Original article

Profiling of toxic metals from fish (*tor putitora*), water and sediments with microbial and chemical water quality appraisals

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ABSTRACT

The current study was focused on selected quality parameters of water, heavy metal contents of water, sediments and fish from Poonch river. Sediments, water and fish samples were gathered including six different sites in the period of April to June 2015. During the study period the mean value recorded for water quality parameters were pH 9.20, mv Oxidation Reduction Potential (ORP) 32.2, Dissolved (Oxygen) DO 6.95 mg/l, Electrical Conductivity (EC) 252.96μS/cm and 271.91 μg/cm, Total Dissolved Solids (TDS) 197.03, Salinity 0.77, Turbidity 3.02, Temperature 31.65 and Pressure 13.37. Mean values recorded for heavy metal absorption in river water, sediments and fish were below the maximum permissible levels. The aim of current study was to show that water is good for the existence of fish survival and its growth. So, this study exhibits that the part of Poonch River, Azad Jammu and Kashmir having water quality parameters and heavy metals were within the tolerable range and no harmful effects on the fish growth and reproduction.

1. Introduction

Toxicity of heavy metals in aquatic environment is inclined by numerous abiotic aspects such as hydrogen, pH, oxygen, alkalinity or fluctuation in water temperature (Mahmood et al., 2013; Ghillebaert et al., 1995; Adhikari et al., 2009). Enormous evolution of industrial and agriculture development resulted in increased pollution for aquatic ecosystem through heavy metals, which is very hazardous (Uluturhan and Kucuksezgin, 2007). Numerous researchers conducted studies on riverine water quality statuses and reported heavy metals in aquatic flora and fauna (Gupta et al., 2007). Heavy metals if present in greater concentration might produce harmful outputs on aquatic organisms (Keepax et al., 2011; Tanee and Albert, 2013).

These heavy metals first accumulated in the fresh water and then transferred to organism by different means which are present in fresh water. When concentration of any heavy metal is surpassed the required level, it become contaminated and poisonous which can cause several health problems (Goldstein, 1990; Malik et al., 2010).

Industries discharges hazardous waste containing heavy metals in different aquatic sources without any safety measures (Mahmood and Malik, 2014). Domestic wastes, industrial run-offs, atmospheric deposition, discharge from landfills, and storm runoff are the main causes of heavy metal pollution in water (Kumar, 2011; Keskin et al., 2007; Storelli et al., 2006). The heavy metals from river water can attain the addition of pollutants (WHO, 1985).

Heavy metals are stable and insistent ecological pollutants in aquatic environment and are categorized as possibly poisonous, probably important and essential (Muñoz-Olivas and Cámara, 2001). Moreover, sediment associated metals pose a straight threat to the benthic aquatic life forms such as fishes which are deposit feeding (Luoma, 1983; Eimers et al., 2001). The aquatic life completely depends on the physico-chemical features of water (Mahmood et al., 2020a).

There are various methodologies to check the pollution level of heavy metals (Mahmood et al., 2020b; Roux et al., 1991; Nussey et al., 2000). The toxicity of much aquatic stuff was qualified by

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water quality. For example, fish generally gets more harm in soft water than hard water due to heavy metals. Owing to offhand dumping of developed and domestic waste, failed leaching system in cities has disturbed river water quality (Ugochukwu et al., 2004; Furtado et al., 1998; Emongor et al., 2005; Chindah et al., 2004). The current study was planned to evaluate the quality of water and analysis of heavy metals in water sediments and fish from Poonch River, Azad Jammu & Kashmir (AJK), Pakistan.

2. Methodology

2.1. Study area:

Poonch River is a tributary of river Jhelum and Mangla Lake, which passes through district Kotli and Mirpur AJK. The Rangpur Nala is a significant western tributary of the Poonch river, while two other vital watercourses known as Ban Nala and Mahuli Nala join it from the East (Fig. 1). This river is not much deep, it is shallow, open, flat and the speed of water is very moderate. Fish fauna is distributed in these water bodies is according to its ideal necessities regarding to temperature and other physico-chemical aspects. Atmospheric condition of region is hotter in summer and colder in winter. The annual rainfall is about 1300 mm, it is more

than half of which happens during month of August and July. It is main source of irrigation, generating electricity, drinking water and food. Its flow is contributed during the great run off or flood period. Less amount of water contributed during the dry weather period (Hymavathi et al., 1999). Characteristically the monsoon is a period of great sediments load in the river. Due to greater erodibility of rocks it is rich in sediments.

2.2. Sampling:

Sediments and water samples were collected from six different sites of Poonch river (district Kotli) AJK. Fish samples were collected from three sites. Samples were collected in the month of June 2015. From each site one sample of water and sediments were collected. Water samples were collected in plastic bottles washed with distilled water. Before further analysis of water samples, they were kept in a dark and dry part of laboratory.

At the depth of 5–10 cm of river, sediments were collected. Sediments were also taken in plastic jars prewashed with distilled water. Sediments were air dried before taking them to the laboratory for further analysis.

Fig. 1. Map of district Kotli AJK showing different sampling sites of the Poonch River (source: ajktours.com).

Fish samples were collected using net by getting help from a local fisher man. Immediate after collection transfer to the laboratory for further analysis.

2.3. Analysis for water quality

To analyze different quality parameters (pH, mvORP, DO, specific conductivity, absolute conductivity, TDS, salinity, turbidity, temperature, pressure) of water Multiparameter (HANNA HI 9829) was used. Simply the nozzle of multiparameter was dipped in the river at six random sites. The readings of all water parameters were obtained.

3. Digestion PROCEDURE:

3.1. Digestion of water

For liquefied metal analysis 250 ml of each water samples were filtered using Whatman filter paper No.45 (APHA,1998) and pH was accustomed by adding 1–2 ml of ultrapure HNO_3 kept at 4^0C till more examination (DeCarlo et al., 2004).

3.2. Digestion of sediments

Powdered sediments sample of 1gm each were processed in 15 ml of aqua regia 3:1 (HCL- HNO_3), left overnight and heated near about 150^0C until brown fumes turnoff and made addition of 5 ml $HCLO_4$ (70–72%) and heated it near to waterlessness. The residual solution was filtered through Whatman filter paper No.42 and volume was upraised to the mark 50 ml with twice distilled water.

3.3. Digestion of fish

After weighing (Electronic balance HYTEK SF-400) one-gram wet soft tissue (muscles, liver, gills, kidney, & Intestine) of fish (*Tor putitora*) locally known as Mahsheer were digested by using 1 ml $HCLO_4$ and 5 ml of HNO_3 in a flask. Soft tissue digestion were done on hot plate (200 to 250^0C) until digested samples were converted into colorless liquid and its volume were elevated up to 50 ml. Each digested sample was clarified through 0.42 μm filter paper, and then moved to polythene bottles and airtight to avoid impurity and vaporization. Samples were stored at 4^0C, after that process of heavy metal analysis should be done .The arranged samples were now evaluated for heavy metals using FS-AAS. Metal concentration was determined in microgram/gram (μg/g).

4. Laboratory Analysis

More recognition of heavy metals (Na, Cu, Zn, Ni, Pb, Fe, Cr, Cd, Mn) in samples remained analyzed in Fast Sequential Atomic Absorption Spectrophotometer (FS-AAS) (Mahmood et al., 2015). Air acetylene gas was used.1 g from each sample including sediments, tissues and water were occupied in a titration flask and from each stock solution of heavy metals 1 ml was reserved into a tube and protected with aluminum foil and also 9.9 ml concentrated water mixed and protected again. Already prepared standard solution of stated heavy metals 0.2 ml was taken and 9.8 ml sanitized water added to the tube. Similarly other three dilutions (1, 0.8, 0.6, 0.4 ml from standard solution of heavy metals) were primed by adding cleaned water so that the ending dimensions raised up to 10 ml.The prepared dilution were sited in Flame Atomic Absorption Spectrophotometer for examination and afterwards results were obtained.

4.1. Statistical analysis

Statistical package program SPSS (version 16.0) and MS Word Excel were used for statistical analysis of data. One-way analysis of variance (ANOVA) followed by LSD comparison test was applied to determine significance of difference among various data sets.

5. Results

5.1. Water quality parameters

Physiochemical characteristics of water samples collected from six random sites of Poonch river are shown in Table 1. Sites were named as station 1, 2, 3, 4, 5, 6 (Tata Pani, Sawar, Kotli, Nar, Rajdhani and Plak respectively). pH values of water at 1–6 sites were 8.73, 9.13, 9.17, 9.53, 9.38 and 9.29 respectively. mvORP count as 59.3, 34.6, 27.0, 20.3, 18.8 and 33.2 at station 1,2, 3, 4, 5 and 6 respectively. DO (mg/l) was not detected at station 1 because there was a hot water spring, rest of all the sites are within the range of 5.5–7.66. Specific EC and Absolute EC were ranged as 190–387 lowest at station 1, highest at station 3 and 115.3–422.3 lowest at station 1, highest at station 3 sites respectively. TDS at all six sites were 331, 251.3,199, 118,118.6 and 164.3 (mg/l). Salinity was highest at station 3 as 2.17 and lowest at site 4 as 0.15 but rest of four sites had the range between 0.1 and 1.4. Turbidity NTU (5.3, 3.5, 2.53, 3.6, 2.9 and 0.1) and Pressure Psi (12.5, 13.6, 13.5, 12.6, 14.5 and 13.5) were found in all six sites. Mean temperature of water recorded highest at station 1 (Tata Pani) was 45C because of hot water spring rest of all sites had almost same range of temperature (27- 29C).

5.2. Heavy metal concentration in water

Mean metal concentration in water samples were shown in Fig. 2. Highest concentration of Pb was 0.09 mg/l detected at site 3 (Kotli) and lowest 0.03 mg/l at site 2 (Sawar). Fe was not detected at site 1, 3 and 6. Rest of sites have mean concentration of Fe is 0.06, 0.11 and 0.14 mg/l at site 2, 4 and 5 respectively. Ni was not detected at site 5 and 6. The mean concentration of Ni at rest of sites ranges from 0.005 to 0.05 mg/l. Mean concentration of Cu was 0.01 mg/l at site 3 and 5, 0.35 and 0.48 ml/l at site 4 and 6 respectively. Cu was not detected in water samples at station 1 and 2. Mean value of Zn at all sites were 0.24, 0.09, 0.01 1.00, 1.09 and 0.02 at site 1, 2, 3, 4, 5 and 6 respectively. Cr were not detected at site 2, 5and 6. Mean concentration of Cr were 0.05, 0.14 and 0.08 at site 1, 2 and 4. Mn ranges between 0.004 and 0.009 mg/l at site 1, 4, 5 and 6. Mn was not detected at site 2 and 3. Mean concentration of Pb were ranges 0.03–0.09 mg/l at all sites.

5.3. Heavy metal concentrations in sediments

In sediment sample mean concentration were shown in Fig. 3. Mean concentration of Pb were 0.06, 0.08, 0.28, 0.22, 0.07 and 0.14 μg/g at station 1, 2, 3, 4, 5 and 6 respectively. Fe were 8.22, 6.12, 3.75, 8.66, 7.72 and 7.76 at station 1, 2, 3, 4, 5 and 6 respectively. Mean concentration of Ni were 0.56, 0.77, 0.5, 0.59, 0.65 and 0.6 at site 1, 2, 3, 4, 5 and 6 respectively. Cu concentration were 0.57, 0.01, 0.51 and 0.46 at station 1, 4, 5 and 6. Cu were not detected at station 2 and 3. Mean concentration of Zn were 1.06, 0.03, 0.03, 1.68, 1.1 and 0.02 at stations 1, 2, 3, 4, 5 and 6. Cr concentration ranges between 1.90 and 2.82 highest at station 2 and lowest at station 4. Mean concentration of Mn were7.53, 8.88, 8.41, 8.22, 7.72 and 8.75 at station 1, 2, 3, 4, 5 and 6 respectively.

Table 1
Results for water quality parameters.

Parameters	Sites						
	1	2	3	4	5	6	Average
pH	8.73	9.13	9.17	9.53	9.38	9.29	9.20
mvORP	59.3	34.6	27.0	20.3	18.8	33.2	32.2
DO(mg/l)	ND	5.5	7.10	7.08	7.45	7.66	6.95
EC(μS/cm)	106.3	233	387.3	228.6	234.6	328	252.96
EC(μg/cm)[A]	115.3	227.6	422.3	253.3	258	355	271.91
TDS(mg/l)	331	251.3	199	118	118.6	164.3	197.03
Salinity	0.56	0.16	2.17	0.15	1.43	0.17	0.77
Turbidity	5.33	3.5	2.53	3.66	2.93	0.19	3.02
Temperature	45	29.27	29	29.3	29.33	28	31.65
Pressure	12.5	13.63	13.5	12.6	14.5	13.5	13.37
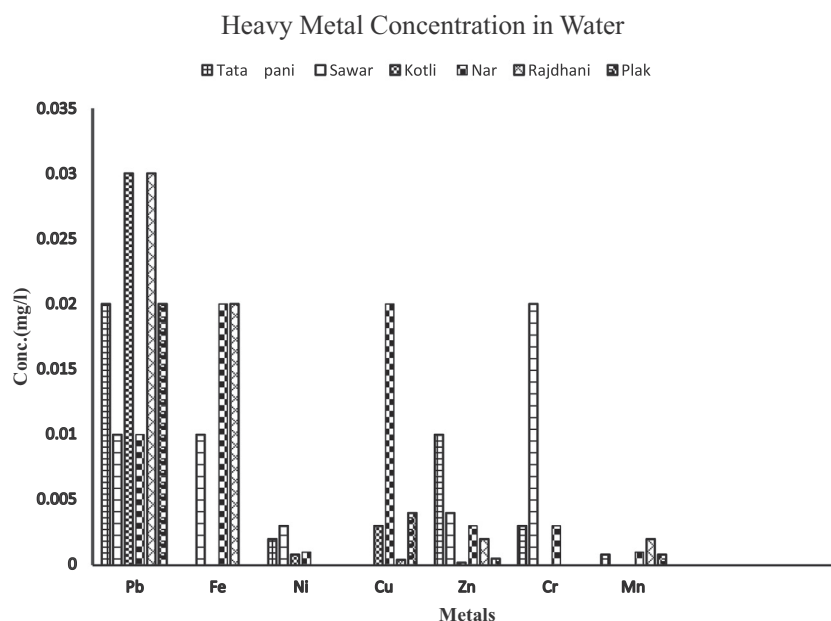
Fig. 2. Heavy metal concentrations in water of the Poonch river.
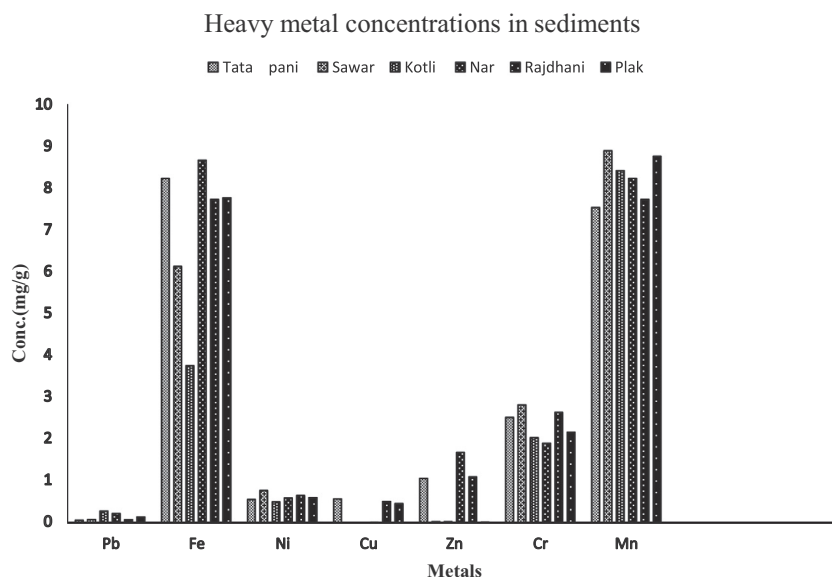
Fig. 3. Heavy metal concentration in sediments of the Poonch river.

5.4. Heavy metal concentration in muscles

In muscle tissues sample mean concentration of metals were shown in Fig. 4. Fish samples were Figure 1 collected from three sites that is site 1, 3 and 5 respectively. Pb were not detected in any sample of fish muscle at any site. Mean concentration of Fe were 0.08, 0.26 and 0.16 μg/g at site 1, 2 and 3 respectively. Ni concentration were 0.03 μg/g at site 1 and 2, 0.008 μg/g at site 3. Cu were not detected in muscle of fish at site 1 and 3, 0.001 μg/g were detected at site 2. Mean concentration of Zn were followed in muscle tissue as 0.02, 0.01 and 0.18 μg/g at site 1, 2 and 3. Cr and Mn were 0.09, 0.07, 0.22 and 0.06, 0.08, 0.07 μg/g at site 1,2 and 3 respectively.

5.5. Heavy metal concentration in intestine

Mean concentration of Pb, Fe, Ni, Cu, Zn, Cr and Mn in intestine were 0.06, 0.30, 0.003, 0.01, 0.02, 0.06 and 0.21 μg/g at site 1 respectively. Pb was not detected in intestine of fish at site 3, remaining mean concentration of Fe, Ni, Cu, Zn, Cr, Mn were 0.25, 0.006, 0.03, 0.04, 0.14, 0.55 μg/g respectively. Mean concentration of metals were 0.1, 0.17, 0.02, 0.02, 0.01, 0.11, 0.16 μg/g in Pb, Fe, Ni, Cu, Zn, Cr, Mn respectively at site 5.Concentration were shown in Fig. 5.

5.6. Heavy metals concentration in gills

Mean concentration of Pb, Fe, Ni, Cu, Zn, Cr and Mn in gills of fish were 0.07, 0.28, 0.005, 0, 0.001, 0.06, 0.05 at site 1, 0.08, 0.46, 0.02, 0, 0.03, 0.02, 0.26 at site 3, 0.1, 0.31, 0.005, 0.01, 0.01,

0.31 and 0.15 μg/g at site 5 respectively. Mean concentration were shown in Fig. 6.

5.7. Heavy metal concentration in kidney

Mean concentration of Pb and Fe in kidney of fish were 0.11, 0.08, 0.2 μg/g and 0.12, 0.25, 0.1 μg/g at site 1, 3 and 5 respectively. Ni, Cu, Zn, Cr and Mn were 0.03, 0.01, 0.004, 0.22, 0.06 μg/g at site 1, 0.003, 0.03, 0.02, 0.09, 0.06 μg/g at site 3 and 0.2, 0.1, 0.01, 0.11, 0.03, 0.24, 0.09 μg/g at site 5 respectively. Metal concentration were shown in Fig. 7.
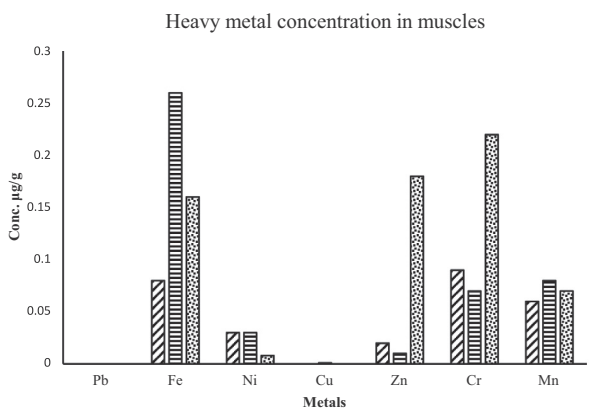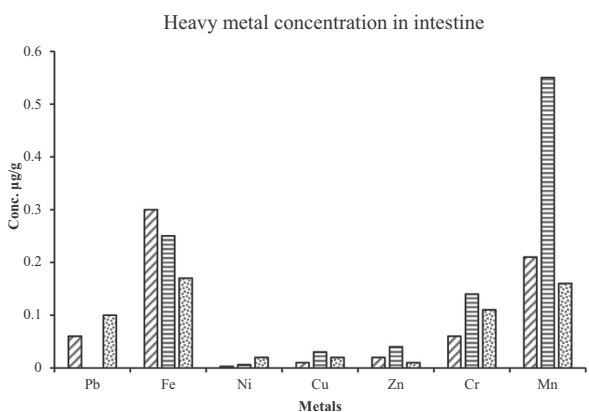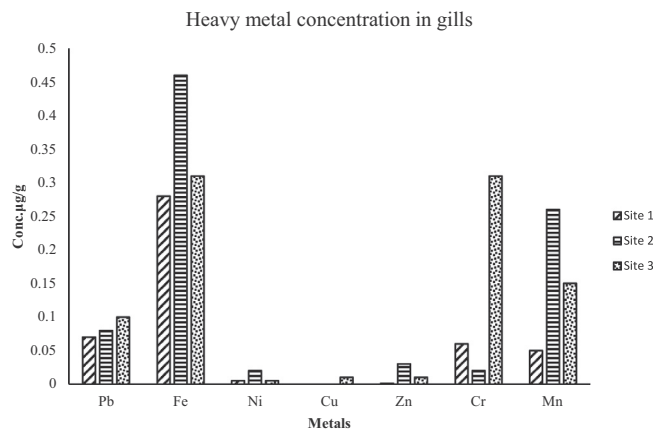

Fig. 6. Heavy metal concentration in the fish gills.

Fig. 7. Heavy metal concentration in fish kidney.

Fig. 4. Heavy metal concentration in the fish muscles.

Fig. 5. Heavy metal concentration in the fish intestine.
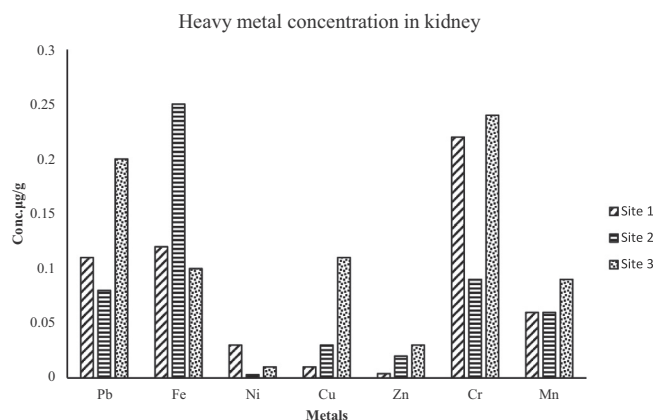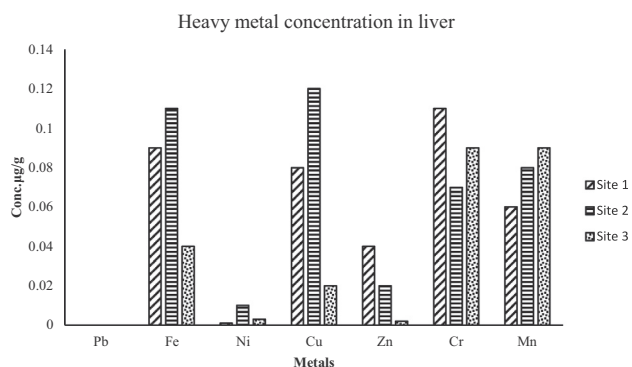

Fig. 8. Heavy metal concentration in the fish liver.

5.8. Heavy metal concentration in liver

Pb were not detected in liver tissues at any site of river. Remaining metals Fe, Ni, Cu, Zn, Cr and Mn were 0.09, 0.001, 0.08, 0.04, 0.11, 0.06 µg/g, 0.11, 0.01, 0.12, 0.02, 0.07, 0.08 µg/g and 0.04, 0.003, 0.02, 0.002, 0.09, 0.09 µg/g at site 1, 3 and 5 respectively. Metal concentration were shown in Fig. 8.

6. Discussion

Different species of water bodies have found strong habitation in river Poonch and functions as their upbringing. River Poonch was within the desirable parameters according to its all chemical and physical properties. The pH of the river water was found 8.73–9.38. The water sample at station 5 was collected have highest range 9.38 of pH after addition of waste from city in the river confined by high population density, indicated pH towards basic side might be because of human activity. The pH of water at station 1 was slightly towards the acidic side 8.73 as there is a hot water spring. However, the average value of river samples indicated pH values within the edge limits of WHO for consuming water. A significant difference was not observed in the water pH at different sites.

mvORP and DO ranges from 18.8 to 59.3 and 5.5 to 7.66 mg/l respectively (Table 1). ORP was highest in water sample at station 1. As hot water spring emerges at site 1 Tata Pani and no human activity surrounds the site results in high percentage of ORP. The lowest ORP in water were recorded at station 5 due to moderate flow of river. Human activities were high at adjacent sideways. Human and animal waste polluted the nearby side's results in lesser amount of mvORP.

The dissolved oxygen indicated the variation within 5.5 – 7.67 mg/l. The oxygen content was not detected at station 1 as it has a hot water spring (Tata Pani) and DO was not detected due to less solubility of oxygen at high temperature. The lowest oxygen content was detected at station 2 may be due to the effects of hot water spring from station 1 as the river flows from station 1 to ahead . Also feasibly because of less solubility due to pollution content. Dissolved oxygen (DO) in the water body is required to prevent odor and is suitable for use by aquatic plants and other water bodies. A significant difference was not observed in DO of water at all stations.

EC and TDS were varied within range of 106–387 µS/cm, 115–422 µg/cm and 118–331 mg/l respectively. Electrical conductivity is a measure of how well a solution conducts electricity and it is correlated with salt content. The higher values of specific, absolute EC and TDS in water sample were at site 3 and site 1 respectively may be suggested because of mixing of domestic waste from surrounding area and waste from slaughter house in river water at station 5. There was a hot water spring at station 1, the sulphur eruption along with hot spring continuously dissolved within water bed and may suggested the cause of higher range of TDS in river water at station 1.

Salinity and pressure range were higher at site 3 and site 5 as 2.17 PSU and 14.5 Psi respectively. Higher salinity range was expected due to heavy human activity and mixing of city waste. The pressure range was higher at site 5 due to the reason that river flow from that site was slightly slow. The river flows from backward creates pressure to move the river water from station. Turbidity and temperature, 5.33 NTU and 45C respectively both were high at station 1. The hot water spring named Tata Pani at station 1 continuously erupt sulphur from ground when river water run from that sulphur erupting area the temperature rise and ultimately turbidity also increased due to continuous mixing of hot sulphur in river water. These results are in accordance to previous reports revealing fluently disposal of waste effluents as a major source of increased pollution in certain river of the country (Nargis, 2013).

The main objective of this study was the determination of selected heavy metals in water, sediments and fish tissues at different sites of Poonch river. At all six sites of river, concentrations of these metals in the water were within the permissible limits of WHO for drinking water. Results from analysis have shown that these heavy metals: were all are not present in all the six sampling site of water that were analyzed. Some metals are absent or not determine in samples at some stations, which predicts that Poonch river is not highly contaminated with heavy metals. At all six sites of the river the concentration of selected heavy metals in water were within the permissible limits of WHO (2, 0.02, 3, 0.3, 0.5, 0.01 and 0.05 mg/l for Pb, Fe, Ni, Cu, Zn, Mn, and Cr respectively).

Fe in the sediment found in higher concentration may be because of the clayey material that forms the river bed in the area sampled. Sediment is the major depository of metals holding more than 99% of total amount of metal present in the aquatic system. In sediments the permissible level present for some metals are 36, 35, 140, 85 and 100 mg/kg for Cu, Ni, Zn, Pb and Cr respectively as recommended by Dutch Target Limits (Tabinda et al., 2013). In the present study Pb, Fe, Ni, Cu, Zn, Cr and Mn concentrations were below the permissible level reported by Ontario Ministry of the Environment and were accepted by Saeed and Shaker (2010) and Olubunmi (2010).

Heavy metal concentrations in tissues of fish collected from Poonch river are within the permissible limit of WHO, because the Poonch river as compared to other rivers in literature is less contaminated (Ullah et al., 2019). The reason is less populated area and industries at nearby area. Metals bioaccumulation were considered less in fish biota. The metals bioaccumulation were found less in water following to sediments then in tissues.

7. Conclusion and recommendation

The misleading causes of pollution in the area are anthropogenic undertakings like improper agricultural practices and disposal of waste effluents. The proportion concentration of heavy metals is highest in the soil and least in water samples. At present, the water of Poonch river fulfill the drinking water criteria of WHO. In sediments of Poonch river Fe boundaries was in complex limits as compared with that of tissues. At present, the quality of water in Poonch river is good for survival, growth and population of fish. Suitable supervision is required to endure the quality of the river for the coming peers. In addition, people living along water ways should be alerted of the conceivable danger and health threat linked with the use of unprocessed water with a view of ominous expulsion of sewage and other developed, agrarian or local waste into water courses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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