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Research Article

Analysis of Heavy Metal Concentrations and Haematological Parameters of Two Fishes, *Channa punctatus* and *Heteropneustes fossilis* from a Fish Market, Aligarh, India

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Abstract

Aim of this study was to investigate the contamination level and health status of two fish, *Channa punctatus* and *Heteropneustes fossilis* collected from a local fish market (Rasalganj), Aligarh. To evaluate the contamination level, concentrations of different heavy metals (Pb, Mn, Fe, Cr, Cu, Zn) in different tissues like muscle, liver, skin and gill were analyzed. The obtained results were compared with their permissible limit suggested by FAO, 1983 for food and agricultural products. In *C. punctatus*, liver showed maximum concentration of Fe and Cu followed by muscle, gill and skin while in case of Zn it was followed by skin, gill and muscle. *H. fossilis* showed maximum concentrations of Fe, Cu and Mn in liver followed by gill, muscle and skin but the trend of Zn was liver > gill > skin > Muscle. Similar trend was observed for Cr concentration in both the fish and was found to be maximum in gill. Gill also showed higher concentration of Pb in both the fishes. Metal Pollution Index was also determined and found to be higher in gill of *H. fossilis* and in liver of *C. punctatus*. Haematological parameters such as total erythrocyte count (TEC), total leucocyte count (TLC), Haemoglobin, Haematocrit, Oxygen carrying capacity and calculated indices (MCV, MCH, MCHC) were examined and compared with controls in both the fish. Hence it was observed that metal contaminations were higher in both fish species than their maximum permissible limit suggested by FAO (1983) which shows that these fishes are unsuitable for consumption purpose. It was also found that *H. fossilis* was more contaminated than *C. punctatus* collected from same fish market, Aligarh.

Keywords: *Metal Accumulation; Haematological Parameters; Metal Pollution Index*

Introduction

Nearly 30% of humankind in the world is suffering from malnutrition [1] and India contributes about one third of it [2]. Fish are the excellent source of protein and low fats especially polyunsaturated fatty acids (PUFA) which are essential for proper growth of children [3,4]. Hence fish can be an economical option to counteract the problem of malnutrition.

In recent years, there has been an increasing ecological and public health concern associated with contamination of food with heavy metals [5]. Aquatic ecosystem is the final recipient of almost all the pollutants coming from different anthropogenic activities like accidental spillage of chemical wastes, dumping of hospital remains, recreational activities, drainage of sewage, idol immersion etc. Therefore, aquatic organisms are always remained in a risk affected by different pollutants like heavy metals. Contamination of these metals may have demolishing effects on the ecological balance of the recipient water bodies and diversity of aquatic organisms [6-8]. Heavy metals constitute a core group of aquatic pollutants due to their toxicity, long persistence, bioaccumulative and non-biodegradable properties in the food chain [9,10]. It has been reported that most fishes are capable of accumulating heavy metals in their tissues several times higher than their ambient levels in water by absorption through the gills or by consumption of contaminated food and sediments [8,11]. The heavy metals concentration in different tissues of fish can be used to assess the pollution in water bodies. Many studies have also reported the accumulation of toxic heavy metals in fishes above than their permissible level suggested by different International agencies for food consumption [12-14]. Consumption of such fishes contaminated with heavy metals may lead to deteriorating effects by causing various problems like cardiovascular disease and other health hazards like chromate nephropathy, neurodegenerative diseases, hepatic failure etc. [15,16]. Many authors have determined the heavy metal content in different fishes collected from various markets of many countries and found them contaminated with many harmful effects [17-19]. As India becomes second largest fish producing country in the world [20] and consumption of fish is also very high in India, It is very important to know the contamination level of heavy metals in fishes which are sold in our local markets. Hence, it was decided to evaluate the level of different heavy metals (Cr, Zn, Fe, Cu, Pb and Mn) in two fish species i.e. *Channa punctatus* and *Heteropneustes fossilis* from a local fish market Aligarh, as both these fishes are of great importance due to their food value, medicinal value and rejuvenating properties [21].

Haematological profiles of fishes are broadly being used for the monitoring of aquatic pollution as well as the health status of the fishes [22]. As any physiological changes caused by different pollutants can be reflected into the blood parameters [12,23], it was decided to analyse different haematological parameters like total erythrocytes count (TEC), total leucocytes count (TLC), haematocrit (Ht), haemoglobin (Hb), and other haematological indices like Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and O_2 carrying capacity of blood in both the fish species collected from market of Aligarh. Haematological parameters were also compared with the obtained results from fish maintained in healthy environment of laboratory condition.

Objective of the Study

Objectives of this study are (1) to evaluate the contamination of heavy metals in different tissues like muscle, gill, skin and liver of edible fish, *C. punctatus* and *H. fossilis* collected from local fish market Rasalganj, Aligarh, (2) to compare the obtained results with the permissible limits suggested by FAO, 1983 (Food and Agricultural Organization) for the food and agricultural products [24], (3) to compare the total metal accumulation i.e. metal pollution index (MPI) in different tissues of *C. punctatus* and *H. fossilis,* (4) to weigh up the haematological parameters of *C. punctatus* and *H. fossilis* collected from local fish market Rasalganj, Aligarh.

Materials and Methods

Study area

The study area was a local fish market Rasalganj, Aligarh, a city in Uttar Pradesh, a north province of India. Aligarh is located at the coordinate 27.88° N 78.08° E. The city is in the middle portion of the daob, the land between Ganges and the Yamuna rivers.

Sample collection

Irrespective of their sex, eight fish of each species approximately of same weight and length (weighing 57.2 ± 10.78 gm and with a length of 20.6 ± 1.51 cm) were purchased from a local fish market Rasalganj, Aligarh.

Sample analysis

The fishes were cold anaesthetized and sacrificed after bringing to the laboratory and then tissue samples like muscle, liver, skin and gills were collected and kept in -80°C for further analysis. For metal analysis tissues were then put into petri-dishes and incubated to dry at 100°C until there was no weight loss. Dried tissue samples were digested with diacid (HNO_3 and $HClO_4$ in 2:1 ratio) until all materials were dissolved. After cooling of the digested sample, distilled water was added into it. Metals concentration in different tissues of fishes was analyzed using flame atomic absorption spectrophotometer (AAS) (Perkin-Elmer Model 2380, Inc., walk, CT, USA).

Metal pollution index (MPI)

The metal pollution index (cumulative metal load) was calculated to determine total accumulation of the metals in different tissues of fishes. The MPI values were calculated following the equation [25].

$MPI = (Cf_1 * Cf_2 * Cf_3 * \ldots\ldots * Cf_n)^{1/n}$

Where Cf_n is the concentration for the metal n in the tissue sample.

Collection of blood

Blood was collected from the tail region of fish by ablating the tail and immediately taken into the potassium EDTA vial.

Haematological analysis

Blood samples from the market fish as well as acclimatized fish were subjected for the haematological analysis. The TEC and TLC were measured by Neubaure's improved hemocytometer using Hayem's and Tuerk's solution, respectively, as diluting fluids [26]. Haemoglobin was estimated using Sahli's haemoglobinometer. Wintrobe's method was used for measuring haematocrit value. The oxygen- carrying capacity of the fish blood was calculated by multiplying the content of haemoglobin by 1.25 oxygen combining power of Hb/gm [27]. The haematological indices like MCV, MCH, MCHC were calculated using the standard formulae of [28].

Statistical analysis

For statistical analysis, Student's t-test was performed to confirm the variability ($p < 0.05$) in the data obtained from both fish species. This test was applied to determine the significance differences ($p < 0.05$) among different haematological parameters of the fish.

Results and Discussion

The heavy metal concentrations in different tissues like muscle, liver, skin and gills of two fish i.e. *Channa punctatus* and *Heteropneustes fossilis* collected from local fish market Rasalganj, Aligarh have been shown in table 1 and table 2. From the tables it can clearly be seen that all the tissues of *C. punctatus* and *H. fossilis* showed higher concentrations of Pb above its maximum permissible limits (0.05 mg/kg) of FAO (1983) [24]. The highest concentration of Pb was observed in gills among all the tissues of both the fishes that probably are due to their close relation with the external environment [29]. The higher concentration of Pb beyond the permissible limits is having detrimental effects to the body condition, health and life span of fish as it is able to cross the blood-brain barrier [30-32]. In human being also excessive consumption may lead to permanent brain damage and eventually death [33].

Tissue Metal (mg/kg)	FAO (1983) (mg/kg)	Muscle	Liver	Skin	Gills
Pb	0.05	**5.212 ± 1.156**	**5.512 ± 0.832**	**1.070 ± 0.347**	**6.587 ± 2.165**
Mn	1.00	**2.625 ± 0.154**	**4.645 ± 0.400**	**2.066 ± 0.138**	**21.279 ± 0.312**
Fe	5.60	**10.187 ± 0.117**	**32.76 ± 0.117**	3.75 ± 0.010	**7.291 ± 0.086**
Cr	1.00	**2.52 ± 0.58**	0.47 ± 0.356	**1.42 ± 0.54**	**2.591 ± 0.14**3
Cu	10.00	1.358 ± 0.286	**12.845 ± 0.39**	1.212 ± 0.137	1.316 ± 0.281
Zn	50.00	6.362 ± 000	9.225 ± 0.017	7.545 ± 0.005	6.904 ± 0.011

Table 1: *Heavy metal concentrations in the various tissues of Channa punctatus.*

Data are given in mean ± SD

Metal concentrations higher than their permissible limits are written in bold letters

Tissue Metal (mg/kg)	FAO (1983) (mg/kg)	Muscle	Liver	Skin	Gills
Pb	0.05	**1.929 ± 0.835**	**1.608 ± 0.622**	**1.883 ± 0.604**	**3.892 ± 1.699**
Mn	1.00	**3.7 ± 0.150**	**4.283 ± 0.109**	**2.337 ± 0.195**	**3.892 ± 0.103**
Fe	5.60	**74.75 ± 18.29**	**22.41 ± 10.80**	**63.73 ± 13.68**	**16.75 ± 4.449**
Cr	1.00	**1.383 ± 0.443**	0.696 ± 0.169	**1.279 ± 0.985**	**7.212 ± 3.583**
Cu	10.00	1.217 ± 0.130	**17.087 ± 0.071**	**0.85 ± 0.209**	1.846 ± 0.069
Zn	50.00	6.220 ± 0.006	7.283 ± 0.006	6.95 ± 0.010	6.958 ± 0.006

Table 2: *Heavy metal concentrations in the various tissues of Heteropneustes fossilis.*

Data are given in mean ± SD.

Metal concentrations higher than their permissible limits are written in bold letters

Like Pb, Mn accumulation was also higher in all the tissues of both species than its maximum permissible limit (1.00 mg/kg) recommended by FAO (1983) [24]. The highest accumulation of Mn was observed in the gills of *C. punctatus* that might be due to increase of chloride cells in contaminated water resulting in increased accumulation of metal [34,35]). In case of *H. fossilis* its maximum accumulation was observed in liver followed by gills, muscle and skin. Mn is an essential metal but its higher concentration is responsible for disturbance in metabolism of carbohydrates and impairing of the immunological functions of fish [36]. It may also cause hallucinations and nerve damage in humans [37].

All tissues were having higher concentration of Fe except in skin of *C. punctatus* when compared to their maximum permissible limit (5.60 mg/kg) of FAO (1983) [24]. But its concentration was found to be higher in all the tissues of *H. fossilis*. Liver had the highest concentration of Fe in both the fishes that might be due to affinity of Fe in the liver of fish as reported by many studies [38]. By the production of large amount of metallothionein particularly in liver, fish respond to heavy metal exposure [39,40]. Iron is an essential element of respiratory system but its higher concentration may cause gill damage, disrupt metabolism and osmoregulation [41]. This excess Fe in the body is usually associated with some iron-overloading disorders, such as hereditary hemochromatosis (HH) and thalassemias [42,43].

It was found that Cr concentration was higher in all the tissues except liver in both the fish species in comparison to maximum permissible limit (1.00 mg/kg) Suggested by FAO (1983) [24]. Gills were having the highest accumulation of Cr in both fish probably because it always remained in direct contact with the aquatic environment and to have large surface area which enhanced bioaccumulation of metals in gills. Cr is an essential nutrient and plays an important role in glucose metabolism but its higher concentration may lead to decreased in growth, altered metabolism and chromosomal aberrations [44,45].

It is well known that copper is essential part of several enzymes and plays an important role in the synthesis of haemoglobin [46] but its higher concentration may cause impairment of gill functions. The concentrations of Cu in the sample of tissues like muscle, skin and gills were within the permissible limits recommended by FAO (1983) [24] except liver of *C. punctatus* but it was found to be lower in skin of *H. fossilis*. The higher concentration of copper was in the liver of both the species might be due to its ability to retain Cu. It has been reported that liver is the selective storage organ of Cu [47]. Due to accumulation of more Cu, the gills become frayed and it lose its ability in regulation and transport of salt between the body and surrounding environment that leads to reduced food intake and resulting in reduced growth of fish [48]. It has been reported that ingestion of drinking water with 3 mg Cu/L will produce gastrointestinal symptoms including vomiting, nausea and diarrhea [49].

Zinc concentrations in all the tissues like muscle, liver, skin and gills were below their maximum permissible limits (50 mg/kg) suggested by FAO (1983) [24] in both the species of fishes. Liver had the highest concentration of Zn in both the fishes probably because it is the metabolic centre of the body. Zinc is an essential trace element and involved in various metabolic activities, but higher concentration of zinc in fish may result in wreckage of gill epithelium and causing mortality, growth retardation and respiratory changes [50,51].

Heavy metal pollution index is an effective tool to assess the quality of fish with respect to heavy metals. It tells about the total metal load in a tissue or in entire body. In our study it was found that metal pollution index was higher in all the tissues of *H. fossilis* than those of *C. punctatus*. Hence, *H. fossilis* was more contaminated than *C. punctatus* collected from the same market, Aligarh (Figure 1).

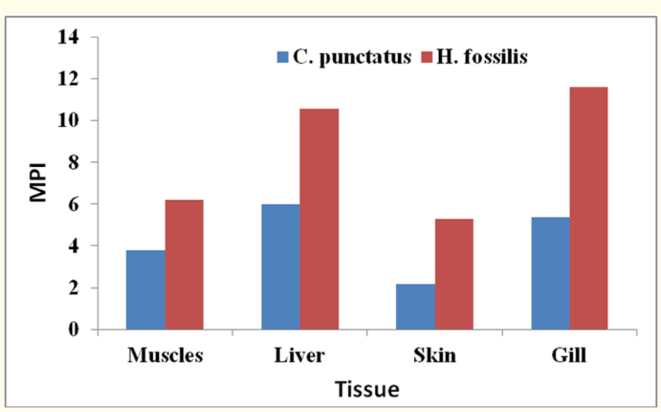

Figure 1: *Metal Pollution Index (MPI) of C. punctatus and H. fossilis.*

Table 3 shows the haematological profiles of both fish species collected from local fish market, Aligarh and its comparison with their respective control ones (kept in healthy environment and given proper and nutritive food). Most of the haematological parameters of the market fishes showed significant alteration from their control fishes. In case of *C. punctatus*, RBC count, Hb and MCHC value showed significant differences from control while in *H. fossilis* RBC count, WBC count, Hb and Ht% values were varied from controls. The differences in the haematological parameters of market fish showed their pathetic condition in comparison to control groups.

Parameters	Control	*Channa punctatus*	Control	*Heteropneustes fossilis*
Total RBC count ($\times 10^6$ mm^3)	5.51 ± 1.26	7.65 ± 0.70*	3.11 ± 0.53	4.38-10.09*
Total WBC count ($\times 10^5$ mm^3)	14.75 ± 0.62	14.94 ± 0.37	12.36 ± 1.3	10.14 ± 0.24*
Hb (g/dl)	9.16 ± 0.29	7.66 ± 0.58”	11.5 ± 0.86	10.16 ± 0.28*
Ht (%)	33.45 ± 0.97	32.95 ± 5.22	56.64 ± 0.67	48.29 ± 0.78*
MCV (fl)	62.56 ± 12.35	46.56 ± 2.95	186.31 ± 36.13	112.44 ± 18.63
MCH (pg)	17.26 ± 4.18	10.04 ± 0.87	38.19 ± 10.27	23.57 ± 4.29
MCHC (g/dl)	27.43 ± 1.31	21.56 ± 1.12*	20.29 ± 1.49	20.92 ± 0.33*
Oxygen carrying Capacity (ml O_2/g^{-1}/Hb)	11.45 ± 1.75	9.58-11.78	14.37-11.07	12.70 ± 0.35

Table 3: *Haematological parameters of the blood of fishes collected from local fish market.*

Data are given in mean ± SD.

**Denotes significant difference (p < 0.05) among haematological parameters of fish collected from local fish market, Aligarh.*

Conclusion

The concentrations of all the six metals (Pb, Mn, Fe, Cu, Cr, Zn) in both the fish collected from market were higher than their permissible limit suggested by an international organization, FAO (1983). Hence these fish species are presently unsuitable for human consumption from this market. It was also found that *H. fossilis* were more contaminated than *C. punctatus* collected from same fish market, Aligarh. Significant alterations in haematological parameters such as RBC count, Hb and MCHC values in *C. punctatus* and RBC count, WBC count, Hb and Ht% values in *H. fossilis* from control ones are showing their pathetic condition. If obstructive measures are not taken to withhold the input of heavy metal and other pollutants, the condition may derange in the future.

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