Bioaccumulation of heavy metals and their toxicity assessment in Mystus species

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Abstract

This study was conducted on two native fish species namely Mystus vittatus and Mystus tengara inhabiting challenging environment of Yamuna River. The heavy metals concentrations in the river water were found to be as follows: Fe > Mn > Zn > Cu > Ni > Cr > Cd, all above the Bureau of Indian Standards (BIS) and World Health Organization (WHO) guidelines. The high metal pollution index in gill, liver, and kidney of M. vittatus was recorded compared to M. tengara. The pathology caused by the accumulation of heavy metals resulted significantly (p < 0.05) higher enzyme activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine kinase (CK) in M. vittatus as compared to M. tengara. However, albumin: globulin ratio was found to be below 0.8 in both fishes. Higher total leukocyte (TLC) (48.5 \times 10^3/mm^3), lymphocytes (40%), respiratory burst activity (1.9), and nitric oxide synthase (NOS) activity (13.11 U/L) in M. vittatus reflect high immune response. In addition, chromosomal breakage study showed significantly (p < 0.05) low micronuclei frequency, lobed nuclei, and kidney-shaped nuclei (KSN) in M. vittatus. These results indicate that under the same challenging conditions M. vittatus have more capability of resistance and its continuous survival points towards its suitability to serve as a bioindicator than M. tengara.

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1. Introduction

Freshwater lotic resources are contaminated with numerous pollutants, which has become a matter of great concern lately (Abiona et al., 2019). Among the pollutants, heavy metals are the main culprits because of their ubiquitous presence, non-biodegradability, and persistency (Chauhan et al., 2019). These heavy metals constantly challenge the ecological balance of the recipient water body, diversity of aquatic fauna, quality of seafood, and health of fish consumers (Abiona et al., 2019). Besides, in vivo studies of fish exposed to heavy metals reveals that the interac-

tions between these toxicants and biological systems could lead to biochemical disturbances (Carvalho and Fernandes, 2019).

Enzymes are involved in every physiological process and could serve as a valid biomarker of pollution (Tabrez and Ahmad, 2009a, b, 2010, 2011a,b). The assessment of enzyme activities could be an indicator of pathology. When any damage occurs in the body of an organism then these intracellular enzymes exude into the blood. The more the injury the more is the discharge of enzymes. Therefore, analysis of intracellular enzymes like alkaline phosphatase (ALP), aspartate transaminase (AST), and alanine transaminase (ALT) could serve as a valid indicator of the pollution magnitude (Javed and Usmani, 2019). The injury caused due to heavy metals could also have a toll on the albumin and globulin levels consequently altering their ratio. Nevertheless, fishes counter the adverse effect caused by heavy metals by the use of innate immunity that forms the basic defense system (Kumar et al., 2019). Leucocytes are the key player of the immune system which are estimated through total leukocyte (TLC) and differential leukocyte count (DLC). In addition, respiratory burst activity and nitric oxide synthase are also employed as a good immunological indexes in fish (Chakrabarti et al., 2014; Kumar et al., 2019). Among various
techniques developed for the determination of genotoxic damage micronuclei test is an easy, sensitive, and reliable tool (Bolognesi and Hayashi, 2011; Ahmad and Ahmad, 2016; Javed and Usmani, 2019). The incidence of micronuclei valuable to judge the mutagenic, clastogenic, and aneugenic potential of environmental agents (Bolognesi and Hayashi, 2011). River Yamuna (Agra, India) (27° 11’2.59”N and 78° 14’7.58”E) is one such freshwater body, which receives untreated and partially treated effluents from factories and sewage from the domestic establishments. At this stretch of river, the Mystus vittatus and Mystus tengara were found to be dominant and abundant species. They belong to the group of cat fish and therefore, their food and feeding habits are also similar. Their food mainly consists of crustaceans, insect’s parts, other fishes, molluscs, and little plant materials. The present study aims to highlight the adaptive differences of the two fish species in the context of enzymes, proteins, leukocytes, immunity, micronuclei, and other erythrocyte abnormalities.

2. Material and methods

2.1. Procurement of fish species and water quality analysis

Fishes (Mystus vittatus and Mystus tengara) [n = 15] were captured from the stretch of river Yamuna at Agra city, India. Length and weight of both species were 12.5 ± 0.5 cm, 20 ± 0.7 g and 14.4 ± 0.3 cm and 22 ± 0.86 g respectively. Fishes were carefully selected bearing in mind the size and age to minimize size and age-specific effects. Gills, liver, and kidney of both species were digested in HClO4 and HNO3 for heavy metals analysis (Javed et al., 2018). Moreover, metal pollution index (MPI) was calculated as mentioned below:

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

where \( C_{f1}, C_{f2}, \ldots \) up to \( C_{fn} \) is the measure for the metal ‘n’ in the sample.

Water samples were stored in plastic bottles and acidified with HNO3 for heavy metals analysis. The concentration of heavy metals (Cr, Mn, Fe, Ni, Cu, Zn, and Cd) was estimated by using atomic absorption spectrophotometer as described in APHA (2005). While dissolved oxygen, temperature, conductivity, and pH were determined using digital meters.

2.2. Enzyme assay

Fishes were anesthetized in quinaldine (10%) and blood samples were withdrawn from the posterior caudal vein. The enzymatic activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine kinase (CK) were performed with the help of Randox commercial kits (RANDOX Laboratories Ltd., Crumlin, United Kingdom), strictly adhering to the manufacturer instructions.

2.3. Albumin and globulin level in serum

The concentration of albumin was determined by a commercially available kit (Siemens Ltd., Gujarat, India) and the absorbance was measured at 628 nm on a spectrophotometer (UV–VIS Systronics, 118). Globulin level was obtained by subtracting the albumin concentration from the total proteins.

2.4. Innate immune parameters

2.4.1. Total leukocyte count and differential leukocytes count

TLC (10³ mm⁻³) was counted by neubauer hemocytometer. For DLC, a blood smear was prepared using Giemsa stain and different leukocyte cells were counted and recorded in percentage.

2.4.2. Respiratory burst activity

The respiratory burst activity was assessed as per the method described by Kumar et al. (2019).

2.4.3. Nitric oxide synthase

The NOS activity was measured as per the procedure described in Kumar et al. (2019) with slight modifications.

2.5. Micronuclei test

Blood smears of both fish species was collected to analyze micronuclei (MN) and other nuclear abnormalities in red cells as per the method described by Ahmad and Ahmad (2016). From both fish species, 1000 erythrocytes were scored to calculate the recurrence of micronuclei.

2.6. Quality control

All the chemicals and reagents used in this study were of analytical grade. The used glasswares were soaked in 10% HNO3 and chromic acid overnight and then washed with double distilled water. For the calibration of instruments and their precision and accuracy, respective blanks and standards were used.

2.7. Statistical analysis

All the studied parameters were analyzed in doublets and values are reported as mean ± SEM (standard error of the mean). Statistical analysis was performed with the help of t-test, two-way ANOVA, and Duncan’s multiple range test through SPSS software.

3. Results

Physicochemical characteristics, the heavy metal concentration of river water, and bioaccumulation are shown in Table 1. The temperature, pH, conductivity, and dissolved oxygen were found to be 28 °C, 7.5, 55 Us/cm, and 6 mg/L respectively. Furthermore, the concentration of heavy metals was found to be in the following order: Fe > Mn > Zn > Ni > Cu > Cr > Cd. In M. vittatus, Cu, and Ni showed highest degree of accumulation in gills, liver, and kidney respectively, whereas Ni in gills and Fe in both liver and kidney showed high bioaccumulation in M. tengara. The trend of metal pollution index (MPI) kidney > gills > liver and gills > liver > kidney in M. vittatus and M. tengara respectively (Table 2). The enzymatic levels of AST, ALT, ALP, and CK were found to be 5.87 U/L, 10.31 U/L, 1.7 U/L, and 54 U/L respectively in M. vit-

Table 1: Physicochemical characteristics, heavy metal concentration of river water, and bioaccumulation.

| Parameter       | Value (±SEM)    |
|-----------------|-----------------|
| Temperature     | 28 °C (±0.5 °C) |
| pH              | 7.5 (±0.2)      |
| Conductivity    | 55 Us/cm (±5 Us/cm) |
| Dissolved Oxygen| 6 mg/L (±1 mg/L) |

Table 2: Enzymatic levels of AST, ALT, ALP, and CK in M. vittatus and M. tengara.

| Fish Species | AST (U/L) | ALT (U/L) | ALP (U/L) | CK (U/L) |
|--------------|-----------|-----------|-----------|----------|
| M. vittatus  | 12.9      | 40        | 3.5       | 70       |
| M. tengara   | 5.87      | 10.31     | 1.7       | 54       |

TLC was found to be 48.5 × 10³/mm² and 40.62 × 10³/mm² in M. vittatus and M. tengara respectively (Fig. 3a). The percentage of neutrophils, lymphocytes, eosinophils, monocytes were: 73%, 40%, 2%, 3.6% and 85%, 15%, 1.8%, and 4% in M. vittatus and M. ten-

40% 2% 3.6% and 85% 15% 1.8% and 4% in M. vittatus and M. ten-

M. vittatus 40% 2% 3.6% and 85% 15% 1.8% and 4% in M. vittatus and M. tengara respectively. The percentage of neutrophils, lymphocytes, eosinophils, monocytes were: 73%, 40%, 2%, 3.6% and 85%, 15%, 1.8%, and 4% in M. vittatus and M. tengara respectively. The percentage of neutrophils, lymphocytes, eosinophils, monocytes were: 73%, 40%, 2%, 3.6% and 85%, 15%, 1.8%, and 4% in M. vittatus and M. tengara respectively.

M. vittatus 40% 2% 3.6% and 85% 15% 1.8% and 4% in M. vittatus and M. tengara respectively.
respectively (Fig. 3b). Surprisingly, the basophils were not at all observed in both fish species. In addition, respiratory burst activity was found to be high (1.982) in M. vittatus compared with M. tengara (0.79) (Fig. 3c). Similarly, NOS activity was also found to be high (13.11 mol/ml) in M. vittatus compared with M. tengara (10.83 mol/ml) (Fig. 3c).

The percentage of nuclear damage in both fishes are illustrated in Fig. 4. In M. vittatus the percentage of micronuclei, lobed nuclei, and kidney-shaped nuclei were found to be 0.86%, 0.37%, and 0.16% respectively. Whereas the micronuclei, lobed nuclei, and kidney shaped nuclei were 1.02%, 0.69%, 0.95% respectively in M. tengara. The MPI calculation shows kidney as the target organ followed by gills and liver in M. vittatus (Table 2). However, gills followed by liver and kidney were found to be the target organ in M. tengara.

### Table 1

|          | Cr   | Mn  | Fe  | Ni  | Cu  | Zn  | Cd  |
|----------|------|-----|-----|-----|-----|-----|-----|
| MV Gill  | 36.95 ± 0.72 | 9.3 ± 0.11 | 95.75 ± 1.2 | 44.65 ± 0.3 | 102.95 ± 1.8 | 72.95 ± 0.96 | 49.2 ± 0.1 |
| Liver    | 24.7 ± 0.53 | 23.75 ± 0.31 | 104.03 ± 1.8 | 52.65 ± 0.47 | 20.5 ± 0.21 | 15.82 ± 0.32 | 46.95 ± 0.1 |
| Kidney   | 62 ± 1.1 | 33 ± 0.32 | 97 ± 0.89 | 101.8 ± 1.5 | 67 ± 1.7 | 18.9 ± 0.01 | 94.6 ± 0.87 |
| MT Gill  | 25.95 ± 0.15 | 11.7 ± 0.1 | 68.45 ± 1.3 | 69 ± 1.2 | 48 ± 0.60 | 62.8 ± 0.87 | 64.27 ± 1.4 |
| Liver    | 26.6 ± 0.21 | 9 ± 0.1 | 88.93 ± 1.9 | 75.5 ± 1.7 | 5.9 ± 0.1 | 48.45 ± 0.5 | 44 ± 0.85 |
| Kidney   | 32.65 ± 0.19 | 4.75 ± 0.1 | 64.8 ± 0.97 | 46.95 ± 0.8 | 11.32 ± 0.13 | 52.25 ± 1.0 | 38.9 ± 0.37 |
| Yamuna water | 5.3 ± 1 | 18 ± 0.41 | 46 ± 0.83 | 11 ± 0.1 | 8.5 ± 0.1 | 14 ± 0.2 | 3.7 ± 0.1 |

All values are given as mean ± SEM (n = 15); MV: M. vittatus; MT: M. tengara; Different superscripts and subscripts indicate significant differences at p < 0.05.

### Table 2

| Fish species | Tissues | MPI |
|--------------|---------|-----|
| M. vittatus   | Gills   | 47.46 |
|               | Liver   | 33.65 |
|               | Kidney  | 58.76 |
| M. tengara    | Gills   | 43.13 |
|               | Liver   | 29.06 |
|               | Kidney  | 27.14 |

### 4. Discussion

The physicochemical characteristics of river water were found to be within the permissible limits of BIS (1992) and WHO (UNEPGEMS, 2006). However, all the measured heavy metals were found to be above the permissible limits creating a burdensome situations for the survival of fish species, their health, and growth. To study bioaccumulation of multiple heavy metals in different tissues/species of fishes, MPI calculations is done to know the total metal load in a tissue. Recently, Ahmed et al. (2019) reported accumulations of As, Pb, Cd, Cr, and Cu in six commercially important fishes. Among the heavy metals Cd, Pb, and Hg have been reported as highly toxic followed by Cu, Cr, Ni, Mn, and Zn (Fernandes et al., 2008). Chronic exposure to a very low concentration of these metals could result in biochemical alterations, immunotoxicity, genotoxicity in different tissues of fish (Islam et al., 2019). Hence, time to time investigations is required to verify the toxicities associated with these heavy metals. Recently, Naz et al. (2020) reported toxic effects of Cu and Cd on hematological parameters of freshwater fish Catla catla. In another study, Fe and Al have been noted to results into lower blood count, histological alterations, and bioaccumulation in Oreochromis niloticus tissues (Abdel-Khalek et al., 2020).
Recently, Barisic et al. (2019) reported higher AST, ALT, and ALP serum level of salmonid that was related to exposure period ultimately resulting liver damage. The lower activities of above-mentioned enzymes in *M. vittatus* indicates lesser damage. Several studies also reported increased activities of serum AST, ALT, and ALP in *Tilapia zillii*, *Cyprinus carpio*, *Mugil cephalus*, *Oncorhynchus mykiss*, and *Barbus luteus* exposed to heavy metals (Zorriehzahra et al., 2010; Parvathi et al., 2011). The higher level of CK is indicative of heavy metals-induced kidney damage (Adham et al., 2002). In an earlier study, Schjolden et al. (2007) reported marked alterations in the osmoregulatory behavior in response to Cu exposure leading to kidney damage. Creatinine kinase has been also suggested as a marker to both liver and kidney function (Banaee et al., 2003; Barisic et al., 2019). It catalyzes the transformation of creatine into ADP and phosphocreatine in the presence of ATP. The higher CK activity could result greater tissue damage causing rhabdomyolysis, muscular dystrophy, myocardial infarction, acute kidney injury, and autoimmune myositides (Luckoor et al., 2017; Oitani et al., 2018).

Albumin and globulin form nearly all of the proteins and any modification in their concentration leads to imbalance in A:G ratio. Heavy metals have been reported to affect the levels of serum albumin and globulin. In the present study, more albumin in *M. tengara* and excessive globulin in *M. vittatus* were observed consequently leading to a lower A:G ratio in *M. vittatus* (0.15) than *M. tengara* (0.38) (Fig. 2). Corroborating results were reported by another study using *M. cavasius* (Palanisamy et al., 2011). Recently, Barisic et al. (2019) reported a decrease in serum total protein and albumin levels as a result of heavy metals exposure in *Oncorhynchus mykiss*. A similar reductions in the aforementioned parameters was observed in response to heavy metals exposure in *Oreochromis niloticus* (Yacoub and Gad, 2012). A:G ratio indicator is widely accepted to monitor differences in the framework of serum or plasma with the reference value in the range of 0.8 to 2.0 (Mazeoud et al., 1977). In both fish species, A:G was found to be much lower than 0.8 with the highest value of 0.38 in *M. tengara* indicating higher liver damage in this fish species. Subsequently, synthesis of more albumin may have occurred in liver of *M. tengara*

![Fig. 3. a. TLC (1000/mm³), b. DLC (mean frequency %), c. respiratory burst and nitric oxide synthase activity (mol/ml) in *M. vittatus* and *M. tengara*. Significance was established at *p < 0.05.*](image-url)
Since both fish species are served as delicacy so these heavy metal particip-
atory burst (Fig. 3c) in phagocytes in fish discharge superoxide anion and its deriva-
tives during a phase of vigorous oxygen consumption, called the respira-
tory burst (Chakrabarti et al., 2014; Secombes, 1996). Elevated respira-
tory burst activity and the higher activity of NOS enzymes. Generally, it is assumed that sensitized
phagocytes in fish discharge superoxide anion and its derivatives through the production of antibodies. There has been reports of a significant correlation between heavy metals exposure and lymphocyte proliferation (Lawrence, 1981). On the contrary, a higher
neutrophil percentage may be because of the activity of myeloperoxidase enzyme that eliminates invaders from the body of the fish (Kumar et al., 2019; Dalmo et al., 1997). Furthermore, immune reciprocation was checked by respiratory burst activity and the activity of NOS enzymes. Generally, it is assumed that sensitized phagocytes in fish discharge superoxide anion and its derivatives during a phase of vigorous oxygen consumption, called the respiratory burst (Chakrabarti et al., 2014; Secombes, 1996). Elevated respiratory burst (Fig. 3c) in M. vittatus than M. tengara indicates heightened phagocytic action to counter the toxic effect brought about by heavy metals. Other researchers have also reported higher respiratory burst against a variety of xenobiotic substances (Chakrabarti et al., 2014; Sakai et al., 2001; Secombes, 1996). NOS catalyzes the generation of cellular signaling molecule nitric oxide that participate crucially in defense mechanism of fish (Kumar et al., 2019; Rombout et al., 2005). In the current study, high frequency of micronuclei, lobed nuclei, and kidney-shaped nuclei was observed in M. tengara than M. vittatus (Fig. 4). This indicates the lower immune response in M. tengara and better immune response in M. vittatus. It has already been mentioned that metal load was high in M. vittatus hence it was protected by better immunity from further damage in nucleus of erythrocytes. Moreover, existence of micronuclei is an irreversible and unrepairable change leading to mutagenesis (Andriani et al., 2016; Braham et al., 2017). Some investigators suggested that these nuclear abnormalities are caused by impaired DNA synthesis, as heavy metals are reported to break DNA strands (Kehinde et al., 2016; Braham et al., 2017). At the time of cell division, heavy metals could lead to mutations which pass on to next generations consequently leading to aneuploidy, diminish reproduction, reduces survival, and may endanger the species (Bolognesi and Hayashi, 2011; Hwang and Kim, 2007). Recently, Khan et al., (2020) reported alteration in serum biochemistry, histology, and DNA damage as a result of heavy metal pollution in Oreochromis niloticus. The present study corroborates well with earlier findings reported on different organisms (Abdel-Khalek et al., 2020; Khan et al., 2020; Naz et al., 2020).

5. Conclusion

The heavy metals contaminated river water affects both species of Mystus. Despite, high bioaccumulation, M. vittatus, immune response showed resistance and helped it to cope better in the prevailing conditions. Therefore, this species could be used as a better indicator organism because it show continuous survival, which is one of the important characteristic features of the indicator organ-
ism. Since both fish species are served as delicacy so these heavy metals may enter the consumers’ body and may cause similar damage as in indicator organism. Hence, we need to consider the point of collection of these fishes before having it in our eatery.

6. Availability of data and material

Data will be available upon the request to corresponding author.

7. Consent to participate

Not applicable.

8. Consent to publish

All authors have given their consent to publish this research article.

Author contributions

S.T and M.J, conceived, designed, and executed the work. T.A.Z. did statistical analysis and also made first draft of this article. All authors reviewed the final draft of manuscript.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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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