 ± 0.012	BDL	BDL
34.336 ±1.785	0.044 ± 0.015	BDL	BDL
0.007 ± 0.002	0.024 ± 0.012	BDL	BDL
18.38±14.47	0.02±0.026		
4.129	N.S.		
7.222 ± 1.158	0.536 ± 0.467	BDL	BDL
2.490 ± 0.151	1.773 ± 0.409	BDL	BDL
9.399 ± 7.273	0.553 ± 0.387	BDL	BDL
19.856 ±5.560	2.524 ± 0.444	BDL	BDL
10.15±0.19	1.13±0.027		
N.S.	1.294		
24.58 ± 0.193	0.042 ± 0.007	BDL	BDL
6.034 ± 0.019	0.005 ± 0.005	BDL	BDL
9.852 ± 0.221	0.030 ± 0.010	BDL	BDL
74.92 ± 3.636	3.059 ± 0.475	BDL	BDL
28.8±28.4	0.78±1.43		
5.515	0.719		

CD=Critical difference; BDL=below detection limit; N.S= Non significant.

Table 6: Seasonal effect on the comparative concentrations (µg/g) of the heavy metals in tissues of the *Schizothorax niger* without parasites collected from Dal Lake

Tissues			Metals: Copper (Cu)	Zinc (Zn)	Cobalt(Co)	Nickel (Ni)
Liver	Autumn	Mean ±SD	0.900 ± 0.026	0.898 ± 0.041	0.603 ± 0.029	0.004 ± 0.002
	Winter	Mean ±SD	0.073 ± 0.009	0.260 ± 0.027	0.642 ± 0.013	3.106 ± 0.071
	Spring	Mean ±SD	5.156 ± 0.072	2.152 ± 0.061	0.006 ± 0.003	0.812 ± 0.019
	Summer	Mean ±SD	2.380 ± 0.083	1.229 ± 0.079	0.204 ± 0.014	0.063 ± 0.059
	Overall Mean		2.12±1.98	1.13±0.70	0.36±0.27	0.99±1.29
	C.D		0.171	0.168	0.053	0.143
Muscle	Autumn	Mean ±SD	0.005 ± 0.002	1.137 ± 0.052	0.505 ± 0.026	0.325± 0.167
	Winter	Mean ±SD	0.008 ± 0.005	5.960 ± 0.028	0.545 ± 0.033	2.897 ± 0.110
	Spring	Mean ±SD	4.394 ± 0.126	1.680 ± 0.044	0.055 ± 0.008	1.556 ± 0.039
	Summer (Mean± SD)	Mean ±SD	0.746 ± 0.030	2.439 ± 0.145	0.007 ± 0.004	0.002 ± 0.001
	Overall Mean		1.28±1.87	2.80±1.93	0.27±0.25	1.19±1.18
	C.D		0.196	0.246	0.064	0.308

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0.098	0.246	0.237	1.690	2.698 .	0.210		
0.53±0.93	0.09±0.23	0.80±0.61	19.60±12.08	19.56±12.26	1.01±0.97		
2.107 ± 0.064	0.004± 0.003	1.391 ±0.112	25.85± 0.810	29.46± 0.553	2.243± 0.077	BDL	BDL
0.009± 0.004	0.005± 0.002	0.026± 0.024	10.88± 0.608	31.55± 1.652	1.622± 0.114	BDL	BDL
0.002± 0.001	0.003± 0.001	0.478± 0.046	35.52± 0.465	2.400± 0.118	0.025± 0.011	BDL	BDL
0.022 ± 0.010	0.365± 0.163	1.334± 0.098	6.140± 0.085	14.84 ± 0.366	0.188± 0.017	BDL	BDL
0.226	0.024	N.S.	13.308	N.S.	3.085		
1.41±1.56	0.01±0.02	1.96±0.89	28.08±12.7	2.10±3.40	3.65±3.63		
1.211 ± 0.073	0.001 ±0.001	1.344±0.184	25.884±5.775	0.240 ±0.237	7.560± 1.762	BDL	BDL
3.937±0.125	0.006±0.004	1.143±112.4	44.430±5.315	6.388±2.134	1.553±0.756	BDL	BDL
0.009± 0.007	0.003±0.002	2.283±0.174	21.25 ±0.708	1.423±0.215	3.720± 0.696	BDL	BDL
0.484 ± 0.039	0.054± 0.015	3.082± 0.052	17.197±3.922	4.245±3.801	0.133 ±0.034	BDL	BDL
Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron(Fe)	Calcium (Ca)	Cadmium(Cd)	Lead(Pb)	Mercury(Hg)

CD=Critical difference; BDL=below detection limit; N.S= Non significant

Table 7: International guidelines for heavy metals in water and fish

Heavy Metals	Maximum Limit WHO (1984)/ FEPA (mg/L) for water	Maximum Limit WHO (1984)/ FEPA/ IAEA-407(ug/g) for fish
Copper(Cu)	0.84	3.28
Zinc(Zn)	4.65	30 (FAO, 1983)
Cobalt(Co)	0.004	-
Nickel(Ni)	0.1-0.2	0.60
Manganese (Mn)	0.050	0.5
Chromium(Cr)	0.05	0.73
Aluminum(Al)	-	-
Iron(Fe)	0.300	146 (Wyse *et al.*,2003)
Calcium(Ca)	-	-
Cadmium (Cd)	0.003	0.18
Lead (Pb)	0.010	0.12
Mercury(Hg)	0.001	-

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Table 8: Correlation between the concentrations of heavy metals of Fish tissues and ***Adenoscolex oreini*** parasites during different seasons in Dal Lake

Seasons	water	Copper (Cu)	Zinc (Zn)	Cobalt (Co)	Nickel (Ni)	Manganese (Mn)	Chromium (Cr)	Aluminium (Al)	Iron (Fe)	Calcium (Ca)	Cadmium (Cd)	Lead (Pb)	Mercury (Hg)
Summer	Adenoscolex	-.235	-.286	-.263	-.599	-.774	-.578	.174	-.258	.874	-.409	-	-
	LAP	**-.930***	-.027	.147	.142	.175	-.229	**-.922***	-.378	-.193	-.657	-	-
	MAP	.322	-.439	.350	-.490	-.070	.402	-.534	.104	.551	.027	-	-
Spring	Adenoscolex	-.503	.204	-.141	.690	-.003	.633	-.370	**-.929***	.552	-.381	-	-
	LAP	.089	-.139	.784	.791	**-.890***	-.554	-.363	.053	-.123	-.060	-	-
	MAP	-.279	.201	.791	.886*	-.149	.183	.641	-.396	-.183	-.874	-	-
Autumn	Adenoscolex	-.595	.432	.313	.675	-.800	.784	-.207	-.513	-.253	**.984****	-	-
	LAP	-.522	**-.947***	.835	-.717	-.009	.583	-.679	-.557	-.591	-.644	-	-
	MAP	-.798	-.418	.670	.255	.634	.148	-.626	-.274	-.582	-.609	-	-
Winter	Adenoscolex	-.248	-.870	-.005	.190	-.377	-.462	-.316	.388	.192	**.929***	-	-
	LAP	-.253	.091	.096	.295	.373	.283	.285	-.597	.019	-.842	-	-
	MAP	.864	.686	**.909***	-.207	.648	-.164	**.881***	**-.882***	-.554	**.907***	-	-

LAP=liver of fishes infected with *Bothriocephalus*; MAP= muscle of fishes infected with *Bothriocephalus* *. Correlation is significant at the
0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

Table 9: Correlation between physicochemical parameters and heavy metal concentrations of water during different seasons of Dal Lake

Seasons	Summer				Spring				Autumn				Winter			
water	Temp	DO	CO2	pH	Temp	DO	CO2	pH	Temp	DO	CO2	pH	Temp	DO	CO2	pH
Copper (Cu)	-0.706	0.55	-0.672	0.654	0.34	-0.558	0.414	**-.933***	-0.752	-0.258	0.541	-0.698	-0.136	-0.403	0.255	-0.618
Zinc (Zn)	-0.665	-0.402	**.885***	-0.57	0.619	0.218	0.073	0.194	**.982****	0.48	0.754	-0.68	-0.665	-0.402	**.885***	-0.57
Cobalt (Co)	-0.625	0.615	-0.621	0.405	-0.318	-0.704	-0.098	-0.158	0.638	0.175	-0.707	0.441	-0.661	-0.684	0.345	-0.59
Nickel (Ni)	0.056	0.286	-0.135	-0.054	0.69	0.239	0.128	0.11	0.254	-0.436	-0.477	0.275	-0.63	-0.404	0.435	-0.366
Manganese (Mn)	0.832	-0.419	0.471	-0.36	**.879***	-0.275	0.693	-0.625	-0.578	0.748	0.135	-0.306	**.972****	0.852	-0.866	0.832
Chromium (Cr)	-0.269	0.772	-0.726	0.607	-0.479	0.412	-0.046	0.806	**-.928***	0.472	0.856	-0.54	-0.108	-0.363	0.215	-0.607
Aluminium (Al)	.927*	-0.716	0.721	-0.569	-0.598	**.939***	-0.755	0.367	-0.815	0.416	0.869	-0.368	-0.363	-0.518	0.414	-0.356
Iron(Fe)	0.716	**-.953***	**.914***	**-.905***	0.743	-0.795	0.825	-0.666	**.952***	0.324	0.856	-0.65	-0.423	-0.398	0.484	-0.701
Calcium (Ca)	**.957***	-0.829	0.841	-0.723	0.434	0.473	0.304	0.62	**-.936***	0.571	0.878	-0.575	0.162	0.004	0.01	0.094
Cadmium (Cd)	0.211	0.229	-0.106	0.153	0.707	-0.846	**.918***	-0.434	**-.902***	0.478	**.911***	-0.658	0.287	0.037	-0.639	0.286
Lead (Pb)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mercury (Hg)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*. Correlation is significant at the 0.05 level (2-tailed); **. Correlation is significant at the 0.01 level (2-tailed).

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CONCLUSION

In Dal Lake the concentrations of copper (1.25µg/g), zinc (5.69µg/g) and iron (29.3µg/g) were maximum in the muscles of infected fishes which were still lower than the Maximum Limit recommended by WHO/ FEPA/IAEA-407 whereas on the other the concentration of nickel (0.80µg/g), manganese (1.69µg/g), cadmium (0.78µg/g) and chromium (2.86µg/g), aluminum (2.99µg/g) were higher than the permissible limits. Cr, Mn, Cu, Zn, Al, Ca concentrations in water showed positive correlation with water temperature. Cu concentration in *Adenoscolex oreini* showed positive correlation with water temperature. Cd concentration in *Adenoscolex* showed positive correlation with pH of water. The parasite *Adenoscolex oreini* have direct relation with water. *Adenoscolex oreini* was positively correlated with Cd concentration in water during autumn and winter. Therefore, the parasite seem to act as bio-indicators for the corresponding metals.

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