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PHYSICO-CHEMICAL VARIABLES AND FISH PARASITES OF RIVER NEELUM AZAD JAMMU AND KASHMIR, PAKISTAN

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ABSTRACT

The physico-chemical parameters and the fish parasites of the River Neelum were studied at three sampling sites, i.e., Pattika, Tali Mandi, and Domail. Water temperature and rate of flow of water were significantly higher at Domail than Pattika and Tali Mandi. ANOVA showed that dissolved oxygen, total suspended solids, inorganic suspended solids, and organic matter of the bottom mud kept on fluctuating and showed a significant difference between three selected sites. Although, these variables showed variations, they remained within tolerable limits set by WHO. Three species of potentially harmful parasites, i.e., *Dactylogyrus vastator*, *Procamallanus laevionchus*, and *Argulus foliaceus* were isolated from fish population of the River Neelum, however, these parasites do not pose any severe threats to general fish life.

Keywords: Dissolved oxygen, total suspended solids, inorganic suspended solids, parasites, river Neelum.

INTRODUCTION

With the ever-increasing demand for cheaper sources of protein, more and more attention is being focused on fish. For obtaining healthy and quality fish meat, the fish should be free from all types of infections. Our reservoirs, rivers, and lakes are a precious source for fish production but very few of these are now in their natural conditions and have the key alarms in terms of water quantity and quality (Bash *et al.*, 2001; Boyd, 2003).

Suitability of water quality furnishes the source for evaluating the effect on aquatic life and their food chains (Glibert *et al.*, 2006). The mortality of fish species due to severe pollution has been recorded in snow-fed and rain-fed rivers (Mishra and Tripathi, 2007; Ali, 2008; Dongdem *et al.*, 2009; Bu *et al.*, 2010). Similarly, the low level of dissolved oxygen also affected the fish species (Khan *et al.*, 2012). In aquatic

systems, parasites may lead to epidemics and mortalities, resulting in economic losses, as fish are host to many adult helminthes parasites and larval forms of species (Barson, 2006). Various helminthes were recovered from the gills and intestine of freshwater fish, *Mystus vittatus* (Rafique *et al.*, 2002), *Mystus aor* (Bilqees *et al.*, 2012), *Schizothorax nigar* and *Channa striatus* (Shaikh and Bilqees, 2011; Moravec *et al.*, 2010; Ahmed *et al.*, 2007). Up to 94% of infections were reported from *Channa striatus* (Lafferty, 2008). Parasites can also induce morbidity and mortality in captive fish populations (Walker *et al.*, 2008).

The present study was initiated to examine and compare the water quality parameters and parasitic load on fishes of the River Neelum from the upstream rural area (Pattika) as well as the downstream urban sites, receiving increasing levels of

the urban pollution (Tali Mandi and Domail).

MATERIALS AND METHODS

Sampling

Three sampling sites, i.e., Pattika (34°26.769 NL, 73°32.986 E), Tali Mandi (34°22'03.4 NL, 073°27'57.4 E) and Domail (34°21'18.9 NL, 073°28'12.8 E) in the lower reaches of the River Neelum were selected (Figure 1). Each study site was visited during each calendar month at the middle part of the day between September 2006 and August 2007 to record the physico-chemical parameters. We collected water samples (450 ml) from the middle of stream in a plastic sampling bottle (taking due care to avoid air or air bubbles) and bottom mud from the depth of 0.3 m (100 g, packed in polythene bags) and brought back to the laboratory duly labeled for further analysis.

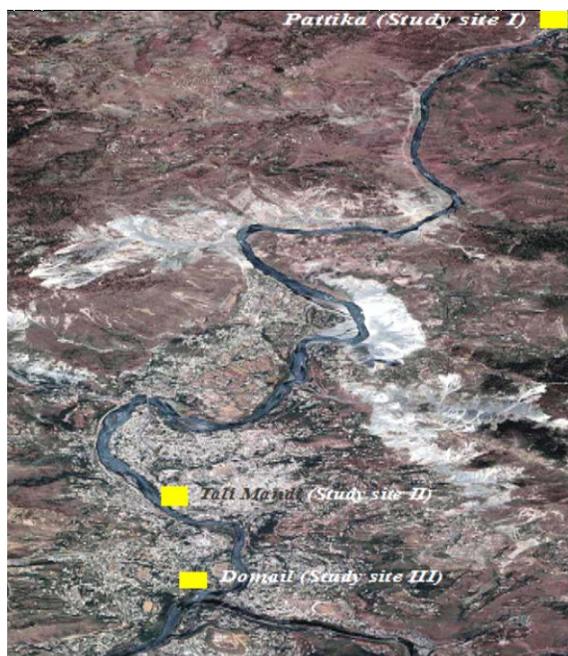


Figure 1: Sattelite imaginery showing location of sampling sites on the river Neelum, AJK.

Fish specimens were collected by using cast nets (mesh sizes: 1 cm×1 cm, circumference 10 m and mesh size 2.5 cm×2.5 cm, circumference 15 m). Each sample was numbered and packed in separate polythene bags, brought back to laboratory, duly labeled for further studies, and identified up to species following Kar *et al.* (2006).

Water Quality

Temperature (digital thermometer; INS -50-1200°C), pH (Model LPM 1.4, Henna), dissolved oxygen (DO meter; NOVA 60, Spectroquent), and electrical conductivity (digital EC meter, model LF 90, WTW) were measured in the field.

Alkalinity was measured by the titration method (2 drops of phenolphthalein indicator mixed in 100 ml water and titrated against 0.02 N sulfuric acids and volume recorded) and levels calculated {alkalinity (mg/L) = ml of sulfuric acid used 10}.

A volume of 2 ml of buffer solution and 8 drops of Erichrome Black T indicator was mixed with 100 ml of the water sample and titrated it against 0.01 N EDTA (Ethylene Diamine Tetra Acetate) solution until the purple color turned into a deep blue. Total hardness (mg/l) = Volume of EDTA used X 10.

For total suspended solids (ppm), 3 ml of the water sample was filtered through an oven dried (80°C), clean, pre-weighed Whatman-4 filter paper, and filter paper re-weighed to work out the weight of filtrate. The filtrate with organic content on filter paper was placed in a pre-weighed clean and dried crucible, and ignited in a muffle furnace (MF-90) for three hours at 500°C. Crucible reweighed after ignition was complete and the difference between the two

weights was regarded as the weight of the organic suspended solids. Inorganic suspended solids were determined by subtracting organic suspended solids from total suspended solids.

Samples of bottom mud were dried in the oven at 80°C overnight. A weighed quantity (10 g) of dried mud was placed into a clean pre-weighted crucible, which was then placed in the muffle furnace and ignited for three hours at 500°C. The crucible was reweighed after ignition and the difference between the two weights was taken as the weight of the organic matter in the bottom mud.

A volume of 300 ml of the filtered water samples was evaporated in a pre-weighted China dish and it was ignited in the furnace at 500°C for three hours. Again weighed in the China dish and the weight of the salts were calculated by subtracting the weight of the dish after ignition from the actual weight of the dish.

Significance of means of physico-chemical variables was judged by ANOVA and the difference between means judged by LSD at ($p < 0.05$) using SPSS.

Parasites

Ectoparasites: Each fish specimen was examined with naked eyes and hand-held lens, with special emphasis on fins, operculum nostrils, and buccal cavity. Gills after removal, were examined under a dissecting microscope (10X-50X, Olympus), smears scanned under a light microscope (40X and 100X objectives Olympus) and pieces of the gills were dipped in 4% formalin (vigorously shaken and deposit observed for parasites). The eyes and oral cavity of sampled fish were also scraped (Aloo *et al.*, 2004) and parasites collected

and examined for parasites under the light microscope. The parasites were transferred to clean or albumenized glass slides (depending on staining regimen), air dried, examined under a light microscope, and identified following Zaidi and Khan (1976).

Endoparasites: Fish were dissected, viscera removed, cut into pieces, and placed in a Petri dish containing physiological saline. Each section of gonads, liver, gall bladder, and intestine was searched for parasites. Parasites were placed in boiling water, allowing them to straighten out, and were measured using an ocular micrometer and measuring scale. Morphological features were studied with special emphasis on scolex, an important taxonomic character (Hassan, 2008).

RESULTS

Water Quality

Figures 2 (a-k) and Tables 1 present a summary of variation in the mean values of different physico-chemical variables during different calendar months. The dissolved oxygen (DO), total suspended organic solids, inorganic suspended solids, salinity, and organic matter in bottom mud were significantly different at 0.05 level at all three sampling sites. However, the changes in pH, alkalinity, and organic suspended solids during different calendar months did not show any difference and exhibited an erratic seasonal variation. ANOVA suggested no significant difference in these variables at different sites.

Parasites

Only 12 (20%) out of 60 fish specimens of 6 different species were infected by three parasites species, viz., *Dactylogyrus vastator*, *Procamallanus laevionchus*, and

Argulus foliaceus (Table 2). *D. vastator*

narrow elongated up to 0.35-0.45 mm, were

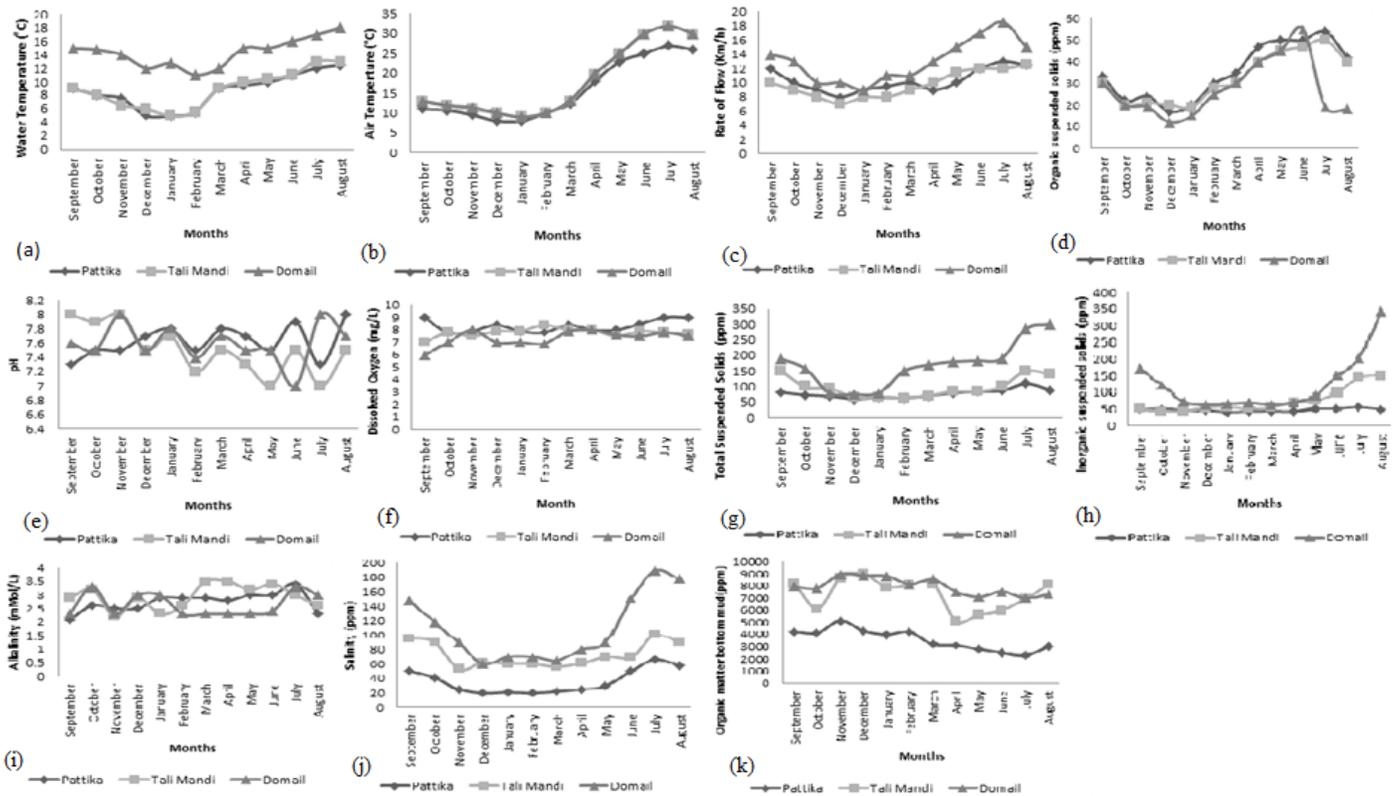


Figure 2: The trend of different physico-chemical parameters at different sites of River Neelum From September 2006 till August 2007. (a): Water temperature, (b): Air temperature, (c):Rate of flow, (d): Organic suspended solids, (e): pH (f): Dissolved oxygen, (g): Total suspended solids, (h): Inorganic suspended solids, (i): Alkalinity, (j): Salinity, (k): Organic matter bottom mud.

Table 1: Variations in physico-chemical water quality variables (Mean±SEM) at different study sites of river Neelum, AJK.

Variable	Sites			P-value
	Pattika	Tali Mandi	Domail	
Water temperature (°C)	8.66±0.74	8.87±0.79	14.39±0.61	0.000
Air temperature (°C)	15.73±2.17	17.89±2.57	17.99±2.56	0.050
Rate of flow (km/h)	10.33±0.47	9.75±0.54	13.04±0.89	0.000
Total suspended Solids (ppm)	77.83±4.08	97.33±9.41	170.1±20.80	0.000
Organic suspended solids (ppm)	35.33±3.76	32.50±3.30	27.33±3.81	0.302
Inorganic suspended solids (ppm)	46.17±1.41	74.08±11.07	123.3±24.22	0.004
Salinity (ppm)	35.67±4.77	72.33±4.87	108.9±13.32	0.000
Bottom mud organic matter (ppm)	3579±250.01	7328±378.73	7955±197.89	0.000
Dissolved Oxygen (mg/L)	8.308±0.13	7.783±0.09	7.350±0.17	0.000
Alkalinity (mMol/L)	2.742±0.102	2.942±0.128	2.650±0.123	0.221
pH	7.625±0.065	7.508±0.100	7.600±0.078	0.582

collected from gills; *A. foliaceus* (8 mm) from the general body surface, and *P. laevionchus* (nematode) exclusively from

the intestine. Three (3) out of 12 (20 %) infected fish specimens were infected by *D. vastator* (Table 2).

Table 2: Relative infestation rate of different parasite species in different organs of the different fish species at three sampling sites between September 2006 and August 2007.

Fish Species	Ecotoparasites						Endoparasite		
	Gills			Body Surface			Intestine		
	<i>D. vastator</i>			<i>A. foliaceus</i>			<i>P. laevionchus</i>		
	I	II	III	I	II	III	I	II	III
<i>S. trutta fario</i>	-	-	-	0.20	-	-	-	-	-
<i>O. mykiss</i>	-	-	-	-	-	-	-	-	
<i>S. plagiostomus</i>	-	0.20	-	0.30	0.10	0.20	-	0.20	-
<i>S. micropogon</i>	-	-	-	-	0.10	0.05	-	0.05	0.15
<i>T. kashmirensis</i>	0.40	0.10	-	-	0.05	0.10	-	0.15	-
<i>G. reticulatum</i>	0.40	-	-	-	-	-	0.20	-	-

I=Pattika, II=Tali Mandi, III=Domail.
Pattika: n = 10, Tali Mandi: n = 20, Domail: n = 20.

DISCUSSION

Three sampling sites have been selected along the length of the River Neelum, keeping in view the physical variations and possible pollution levels. Pattika is located in the upper reaches, and the river does not receive appreciable anthropogenic wastes. Beyond this point, the river receives many small streams pouring warmer water into the river. Most of the human settlements also appear along the river, as it flows downstream of Pattika, and it reaches Domail after it passes through the capital town of Muzaffarabad (AJK). The municipal solid wastes and sewerage is directly or indirectly dumped into the river. Thus these sampling sites have a continuing variation with decreasing altitudes; from Pattika to Domail, increasing organic and inorganic pollutants by increasing the sediments through soil erosion and anthropogenic effect of capital towns. All these factors can potentially affect the physico-chemical quality of water and the

composition of fish fauna, and possibly the parasite load.

Analysis of physico-chemical variables of the water of different sampling sites showed that there was a gradual increase in the water temperature, air temperature, and water flow (Figures 1, 2 and 3). ANOVA showed the significant difference among the dissolved oxygen, total suspended solids, inorganic suspended solid, salinity and organic matter in the bottom mud (Table 1, P-values), along the length of the river, from Pattika to Domail. Salinity was higher at Domail (108.9±13.32ppm) followed by Tali Mandi (72.33±4.8ppm) and Pattika (35.67±4.77ppm). Conversely, there was a gradual decrease in the quantities of the suspended organic solids and dissolved oxygen in the water at Domail followed by Tali Mandi of river Neelum. The alkalinity and pH remained constant at different sampling sites and the rate of the flow of water was significantly higher at Domail. Such an inter-site variation was expected

under the physical change of declining elevation and increasing anthropogenic effects in southern reaches of the river. The increasing human population not only adds the human and animal wastes into the water, but also causes increased deforestation and thence, increased soil erosion. The decreased suspended organic solids is caused by increased break down through metabolic activities and their increased deposition in the bottom, which is indicated by the increased organic contents of the bottom mud and decreased dissolved oxygen. The seasonal changes also affect water quality and increasing air and water temperature during the summer (June, July). Higher levels of rainfall during the summer monsoon cause floods and erosion of soil. Similar seasonal changes in water temperature have been reported by Ali *et al.* (2006) for the northern areas (KPK, Pakistan). Under such a pattern, suspended solids increase, and there are changes in water color and flow.

Fish parasites and their pathogenicity

The parasite prevalence depends on factors like parasite longevity and its life cycle, physical factors, and the quality of water (Ahmad *et al.*, 2007). The study area presents a fast river flowing through unpopulated and densely forested area. Therefore, water was not contaminated except for the lower region (Domail and Tali Mandi to some part). Only this site received sewage water and trash of the capital town.

Nonetheless, three species of parasites, viz., *Dactylogyrus vastator*, *Procamallanus laevionchus*, and *Argulus foliaceus* were isolated from different organs of the different fish specimens (Table 2). *D. vastator* was recovered from gills, *A. foliaceus* (fish lice) from the general body surface, while *P. laevionchus* was recovered

from intestine. Although parasites were recovered from fish species collected during the study period with a different rate of infestation, it appears that the changes in the quality of water under anthropogenic pollution have not resulted in an increase in the parasite load. The physical conditions of the water, particularly dissolved oxygen, stops embryonic development in the eggs of parasites (Schmidt, 1998; Rafique *et al.*, 2002). Moderate changes in physico-chemical factors, like the salinity of water or anthropogenic activity, can also help in checking the parasite infestation, rendering the environment unsuitable for the parasite, especially for the ectoparasites. Although, these species are harmful yet at the present infestation level, these do not pose any severe threats. No previous study is in hand to support these results. Further studies would be required to understand the parasite infestation pattern.

CONCLUSION

The present study observed the variation in the water quality, however, parameters are within the WHO permissible limit. Water quality exhibited changes with increasing pollution and geographical location of the sampling sites, yet at none of the locations, the pollution and associated anthropogenic activities seriously affect the quality of water. Further studies on pollution need to be worked out, and pollution status needs monitored and appropriate curative measures taken to check dumping and discharge of domestic sewage into the river system.

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