Assessment of heavy metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) in rivulet water, their accumulations and alterations in hematology of fish *Channa punctatus*

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The present study was conducted to examine the contamination of rivulet situated at Kasimpur, Aligarh (27.218° N; 79.378° E). It receives the wastewater of Harduaganj Thermal Power Plant (HTPS) containing fly ash and heavy metals. Among the heavy metals estimated in the rivulet water, Fe (8.71 mg L⁻¹) was present in the highest concentration followed by Cu (0.86 mg L⁻¹), Zn (0.30 mg L⁻¹), Mn (0.21 mg L⁻¹), Ni (0.12 mg L⁻¹), Co (0.11 mg L⁻¹) and Cr (0.10 mg L⁻¹). The values for the heavy metals such as Fe, Ni and Mn were beyond the limits set by UNEP/GEMS.

*Channa punctatus* inhabiting the rivulet, bioaccumulate these heavy metals in tissues such as gills, liver, kidney, muscle and integument. Accumulation of Fe (140.2 to 1533.08 mg kg⁻¹.dw) was highest in all the organs. The accumulation of Fe, Zn, and Mn, observed in the tissues were above the values recommended by FAO/WHO. Abnormalities in hematological parameters of exposed *C. punctatus* were also observed. The total RBC count (-51.39%) showed decline over control. Therefore, fall in Hb (-36.98%) and oxygen carrying capacity (37.00%) was also observed over control. On the contrary, the total WBC count (+25.43%) increased over control.

**Key words:** *Channa punctatus*, effluents, heavy metals, bioaccumulation, hematology.

INTRODUCTION

Rapid industrialization in India has resulted in substantial increase in the solid/ liquid waste which is traditionally discharged into nearby natural water causing a number of environmental problems including threat to plants and animal lives (Babalola et al., 2009). Sewage and industrial disposal has greatly enhanced the addition of heavy metals into the aquatic ecosystem. Heavy metals occur in the environment in both ionic as well as bound form. Ionic species are readily available to biota for uptake and cause deleterious changes at various levels of organization of the animals. Amongst animals, fishes are the inhabitants that cannot escape from the detrimental influence of these pollutants (Clarkson, 1998; Dickman and Leung, 1998; Olaifa et al., 2004). Fishes are widely used to evaluate the health of aquatic ecosystems; hence, act as bioindicators. There are very few studies conducted in Aligarh area to focus on this aspect. High concentrations of heavy metals had also been reported in water of sewage-fed aquaculture pond and prevalent fish *C. punctatus* at Panethi, Aligarh (Javed and Usmani, 2012a, 2013a). Similarly, sugar mill effluent (Cu, Ni, Co, Cr) dominated water body at Satha, Aligarh also reported to contained beyond permissible levels of heavy metals (Ni, Cr) in both water and fishes (Javed and Usmani, 2013b). There are many water bodies which lie in vicinity of population have been polluted...
by effluents released by industries, factories, power stations, domestic waste etc. which besides disturbing the quality of water also degrade the protein source in the form of fish food and limits their use (Baki et al., 2011; Qadir and Naseem, 2011; Taweel et al., 2012; Emere and Dibal, 2013; Javed, 2013; Javed and Usmani, 2013c). Therefore, it becomes important to study such water bodies where wastes are released and also populations of fishes are thriving.

The aim of this study was to assess the abnormal presence of heavy metals in rivulet water because it receives effluents from ‘thermal power station’, their bioaccumulation in different tissues and effect on hematology of fish C. punctatus, in order to provide the fish as a bioindicator. For this purpose, the bioaccumulation of Cu, Ni, Fe, Co, Mn, Cr and Zn were investigated in the fish tissues like gills, liver, kidney, muscle and integument. Hemoglobin content, RBC, WBC counts and oxygen carrying capacity were also worked out. C. punctatus being relished by large mass of population, also of its hardy and tolerant nature and adaptability to various conditions and habitats, was chosen as the model for study. Fishes, specially are being the potential indicators of pollution, and clearly indicate the pollution status of the rivulet.

**Description of the study area**

Harduaganj Reservoir (27.218° N and 79.378° E) at Kasimpur (Figure 1), (during late 1990's) Aligarh was quite productive and healthy. Water filled area being 13.5 ha. Now this reservoir has damaged completely because of the discharge of effluents from Harduaganj Thermal Power Station. This has changed the condition of reservoir drastically and it appears more of a waste land (Figure 2a and b). Besides the damage of aquatic ecosystem, the fly ash from the power plant also destroys the terrestrial ecosystem. The area was previously occupied by the lush green crop fields. With time, the greenery has been replaced by waste land having ash deposited over it. This all happens due to unsafe disposal of ash from the power station damaging the soil, making it unfit for agriculture. But now it is the rivulet which becomes the victim of ‘thermal power station’. The pipelines from the ‘power plant’ drain the wastewater into the rivulet flowing through the area (Figure 2c and d).

The rivulet water has also been rendered unsafe for fishes as well as for domestic consumption, irrigation, and other needs particularly consumption by cattle and other domestic animals.

**MATERIALS AND METHODS**

**Sample collection**

Water was collected in pre-cleaned and acidified glass bottles and preserved by acidifying with 6 N HNO₃ (pH maintained to about 2.0, Thomas Baker) to estimate the presence of heavy metals in the rivulet. On spot fixation of water was carried out to measure the Dissolved Oxygen (D.O). Total solids (T.S), total dissolved solids (T.D.S) and total suspended solids (T.S.S) were determined using standard techniques (APHA, 2005). The temperature and pH were recorded on the spot using thermometer (Deluxe, 6) and pH paper (S.D fine chemicals, 0 to 0.1). Samples of C. punctatus (n = 20) were collected with the help of professional local fishermen. Live specimens of C. punctatus were transferred to water buckets and brought to the laboratory for further analysis. Sampled fishes were killed immediately after blood collection, measured (length and weight) and then utilized for analysis of bioaccumulation of heavy metals.

**Estimation of heavy metals in water samples and fish organs/tissues**

Heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) were estimated in rivulet water and fish organs/tissues using atomic absorption spectrometer (Perkin Elmer, Analyst A, 800) as per the standard protocols of APHA (2005).

**Hematological analysis**

Live fishes were used to collect the blood by cardiac puncture in ethylenediaminetetraacetic acid (EDTA, Sigma) vials.

**Total count of RBC**

The RBC counts were made by Neubauer Hemocytometer (Rohem, India). Blood was diluted 1:200 with Hayem’s solution. Counting was done under the binocular microscope in the five smaller squares that is, in the 1st, 5th, 13th, 21st and 25th. The RBC’s on the lower and right sides of a square were added in the total, while those on the upper and left sides were rejected. Total numbers were reported as10⁶ mm⁻³ (Wintrobe, 1967).

**Total count of WBC**

The WBC counts were made by Neubauer Hemocytometer (Rohem, India). Blood was diluted in 1:20 with Turk’s diluting fluid and placed in hemocytometer. Four large (1 mm) corner squares of the hemocytometer were counted under the binocular microscope. The total number of WBC was reported as10⁹ mm⁻³ (Wintrobe, 1967).

**Estimation of hemoglobin**

Hemoglobin (Hb) was determined with hemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethemoglobin method.

**Oxygen carrying capacity**

The oxygen carrying capacity is obtained by multiplying the Hb content with the O₂ combining power of 1.25 ml of O₂ per g Hb (Johansen, 1970).

**Metal pollution index**

The metal pollution index (MPI) is used to compare the total metal accumulation level in various tissues of the fish. It is used to simplify the data to give it a one value instead of many in an organ. The
values were calculated using the equation:

$$\text{MPI} = (C_{f1} \times C_{f2} \times \ldots \times C_{fn})^{1/n}$$

Where, $C_{f1}$, $C_{f2}$, ..., $C_{fn}$ is the concentration for the metal 1, 2, ..., up to 'n' in the sample which raise to the whole power of one by total number of metals 'n' in the sample (Usero et al., 1997).

Bioaccumulation factor

The bioaccumulation of the heavy metals (HM) in fish tissues were quantified using bioaccumulation factor (BAF). BAF is the ratio of the concentration of a specific heavy metal in the tissue of the organism, to the concentration of that heavy metal in the water (Hassan et al., 2003).

$$\text{BAF} = \frac{\text{Concentration of HM in dry fish tissue (mg kg}^{-1})}{\text{Concentration of HM in rivulet water (mg L}^{-1})}$$

Statistical analysis

All values are given as mean ± SD. Statistical differences among the means of heavy metal accumulation in fish tissues were calculated using one way ANOVA and Duncan’s multiple range test (Duncan, 1955). Student’s t test (2-tailed) was used to test the significant differences in hematological parameters.

RESULTS

The average length of fishes measured was 6.5 ± 2.3 cm and average weight was 14.3 ± 1.2 g.

Physicochemical parameters and heavy metals in rivulet water samples

Physicochemical characteristics of rivulet water (temperature = 27.60°C, pH = 6.9, dissolved oxygen = 6.9 mg L\(^{-1}\), total solids = 652 mg L\(^{-1}\), total dissolved solids = 407 mg L\(^{-1}\), total suspended solids = 245 mg L\(^{-1}\)) were comparable with ideal water quality. However, the heavy metal content in rivulet water were in the order Fe (8.71 mg L\(^{-1}\)) > Cu (0.86 mg L\(^{-1}\)) > Zn (0.3 mg L\(^{-1}\)) > Mn (0.21 mg L\(^{-1}\)) > Ni (0.12 mg L\(^{-1}\)) > Co (0.10 mg L\(^{-1}\)) (Table 1); where it was found that Fe, Ni, Mn and Cr exceeded the recommended values set by UNEPGEMS (2006) but Cu and Zn were within the tolerable range.

Heavy metals in fish tissue samples

The results of the levels of concentration of the heavy metals in the fish samples are presented in Table 2. Superscripts shows the accumulation of a heavy metal in various organs of C. punctatus and subscript shows the accumulation of different heavy metals in particular organs. Fe accumulation was highest in all organs. The concentration of Fe in different organs ranged from 140.20 to 1533.08 mg kg\(^{-1}\).dw and it was highest (1533.08 mg kg\(^{-1}\).dw) in liver and least (140.20 mg kg\(^{-1}\).dw) was recorded in muscles. The order of accumulation was liver > kidney > gills > integument > muscle. The Zn concentration fluctuates between 95.09 to 434.59 mg kg\(^{-1}\).dw and its pattern of accumulation was kidney > gills > liver > integument > muscle. Similarly, Cu concentration was highest (236.66 mg kg\(^{-1}\).dw) in kidney and least (13.25 mg kg\(^{-1}\).dw) in muscle and the sequence of their presence in organs/tissues were kidney > gills > liver > integument > muscle. Cr ranged from 9.72 to 200.48 mg kg\(^{-1}\).dw in different organs/tissues. Its accumulation was highest in kidney and least in integument. The pattern of accumulation was kidney > gills > liver > muscle > integument.
Figure 2. Harduaganj thermal power plant draining heavy metal into the reservoir (a); present scenario: degraded condition of reservoir (b); wastewater emerging from power plant polluting the rivulet (c); rivulet water used for different purposes (d).

Mn concentration was highest (103.21 mg kg\(^{-1}\).dw) in gills and least (9.24 mg kg\(^{-1}\).dw) in muscle. The pattern of accumulation was in the order gills > integument > kidney > liver > muscle. Ni concentration was maximum (17.49 mg kg\(^{-1}\).dw) in gills and least in muscle (8.31 mg kg\(^{-1}\).dw). The pattern of accumulation was in the order gills > integument > liver > kidney > muscle. Co concentrations fluctuate between 3.87 to 13.68 mg kg\(^{-1}\).dw. It was recorded highest in gills (13.68 mg kg\(^{-1}\).dw) and least in muscle (3.87 mg kg\(^{-1}\).dw).
Table 1. Heavy metal content in rivulet water compared with quality guidelines and standards by international organization or country.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>WHO (guidelines)</th>
<th>USA (standards)</th>
<th>*Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>2</td>
<td>1.3</td>
<td>0.86 ± 0.01</td>
</tr>
<tr>
<td>Ni</td>
<td>0.02</td>
<td>-</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Fe</td>
<td>-</td>
<td>0.3</td>
<td>8.71 ± 2.88</td>
</tr>
<tr>
<td>Co</td>
<td>-</td>
<td>-</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>Mn</td>
<td>0.5</td>
<td>0.05</td>
<td>0.21 ± 0.10</td>
</tr>
<tr>
<td>Cr</td>
<td>0.05</td>
<td>0.1</td>
<td>0.1 ± 0.02</td>
</tr>
<tr>
<td>Zn</td>
<td>3</td>
<td>5</td>
<td>0.3 ± 0.03</td>
</tr>
</tbody>
</table>

All values are in mg L⁻¹. *Values of heavy metal content in the present study are given as mean ± SD, (n = 4 × 3), samples collected from 4 different zones of rivulet and were analyzed in triplicates. Standard guidelines adapted for Water Quality for Ecosystem and Human Health, 2006 [prepared and published by the United Nations Environment Programme, global environment monitoring system (GEMS)/ Water Programme]. Blank cells indicate that no citable information was available.

Table 2. Accumulation of heavy metals in the organs of Channa punctatus.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Gills</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Integument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>27.09±19.61ab</td>
<td>43.33±19.73c</td>
<td>236.66±03.36</td>
<td>13.25±1.31</td>
<td>14.06±5.31</td>
</tr>
<tr>
<td>Ni</td>
<td>14.49±8.19def</td>
<td>10.37±2.90def</td>
<td>9.22±5.86ef</td>
<td>8.31±0.80edef</td>
<td>11.21±8.36ede</td>
</tr>
<tr>
<td>Fe</td>
<td>488.21±45.12a</td>
<td>1533.08±95.2a</td>
<td>1130.33±65.5a</td>
<td>140.20±51.5a</td>
<td>182.13±18.1a</td>
</tr>
<tr>
<td>Co</td>
<td>13.68±2.24def</td>
<td>7.64±2.23de</td>
<td>6.13±2.63a</td>
<td>3.87±0.50edef</td>
<td>b</td>
</tr>
<tr>
<td>Mn</td>
<td>103.21±7.27c</td>
<td>17.35±7.76d</td>
<td>18.89±5.37c</td>
<td>9.25±5.06edef</td>
<td>33.18±3.32c</td>
</tr>
<tr>
<td>Cr</td>
<td>23.03±1.69de</td>
<td>14.69±3.99de</td>
<td>200.48±4.95</td>
<td>10.83±7.44c</td>
<td>9.72±3.88cdef</td>
</tr>
<tr>
<td>Zn</td>
<td>345.68±90.81ab</td>
<td>206.12±77.89b</td>
<td>434.59±67.25b</td>
<td>95.09±35.5ab</td>
<td>153.01±98.3</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, (n = 12). ND = not detected. Duncans multiple range test was used to test the statistically significant difference among the means. Means with similar letters in a column and row are statistically similar at P < 0.01; superscripts indicate, accumulation of a heavy metal (mg kg⁻¹ dry weight) in various organs, subscripts indicate accumulation of heavy metals (mg kg⁻¹ dw) in particular organs.

Table 3. Metal pollution index value of total metal accumulation in organs/tissues of Channa punctatus.

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>Metal pollution index (MPI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>81.93</td>
</tr>
<tr>
<td>Gills</td>
<td>59.37</td>
</tr>
<tr>
<td>Liver</td>
<td>43.09</td>
</tr>
<tr>
<td>Integument</td>
<td>26.94</td>
</tr>
<tr>
<td>Muscle</td>
<td>17.80</td>
</tr>
</tbody>
</table>

The pattern of accumulation observed was in the order gills > liver > integument > kidney > muscle.

According to the metal pollution index (MPI) calculated for the sampled organs/tissues (Table 3), it was observed that kidney (81.93) of the fish C. punctatus most influenced and had highest metal load followed by gills (59.37), liver (43.09), integument (26.94) and muscle (17.80) was least influenced by heavy metals. This clearly indicates that each tissue had different capacity of accumulation; since muscle and integument are edible part and therefore their quality needs to be monitored prior to consumption. The accumulation of heavy metals needed to be compared with recommended levels (Table 4). It was observed that except for Cu, Ni and Cr, the concentrations of Fe, Zn and Mn are beyond the safe limits. Table 5 revealed the calculated BAF for different heavy metals in fish tissues. It was found that the concentration of the heavy metals in different tissues of the fish were several folds higher than their concentrations in water. Kidney showed high BAF for Cu, Cr and Zn. In gills, the highest BAF was recorded for Ni, Co and Mn while in liver it was Fe. Similarly, in muscle, the highest BAF was recorded for Zn and least for Cu. In integument, highest and least values of BAF were calculated for Ni and Cr, respectively.

Hematological analysis and oxygen carrying capacity

Alterations in blood profile of C. punctatus were presented in Table 6. The total count of RBC in exposed fish was low (2.61 × 10⁸ mm⁻³) than control (5.73 × 10⁸ mm⁻³). Similarly, Hb content in exposed fish was low (8.23 g dL⁻¹) as compared to the control (13.06 g dL⁻¹); but higher counts of WBCs (5.72 × 10³ mm⁻³) were observed in exposed fish as compared to control (4.56 ×
Table 4. Comparative account of heavy metal concentrations in edible part of *Channa punctatus* with standard guidelines.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th><em>Average concentration</em></th>
<th>Recommended levels (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>13.65</td>
<td>30 (FAO/WHO, 1983)</td>
</tr>
<tr>
<td>Ni</td>
<td>9.79</td>
<td>70 to 80 (USFDA, 1993b)</td>
</tr>
<tr>
<td>Fe</td>
<td>161.16</td>
<td>100 (FAO/WHO, 1989)</td>
</tr>
<tr>
<td>Co</td>
<td>5.59</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>21.20</td>
<td>1.0 (FAO/WHO, 1989)</td>
</tr>
<tr>
<td>Cr</td>
<td>10.27</td>
<td>12 to 13 (USFDA, 1993a)</td>
</tr>
<tr>
<td>Zn</td>
<td>124.05</td>
<td>100 (FAO/WHO, 1989)</td>
</tr>
</tbody>
</table>

*At times along with fish muscle, integument is also consumed therefore average of both is taken; blank cells indicate that no information was available.

Table 5. Bioaccumulation factors (BAF)* of heavy metals in the different tissues of *Channa punctatus*.

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Gill</th>
<th>Liver</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Integument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>31.5</td>
<td>50.38</td>
<td>275.18</td>
<td>15.40</td>
<td>16.34</td>
</tr>
<tr>
<td>Ni</td>
<td>120.75</td>
<td>86.41</td>
<td>76.83</td>
<td>69.25</td>
<td>93.41</td>
</tr>
<tr>
<td>Fe</td>
<td>56.05</td>
<td>176.01</td>
<td>129.77</td>
<td>16.09</td>
<td>20.91</td>
</tr>
<tr>
<td>Co</td>
<td>124.36</td>
<td>69.45</td>
<td>55.72</td>
<td>35.18</td>
<td>66.45</td>
</tr>
<tr>
<td>Mn</td>
<td>505.76</td>
<td>82.61</td>
<td>89.95</td>
<td>44.04</td>
<td>158.0</td>
</tr>
<tr>
<td>Cr</td>
<td>230.30</td>
<td>146.90</td>
<td>2004.8</td>
<td>108.30</td>
<td>97.2</td>
</tr>
<tr>
<td>Zn</td>
<td>1152.26</td>
<td>687.06</td>
<td>1448.63</td>
<td>316.96</td>
<td>510.03</td>
</tr>
</tbody>
</table>

*BAF = Concentration of HM in dry fish tissue (mg kg⁻¹) / Concentration of HM in rivulet water (mg L⁻¹).

Table 6. Hematological parameters and oxygen carrying capacity of the fish *Channa punctatus*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Exposed</th>
<th>% Change over control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Channa punctatus</em></td>
<td><em>Channa punctatus</em></td>
<td></td>
</tr>
<tr>
<td>Total RBC (10⁶ mm⁻³)</td>
<td>5.37 ± 0.034*</td>
<td>2.61 ± 0.21*</td>
<td>-51.39</td>
</tr>
<tr>
<td>Total WBC (10³ mm⁻³)</td>
<td>4.56 ± 0.183</td>
<td>5.72 ± 1.23</td>
<td>+25.43</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.06 ± 0.265*</td>
<td>8.23 ± 0.37*</td>
<td>-36.98</td>
</tr>
<tr>
<td>Oxygen carrying capacity (Vol.%)</td>
<td>16.32 ± 0.121*</td>
<td>10.28 ± 0.46*</td>
<td>-37.00</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD, (n = 8); *significantly different at P < 0.01.

The water quality of rivulet was suitable for sustenance of fishes as determined by physicochemical characteristics; however, among the heavy metals examined, some were beyond the maximum permissible limits. The heavy metal content estimated in water were present in the order Fe > Cu > Zn > Mn > Ni > Co > Cr where it was found that Fe, Ni, Mn and Cr content exceeded the recommended guidelines set by UNEPGEMS (2006) due to which the water becomes unfit for the inhabitant fishes. Fe was reported to present in highest amounts in water which could be ascribed due to the use of iron pipes for draining purposes. From other regions of Aligarh also, heavy metals are reported in water bodies beyond maximum permissible limits. Javed and Usmani (2012a) reported that water of sewage fed aquaculture pond at Panethi, Aligarh which is used as a source of commercial food fish also contain heavy metals in the order Fe > Mn > Zn > Co > Ni > Cu = Cr. In another study, the sugar mill effluent dominated river at village Satha, Aligarh also reported to contain heavy metals in the order Ni > Cr > Cu > Co (Javed and Usmani, 2013b). Lake Manzala in Egypt is subjected to the large amounts of raw sewage, agricultural and industrial wastewaters and reported to contain 10³ mm⁻³. Oxygen carrying capacity depends on Hb content therefore lower value (10.28 vol%) was recorded in exposed fish than the control (16.32 vol%).

DISCUSSION

The water quality of rivulet was suitable for sustenance of fishes as determined by physicochemical characteristics; however, among the heavy metals examined, some were beyond the maximum permissible limits. The heavy metal content estimated in water were present in the order Fe > Cu > Zn > Mn > Ni > Co > Cr where it was found that Fe, Ni, Mn and Cr content exceeded the recommended guidelines set by UNEPGEMS (2006) due to which the water becomes unfit for the inhabitant fishes. Fe was reported to present in highest amounts in water which could be ascribed due to the use of iron pipes for draining purposes. From other regions of Aligarh also, heavy metals are reported in water bodies beyond maximum permissible limits. Javed and Usmani (2012a) reported that water of sewage fed aquaculture pond at Panethi, Aligarh which is used as a source of commercial food fish also contain heavy metals in the order Fe > Mn > Zn > Co > Ni > Cu = Cr. In another study, the sugar mill effluent dominated river at village Satha, Aligarh also reported to contain heavy metals in the order Ni > Cr > Cu > Co (Javed and Usmani, 2013b). Lake Manzala in Egypt is subjected to the large amounts of raw sewage, agricultural and industrial wastewaters and reported to contain 10³ mm⁻³. Oxygen carrying capacity depends on Hb content therefore lower value (10.28 vol%) was recorded in exposed fish than the control (16.32 vol%).
Fe > Cu = Mn > Zn > Pb > Cd (Saeed and Shaker, 2008). But the metal content in these waters are lower than the present study. In the present study, possible reason for high levels of heavy metals in rivulet water could be attributed to the fact that this ‘power station’ may not have efficient wastewater treatment plant due to which these metals are present beyond the maximum permissible limits. Although, these metals are essential in traces but their excess becomes toxic and gets accumulated in organs/tissues of fish C. punctatus.

In the present study, the accumulation of Fe was highest in all organs of the fish C. punctatus. It had highest accumulation in liver and least in muscle. Studies reported for Clarias gariepinus (Osman and Kloas, 2009), Tinca tinca (SeldaTekin et al., 2005), Labeo rohita (Javed and Usmani, 2011) also revealed the maximum accumulation of Fe in liver and least in muscle. Saeed and Shaker (2008) reported two fold higher concentrations of Fe in liver, gills and muscle of Oreochromis niloticus than the present case. The high Fe concentrations observed in the liver tissue of these fishes including the present study (C. punctatus) could be due to iron-containing enzymes and the extensive vascular system of the liver, as the hemoglobin in the blood binds approximately three quarters of the Fe in the body (Voynar, 1960) and also because the liver is detoxifying organ. This shows that liver is the target organ for Fe accumulation. The FAO/WHO (1989) has recommended that Fe should not exceed the maximum permissible limits (100 ppm). But in this case, the Fe content in the edible part exceeds the limits.

Fe accumulation was followed by Zn in organs of the fish. It was highly accumulated in kidney and least in muscle of C. punctatus. Javed and Usmani (2011) also reported high accumulation of Zn in kidney of C. punctatus. O. niloticus inhabiting Lake Manzala, Egypt exhibited very high concentrations of Zn in liver, gills and muscle since the lake is subjected to industrial and agricultural wastewaters (Saeed and Shaker, 2008). Same is the case in the present study where much higher values are reported in gills and liver. Tilapia mossambica, Mystus vittatus, Ctenopharyngodon idella, Heteropneustes fossilis inhabiting the Kolliadam River which receives agricultural runoff reported to contain very low levels of Zn in gills, liver, kidney, muscle and integument (Ambedkar and Muniyan, 2011).

In the present study, Zn accumulation in edible part was beyond the permissible limits set for Zn is 100 ppm (FAO/WHO, 1989). Zn is also recognized as an essential element as required by a wide variety of enzymes and other cell components having vital functions in all living things. But excessive Zn will damage the health of animals as well as humans.

In the present study, high Cu accumulation was reported in kidney and least in muscle. Highest accumulation of Cu was also reported in kidney of fishes C. punctatus, L. rohita, C. gariepinus (Javed and Usmani, 2011) and least in muscle of L. rohita (Javed and Usmani, 2011) and C. punctatus (Javed and Usmani, 2013b). High levels of Cu as compared to present study were observed in gills, liver and muscle of Tilapia niloticus (Saeed and Shaker, 2008). In other studies, fishes such as T. mossambica, M. vittatus, C. idella, H. fossilis found to contain very low levels of Cu in gills, liver, kidney, muscle and integument (Ambedkar and Muniyan, 2011) than the present study. But the accumulation in present study was within limits. The permissible limit set for Cu (30 ppm) by FAO/WHO (1983). Copper is essential for good health. However, exposure to higher doses can be harmful. Cu toxicity in natural water arising from pollutants may cause severe damage in gills and necrotic changes in the liver and kidneys. The highest accumulation of Cr in C. punctatus was also observed in kidney and least in integument. This observation corroborates well with our previous study in C. gariepinus (Javed and Usmani, 2011).

In other studies, very low levels of Cr had been reported in fishes such as L. rohita, Cirrhinus mrigala, T. mossambica, M. vittatus, C. idella, Epinephelus morio, Chanos chanos, Channa striata in gills, liver, kidney, muscle and integument (Rauf et al., 2009; Ambedkar and Muniyan, 2011). In the present study, the accumulation observed in muscle was within the recommended guidelines set for Cr (12 to 13) by USFDA (1993a). Cr exists in two forms, Cr (III) and Cr (VI). Cr (III) is an essential nutrient in humans whereas Cr (VI) is danger to human health. Also, in animals, this can adversely affect developmental processes which include post implantation loss, reduced ossification and decreased number of live fetuses (ATSDR, 2008). The highest accumulations of Mn occur in gills and least in integument. Other workers also reported high accumulation in gills of fishes Cyprinus carpio (Jabeen and Chaudhary, 2010) and Clarias anguillaris (Nwajei et al., 2012). Accumulation observed in gills in present study corroborates to L. rohita (Javed and Usmani, 2011). The accumulation of Mn occurred in liver, kidney and muscle were comparable to that in C. punctatus (Javed and Usmani, 2011). But the accumulation in edible part in these studies including the present work exceeds the permissible guidelines set for Mn (1 ppm) by FAO/WHO (1989). Finally, laboratory tests with test animals have shown that severe manganese poisoning should even be able to cause tumor development in animals. Similarly, highest accumulations of Ni occur in gills and least in muscle of C. punctatus.

In other studies as well, the highest accumulations were observed in gills of fishes such as C. punctatus (Javed and Usmani, 2011, 2012a) and comparable results of accumulation in all organs were observed in L. rohita (Javed and Usmani, 2011). In our previous study with C. punctatus from sugar mill effluent dominated water body, Ni had not accumulated in any organs of the fish though it was present in water. Accumulations of Ni in edible part in the present study are much below the permissible limits (70 to 80 ppm) set by USFDA (1993b). Ni exhibits similar chemical behavior as Fe and Co con-
centrations of Ni in water is likely to be of health concern in environments where pH is less than 4.5. As is the case with other essential elements, Ni is also toxic to fish when present in high enough concentrations (Pickering, 1974). The highest accumulations of Co also occur in gills and least in muscle of C. punctatus. Comparable accumulations of Co in all the organs were reported in fishes C. gariepinus and L. rohita (Javed and Usmani, 2011). In another study, the Co accumulations were not detected in organs of fish C. punctatus inhabiting in the sewage fed pond and sugar mill effluent dominated water body though it was detected in water (Javed and Usmani, 2012a). Channa obscura, Hepsetus odoe, Tilapia zilli inhabiting Densu River, Nigeria also revealed very low levels of Co accumulation (Anim et al., 2011). No permissible guidelines/limits have yet been established for Co. Generally, cobalt compounds that dissolve easily in water are more harmful than those that are hard to dissolve in water. Once cobalt enters the body, it is distributed into all tissues, but mainly into the liver, kidney and bones (ATSDR, 2004).

According to MPI, the kidney of C. punctatus had highest metal load followed by gills and least load was observed in muscle. Kidney and gills both are excretory organs of the fish and they function efficiently to remove as much metals as they can. But unfortunately when uptake exceeds the excretion due to some damage in these organs, accumulation occurs; that is why lower levels of accumulation occurred in liver, muscle and integument; but still the accumulated amount is of concern. Bioaccumulation factor (BAF) was used to quantify accumulated metal concentrations in relation to their concentration in water. BAF below 250 is considered as low and above 1000 as high and between these two extremes is considered as moderate. In the present study, the BAF ranged from 9.72 to 2004.8; therefore, it was considered high. It was calculated highest for kidney (55.72 to 2004.8) and least for muscle (15.40 to 316.96) and following the order kidney > gills > liver > integument > muscle. To confirm the adverse effects of heavy metals on health of fish C. punctatus, the hematological parameters were studied since blood is known to exhibit pathological changes before the onset of any external symptoms of toxicity. In the present study, the exposed C. punctatus showed low total RBC count and hemoglobin content than control fish. These results are consistent with our earlier work on fishes such as Mastacembelus armatus collected from the same rivulet (Javed and Usmani, 2012b) and C. punctatus collected from the wastewater aquaculture pond (Javed and Usmani, 2013a). Shaheen and Akhtar (2012) also reported significant decline in Hb content and RBC counts of fish C. carpio when exposed to Cr (VI).

The results are also in good agreement with earlier works that reported a significant decrease in RBC and hemoglobin of fresh water fishes such as L. rohita and C. carpio exposed to heavy metals (Cr, and mixture of Cd, Pb, Cr and Ni), respectively (Vutkuru, 2005; Vinodhini and Narayanan, 2009). In the present study, low erythrocyte count coupled with low Hb content could be ascribed due to the destructive action of accumulated heavy metals on kidney. Since kidney of fish is the hematopoietic organ and any changes in its structure and function clearly reflects in blood profile of the fish. No doubt the Fe was deposited in highest amount in all organs of C. punctatus but still decline in these RBC and Hb was observed due to impaired intestinal absorption of Fe and/or altered hematopoiesis. The oxygen carrying capacity was also low in C. punctatus because it depends on Hb content of fish. Other workers also reported the decrease of oxygen carrying capacity in fishes such as H. fossilis exposed to mixture of Cu and NH₃ (James and Sampath, 1995), Oreochromis mossambicus exposed to copper and zinc (Sampath et al., 1998). This decline could be attributed due to the fact that heavy metals damage the structure of RBC consequently instead of four, less molecules of oxygen bind to hemoglobin. The loss of hemoglobin and consequent reduction in the oxygen carrying capacity of the blood is the most conspicuous feature of anemia.

In the present study, contrary to these parameters, an increase in total WBC count was observed than control fish. Other workers also reported similar observation in fishes such as C. punctatus exposed to lead (Hymavathi and Rao, 2000), Clarias batrachus exposed to mercuric chloride (Joshi et al., 2002), C. gariepinus to metal finishing company effluents (Adakole, 2012), M. armatus exposed to thermal power plant effluents (Javed and Usmani, 2012b) and C. punctatus to sewage water (Javed and Usmani, 2013a). Shaheen and Akhtar (2012) also reported significant increase in WBC count of fish C. carpio when exposed to Cr (VI). Hanan et al. (2013) reported much higher values of WBC count than the present study in fish C. gariepinus inhabiting El-Rahawy delta of River Nile which receive industrial, domestic and agricultural waste. These wastes contained Fe, Mn, Zn,Cu, Pb and Cd. Increase in WBC in all these cases including the present one could be due to their function that they are defensive in nature. And also, leukocytosis is directly proportional to the severity of damage and stress induced by heavy metals which as a consequence result in the stimulation of immunological defense (Javed and Usmani, 2012b). According to Hanan et al. (2013), this relative decrease/increase in hematological indices proved the toxic effect of heavy metals that affect both metabolic and hemopoietic activities of fish C. punctatus. The results of the present investigation confirm that rivulet water contain heavy metals which on one hand affect the quality of water and on another quality of food in the form of fish protein. The accumulations of heavy metals occur in all the organs of fish C. punctatus which consequently affect the physiology of fish and making it weak, anemic, hypoxic and vulnerable to diseases. It can suitably be used as a bioindicator of rivulet.

C. punctatus also serve as a delicacy in and around the region. Therefore, this is apparent from the present study that local population is at a greater risk of Fe, Mn and Zn.
since these were present beyond the recommended standards in the edible part. These metals can cause gastric and esophageal ulceration in humans and animals due to chronic exposure to Fe. Agency for Toxic Substances and Disease Registry (ATSDR) suggests that ingesting high levels of zinc for several months may cause anemia, damage the pancreas and decreased levels of high-density lipoprotein (HDL) cholesterol. High levels of Mn can cause lung, liver and vascular disturbances, declines in blood pressure, reproductive abnormalities and brain damage. Severe manganese poisoning should even be able to cause tumor development in animals. Since 'thermal power plants' are also necessary, therefore it is seen been suggested that the government should make guidelines for the proper treatment before the discharge of wastewater. Use of columns, made of materials which have the property of biosorption of heavy metals for draining purposes should be encouraged.

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