

Effect of anthropogenic activities on aquaculture in north India and consequences for fish health resulting from bioaccumulation of heavy metals and histological alterations

Sadiya Binte Swaleh, Umarah Zahoor Banday and Nazura Usmani*

Aquatic Toxicology Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh, India

*Corresponding author: nazurausmani@gmail.com

Abstract

Investigations were carried out to assess the condition of catfish, *Clarias gariepinus*, cultured in ponds in Aligarh, Northern India. It is indeed a matter of concern to detect Cu, Zn, Ni, Pb, Cr, Fe, Cd and Mg in water as well as fish tissues/organs (gastrointestinal tract, stomach, liver, blood, stomach content and testes). Water and fish samples were collected from four different zones of the pond and analyzed for heavy metal accumulation. Thereafter, alteration in fish tissue histology was examined. Results obtained for water samples followed the order Ni ($0.581 \pm 0.004 \text{Mg L}^{-1}$) > Pb ($0.570 \pm 0.006 \text{Mg L}^{-1}$) > Cd ($0.110 \pm 0.001 \text{Mg L}^{-1}$) > Cr ($0.077 \pm 0.009 \text{Mg L}^{-1}$) > Zn ($0.075 \pm 0.003 \text{Mg L}^{-1}$) > Mn ($0.065 \pm 0.004 \text{Mg L}^{-1}$) > Cu ($0.054 \pm 0.007 \text{Mg L}^{-1}$) > Fe ($0.052 \pm 0.005 \text{Mg L}^{-1}$). Maximum accumulation among tissues was observed in blood and lowest in integument. Fe ($1257.90 \text{Mg kg}^{-1} \text{.dw}$) concentration was recorded highest and Cd ($6.68 \text{Mg kg}^{-1} \text{.dw}$) was the lowest in all the tissues. Values for bio-indices were as follows: Hepatosomatic Index (1.01), Gastrosomatic Index (0.24) and Gonadosomatic Index (0.42). Bioconcentration factor (BAF) was highest in blood followed by stomach content, liver, testes, G.I.T., stomach and integument. Water and fish tissues showed accumulation beyond the permissible limits suggested by WHO and the United States (US) guideline. Histopathological effects included: Necrosis, Pyknosis and Vacuolization in the liver, Macrophage infestation in the stomach, disappearing of spermatocytes and tissue breakage in testes.

Keywords: *C. gariepinus*, Heavy metals, Bioindices, Pyknosis, Vacuolization, Histopathology

Introduction

Fish culture has been in practice since the time immemorial to meet the food demand of growing population. Many culture practices in India include composite culture, integrated culture, cage culture and weed- based carp polyculture. Fish being a cheap and an essential protein source is one of the most preferred foods consumed by the people of different countries. According to the latest data, Indians on an average consume 269 grams of fish per month in rural areas while in urban areas the amount is 238 gram, noticeably, just about 282 of 1000 households in rural areas consume fish, while the number is 209 households for urban areas (Dash, 2014). To obtain a healthy diet fish intake has increased by a greater amount than before. Uttar Pradesh is a populous state in the country with the agrarian economy. Almost 79% of its population lives in the rural areas. Considering the size of the state and availability of water bodies the total inland fish production is not remarkable. As much as 11.52 Lac hectare water bodies can be used for the fish culture (Inland Fishery Report, 2015).

Fish culture not only provides food but also raises the income of poor farmers to support their livelihood. The most important aspect of fish culture is healthy food for human consumption. The fish should be free from any pollutants which may enter the culture ponds. Pollution in culture ponds can possibly be from the point as well as non-

point sources. Anthropogenic activities including waste disposal and agriculture can also create an unhealthy environment for the fish. People engaged in fish culture practices in their local areas are usually unaware of the health risk when handling chemicals. They often overlook the substances which may cause damage to the pond and also the surrounding environment and on the quality of aquatic food products. Because of increasing concern over the potential harm of aquaculture effluents on water bodies, the bioaccumulation problem and human risks associated with the use of chemicals in fish culture practices, FAO has established standards in aquaculture for chemicals that are hazardous to human health and the environment. A number of studies have been conducted by Javed and Usmani (2011a, 2012b) on local water bodies in Aligarh (27.88°N 78.08°E) which were contaminated by anthropogenic activities, power plant discharges and sugar mills. Bioaccumulation in fish tissues and histopathological alterations in different organs have also been observed (Canli et al., 2003; Jezierska et al., 2006; ; Al.Weher, 2008; Vinodhini et al., 2008; Alturiqi et al., 2012). The area chosen for the present study is a culture pond (Figure 1) located at 27.9451°N , 78.1550° , and agricultural runoffs and household wastes were the main cause of contamination, primarily by heavy metals. This study was conducted to investigate their accumulation in different organs (Figure 2) and also the histological changes in fish tissues.

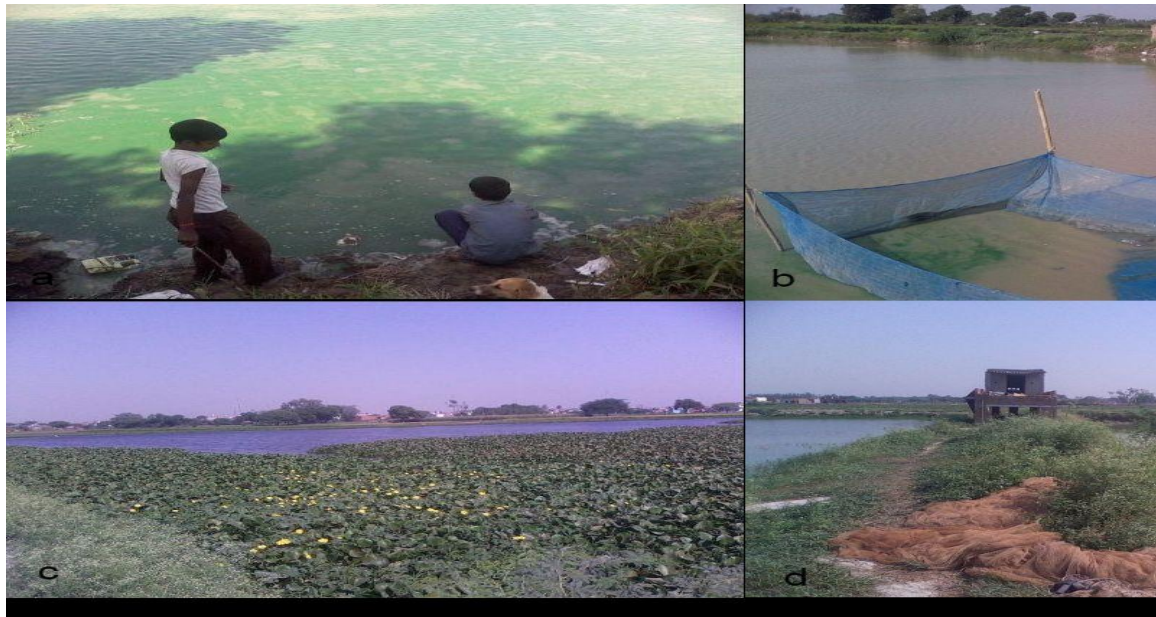


Figure 1. Hardua fish culture pond (27.9451° N, 78.1550° E). Water was sampled from a, b, c, d locations.

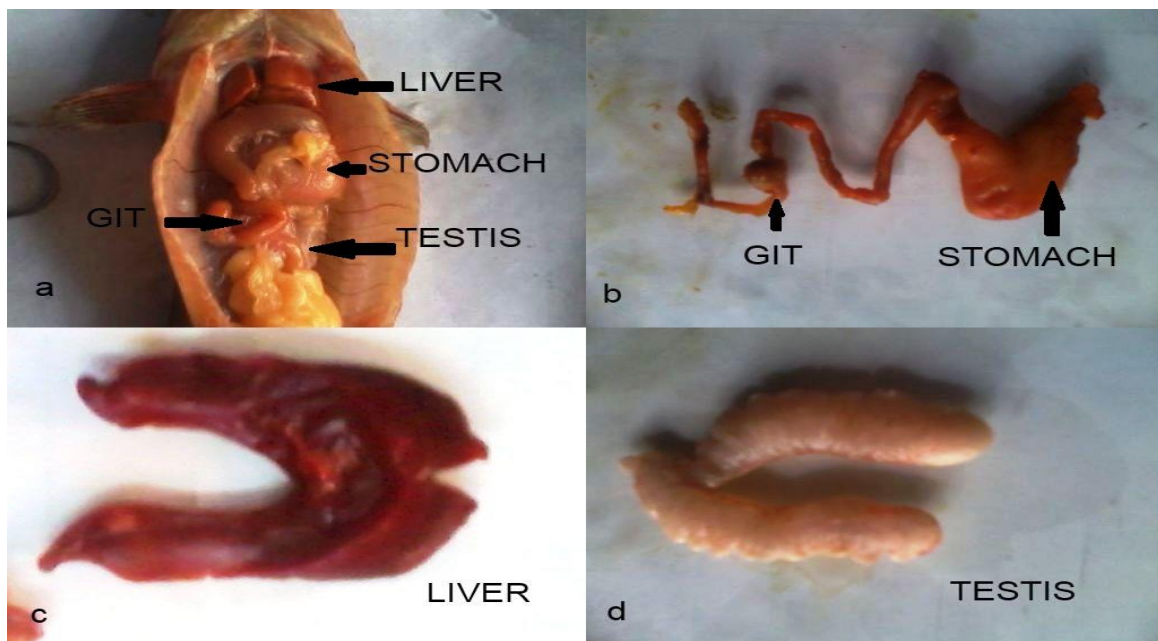


Figure 2. Organs of the fish studied for bioaccumulation a: Dissected *Clarias gariepinus* b: GIT and Stomach c: Liver and d: Testis.

Materials and Methods

Water and fish collection

Water was collected in pre-cleaned and acidified glass bottles and preserved by acidifying with 6 N HNO₃ (pH about 2.0) to estimate the heavy metal levels. Water samples were fixed on the spot to measure the dissolved oxygen (DO), total dissolved solids (TDS) and pH using standard techniques (APHA 2005). The temperature was recorded using the thermometer (Deluxe, 6). Samples of *C.gariepinus* (n=12) were collected from the pond. Live specimens of the fish were kept in water buckets and brought to the laboratory for further analysis. Fish were immediately sacrificed and their

length and weight were measured. Liver, blood, stomach, intestine and testes were removed to estimate the heavy metals (Topping, 1973) and for histopathological observations.

Preparation of standard metal ion solutions

Stock solution (1000mg/ L) of each of the metal ion was prepared using an appropriate metal salt of AR grade quality in dilute nitric acid. The standard solutions were prepared by appropriate dilutions in distilled water.

Estimation of heavy metals in the water sample and fish organs/tissues

Heavy metals (Cu, Cd, Ni, Fe, Mn, Cr, Pb and Zn) were estimated in pond water and fish organs/tissues using Atomic Absorption Spectrometer as per the standard protocols of APHA (2005).

Tissue preparation for histological examination

Freshly sacrificed fish were dissected to remove the organs (stomach, intestine, testes and liver) which were later fixed in 30% formalin. Tissues were processed according to the protocols of Humason (1979). The sections were examined and photographed using light microscope (Zeiss).

Calculation of bioindices**Hepatosomatic Index (HSI)**

Weight of the fish was measured using a weighing balance. Incision was given from the ventral region of the abdomen to carefully remove the liver for measurement of weight. Hepato-somatic index (HSI) was determined by the formula:

$$\text{HSI} = \frac{\text{Weight of liver (g)} \times 100}{\text{Weight of fish (g)}}$$

Gastrosomatic Index (GaSI)

Similarly, the stomach was removed carefully for measurement of weight and calculation of GaSI (Desai 1970):

$$\text{GaSI} = \frac{\text{Weight of the stomach (g)} \times 100}{\text{Weight of the fish (g)}}$$

Gonadosomatic Index (GSI)

Gonads were dissected out for calculation of gonadosomatic index using formula.

$$\text{GSI} = \frac{\text{Weight of the gonad (g)} \times 100}{\text{Weight of the fish (g)}}$$

Bioaccumulation Factor

Bioaccumulation of the heavy metals in fish tissues was assessed using the bioaccumulation factor (BAF) calculated as the ratio of the concentration of the specific heavy metal in the tissue to the concentration that heavy metal in the water.

Histological examination and tissue preparation

For examining the histological changes occurring in the target tissues of exposed fish, the required samples were extracted from the fish. They were fixed in 10% (v/v) phosphate buffered formalin solution (pH 7.4) for 24 h and dehydrated, cleared, embedded in paraffin wax, cut (5 µm thick sections) using a microtome (ERMA, JAPAN) and stained (Haematoxylin and Eosin H&E) following standards histological protocols and studied under light microscope (ZEISS).

Statistical analysis

Quantitative values of the analyzed parameters are given as Mean ± SD. Statistical differences for the mean of heavy metal values in water and in fish tissues were calculated using

ANOVA and Duncan's Multiple Range Test (Duncan software) to determine the significance at 5% probability level ($p < 0.05$).

Results**Physicochemical parameters of water and heavy metal load in pond water**

Contamination of the pond significantly affected the physico-chemical qualities of the water. Dissolved oxygen was 08 MgL⁻¹, pH= 7.5, Temperature= 22°C and total dissolved solids = 22500 MgL⁻¹ (Table 1). Out of the heavy metals (Cu, Zn, Ni, Pb, Cr, Fe, Cd and Mn) examined the Ni(0.581±0.004 MgL⁻¹) was present in the highest concentration followed by Pb(0.570±0.006 MgL⁻¹), Cd(0.110±0.001 MgL⁻¹), Cr(0.077±0.009 MgL⁻¹), Zn(0.075±0.003 MgL⁻¹), Mn(0.065±0.004 MgL⁻¹), Fe(0.052±0.005 MgL⁻¹) (Table 2). International guidelines were used to compare the heavy metals exceeding the permissible limits. According to the World Health Organization (WHO) standard the levels of the metals: Ni, Pb, Cd and Cr were beyond permissible limits. The values of Zn Mn, and Fe were within the acceptable limits. According to USA Standards, Mn exceeded the limit set for it. All the mean values for metal were statistically significant ($p < 0.05$) from each other except between Chromium and Zinc (Table 2).

Table 1. Physicochemical properties of water sample from Hardua pond.

Water	Temperature	pH	Dissolved Oxygen (d.o.) Mg ⁻¹	Total Dissolved Solids (tds) mg ⁻¹
Ideal condition for fresh water	20- 30.0°C	6.8-8.6	6-8	500
Hardua culture pond	22	7.5	8	22500

Table 2. Concentrations of heavy metals (Mg/L) in water samples from the study site. Data are presented as mean±SD with different superscript (a, b, c, d, f, e) indicating values differing significantly. Same superscript denotes insignificant values.

Heavy metals	Hardua culture pond (Mg ⁻¹)	WHO guidelines (Mg ⁻¹)	USA guidelines (Mg ⁻¹)
Cu	0.054±0.007 ^h	2	1.3
Zn	0.075±0.003 ^{ef}	3	-
Ni	0.581 ±0.004 ^a	0.02	-
Pb	0.570 ±0.006 ^b	0.01	-
Cr	0.077±0.009 ^e	0.05	0.1
Fe	0.052±0.005 ^c	1	0.3
Cd	0.110±0.001 ^d	0.003	-
Mn	0.065±0.004 ^g	0.5	0.05

All values are in mg/L 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. Adapted for Water Quality for Ecosystem and Human Health, 2006 (prepared and published by the United Nations Environment Program. Global Environment Monitoring System (GEMS)/ Water Program). Blank cells indicate that no citable information was available.

Concentration of heavy metals in tissues

Cu: Concentration of Cu was in the range of 15.6 - 315.5 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was found in blood (315.5 $\text{Mgkg}^{-1}\text{.dw}$) and lowest in integument (15.6 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed: Blood>Liver> Testis> G.I.T.> Stomach> Stomach contents> Integument. Cu concentration was found to be statistically significant in all the organs except between liver and testis and GIT and stomach.

Zn: Concentration of Zn varied from 34.19 to 349.44 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was noticed in blood (349.44 $\text{Mgkg}^{-1}\text{.dw}$) and lowest in integument (34.19 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed: Blood> Testis> G.I.T.> Liver> Stomach content> Stomach> Liver> Integument. Zn concentration was statistically significant for all organs except between stomach and stomach content.

Ni: Concentration of Ni ranged from 7.55 to 94.28 $\text{Mgkg}^{-1}\text{.dw}$. Maximum amount was seen in the blood (210.15 $\text{Mgkg}^{-1}\text{.dw}$) and minimum in the Integument (7.55 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed: Blood> Testis> Stomach content> G.I.T.> Stomach> Liver> Integument. Concentration of this mineral was statistically significant for all organs except between stomach content, GIT, liver and integument.

Pb: Concentration of Pb was in range of 11.55 to 210.15 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was found in blood (210.15 $\text{Mgkg}^{-1}\text{.dw}$) and lowest in integument (11.55 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed in organs: Blood> Testis> Stomach content> G.I.T.> Stomach> Liver> Integument. Pb concentration was found to be statistically different for all organs except between Stomach, GIT and Stomach content.

Cr: Concentration of Cr was in range 9.73 to 177.95 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was found in Testis (177.95 $\text{Mgkg}^{-1}\text{.dw}$) and lowest was found in liver (9.73 $\text{Mgkg}^{-1}\text{.dw}$).

Following order of accumulation was observed in organs: Testis> Stomach content> G.I.T.> Stomach> Blood> Integument> Liver. Cr concentration was found to be statistically significant for all organs except between Stomach, Integument, Blood and Liver.

Fe: Concentration of Fe was in the range 111.73 to 1257.908 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was found in blood (1257.908 $\text{Mgkg}^{-1}\text{.dw}$) and lowest in integument (111.73 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed in organs: Blood> Stomach content> Liver> Testis> G.I.T.> Stomach> Integument. Fe concentration was found to be statistically significant for all organs.

Cd: Concentration of Cd was in the range of 6.68 to 98.25 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was found in testis (98.25 $\text{Mgkg}^{-1}\text{.dw}$) and lowest in integument (6.68 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed in organs: Testis> Blood>G.I.T.>Stomach>Liver>Stomach content> Integument. Cd concentration was found to be statistically significant for all organs except between GIT and liver.

Mn: Concentration of Mn was in range of 30.99 to 293.01 $\text{Mgkg}^{-1}\text{.dw}$. Highest amount was found in blood (293.01 $\text{Mgkg}^{-1}\text{.dw}$) and lowest in Integument (30.99 $\text{Mgkg}^{-1}\text{.dw}$). Following order of accumulation was observed in organs: Blood> Testis> Stomach content> G.I.T.> Liver> Stomach> Integuments. Mn concentration was found to be statistically significant in all the organs.

Table 3. Concentration of heavy metals in different tissues of *Clarias gariepinus*. Data (n=12) are presented as mean±SD. Values with different superscripts (a, b, c, d, e, f) differ significantly (p<0.05) at 5% probability level. Same superscript signifies insignificant values.

Heavy metals	Integument (Mgkg ⁻¹ .dw)	Blood (Mgkg ⁻¹ .dw)	G.I.T (Mgkg ⁻¹ .dw)	Stomach (Mgkg ⁻¹ .dw)	Stomach content (Mgkg ⁻¹ .dw)	Liver (Mgkg ⁻¹ .dw)	Testes (Mgkg ⁻¹ .dw)
Cu	15.60±0.881 ^e	315.50±13.44 ^a	90.08±2.34 ^c	85.78±3.86 ^c	65.33±2.86 ^d	104.11±3.36 ^b	104.01±7.16 ^b
Zn	34.19±1.20 ^f	349.44±11.82 ^a	133.97±2.99 ^c	78.00±3.10 ^e	79.69±3.06 ^e	101.83±1.50 ^d	237.21±1.30 ^b
Ni	7.55±0.45 ^d	94.28±9.84 ^a	14.04±2.86 ^{cd}	10.26±1.14 ^d	22.05±1.53 ^c	8.43±1.14 ^d	41.40±5.47 ^b
Pb	11.55±0.15 ^e	210.15±8.58 ^a	38.07±1.87 ^c	35.46±0.68 ^c	40.88±0.43 ^c	26.50±2.40 ^d	100.98±3.18 ^b
Cr	17.74±0.58 ^{de}	21.74±0.96 ^{de}	63.62±2.78 ^c	33.88±2.31 ^{cd}	91.11±2.51 ^b	9.73±1.10 ^e	177.95±7.16 ^a
Fe	111.73±1.15 ^g	1257.90±5.98 ^a	234.05±2.34 ^e	126.78±4.75 ^f	1204.72±1.94 ^b	488.06±1.95 ^c	248.15±7.94 ^d
Cd	6.68±0.75 ^f	85.66±4.75 ^b	45.80±3.31 ^c	31.33±2.82 ^d	24.10±2.17 ^e	39.61±0.83 ^c	98.25±6.74 ^a
Mn	30.99±0.76 ^g	293.01±10.07 ^a	66.73±2.81 ^d	44.90±2.12 ^f	121.12±1.56 ^c	55.19±1.36 ^e	179.04±4.17 ^b

Table 4. Bioaccumulation factor of heavy metals in different tissue samples of fish (*C. gariepinus*).

Heavy metals	Integuments	Blood	G.I.T	Stomach	Stomach content	Liver	Testes
Cu	288.8	5842.5	1668.1	1588.0	1209.8	1927.0	1926.1
Zn	455.8	4658.6	1786.2	1062.5	1062.5	1357.7	3162.8
Ni	13.0	162.5	24.2	17.6	38.0	14.5	71.3
Pb	20.1	368.6	66.7	62.2	71.7	46.4	177.1
Cr	230.3	282.3	826.2	440.0	1183.2	126.3	2311.0
Fe	2148.6	24191.9	4500.9	2438.0	23167.3	9385.7	4772.1
Cd	57.0	813.3	258.7	177.0	205.9	223.7	555.0
Mn	476.7	4507.8	1026.6	690.7	1863.3	849.0	2754.4

Bioindices

The values of GaSI, HSI and GSI were 0.246, 1.01 and 0.42, respectively, Bioaccumulation factor was highest for blood (24191.92) and least for integument (13.01) and following was the order of bioaccumulation: Blood > stomach content > liver > testis > G.I.T. > stomach > integument. This shows that the blood had the highest metal load than other organs of the fish (Table 4).

Histopathological observations

Histopathological alterations were seen in all the tissues (liver, stomach and testis) of the fish collected from the Hardua culture pond. Pollutant level recorded from the water collected seemed to have caused marked deformities in the tissue of these organs. Some heavy metals were found to exceed the permissible limits of WHO and USEPA. These increased values have a relation with the observed deformities in the tissues.

Liver: Hepatocytes showed inflammation caused due to necrosis is markedly visible (Figure 3a). Pyknosis was also observed in cells scattered in the tissue sections with vacuolization and dilated blood vessels (Figure 3 b, c).

Stomach: Histopathological deformities were distinct in the mucosa, sub-mucosa, serosa, and inner and outer muscles of the stomach. Microvilli disruption was clearly visible in the mucosal layer (Figure 3d, e). Degeneration of vessels, vacuolization and macrophage infestation in the base lining of gastric folds near the beginning of the inner circular muscle layer was visible (Figure 3 f).

Testis: Tissue sections showed disappearing spermatocytes and vacuolization in sertoli cells. Irregular smooth muscle lining with breakage was visible (Figure 3 g, h, i).

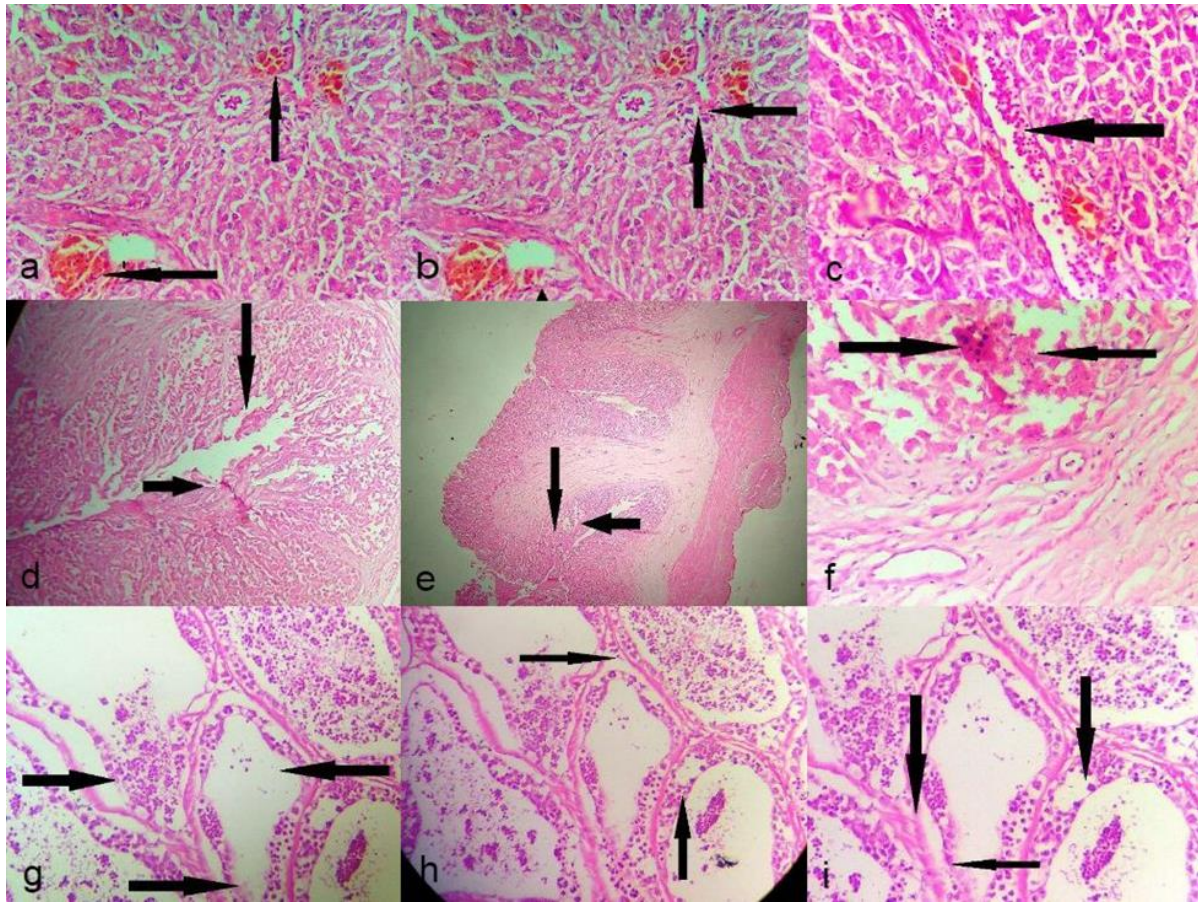


Figure 3. a- Necrosis b-Pyknosis, c-Dilation of vessels, d- Microvilli disruption, e- Vacuolization, f- Macrophage infestation, g-disappearing spermatocytes, h-vacuolization and i-Irregular smooth muscle lining. All images were viewed at 400X, using light microscope (Zeiss).

Discussion

The ponds used for culture practices are considered safe and free from any undesirable substances. However, the findings of the present work conducted on Hardua pond have put a question mark on properties of water and the quality of fishes dwelling therein. Chemicals present beyond limit are considered as major threats and, therefore, alongwith the physico-chemical quality (which was good enough to allow acceptable living conditions for fish), heavy metals, including Cu, Cd, Ni, Fe, Co, Mn, Cr and Zn were also estimated. Highest concentration recorded was that of Ni ($0.581 \pm 0.004 \text{ MgL}^{-1}$) followed by Pb ($0.570 \pm 0.006 \text{ MgL}^{-1}$) > Cd ($0.110 \pm 0.001 \text{ MgL}^{-1}$) > Cr ($0.077 \pm 0.009 \text{ MgL}^{-1}$) > Zn ($0.075 \pm 0.003 \text{ MgL}^{-1}$) > Mn ($0.065 \pm 0.004 \text{ MgL}^{-1}$) > Cu ($0.054 \pm 0.007 \text{ MgL}^{-1}$) and Fe ($0.052 \pm 0.005 \text{ MgL}^{-1}$). Statistical analysis showed significantly different concentration of heavy metals in water samples except between Chromium and Zinc. Seepage of heavy metals into the pond can be attributed to mishandling of both domestic and agricultural waste discharged near the site. Plantations near the pond may also be a source of heavy metals as pesticides sprayed on them could contain compounds (salts) of heavy metals. Many toxic substances (inorganic and organic compounds, and heavy metals) deposited and buried in the soil are also washed by water

and enter the aquatic system. These heavy metals make their way into the food chain by the uptake of plants near the site and elsewhere taken by the consumers and also via the dwellers of the aquatic body. Realizing similar influence, fish tissues (stomach, gastrointestinal tract, liver, gonads (testes/ovaries), kidneys, blood and integument) were analyzed. Metals have a tendency to accumulate to various degrees in different tissues causing specific yet correlating effects on the organs. They tend to alter the proteins, form salt bridges and increase the oxidative stress. In the study conducted the high concentrations of Iron (Fe), Copper (Cu), Zinc (Zn), Nickel (Ni), Lead (Pb) and Manganese (Mn) were found in blood, while lowest in integument. Fe accumulation has been reported to be fatal by many workers. Ferrous iron (Fe^{2+}) is considered to be more toxic than ferric (Fe^{3+}) (Barjhoux, 2012). It tends to accumulate more in liver and gonads, least values have been recorded for brain, muscle and heart (Decker et al, 1974; Rensburg, 1989). Omar et al. (2014) also observed fish liver to be the target organ. Respiratory disruption caused by physical clogging of the gills could also be related to iron toxicity (Grobler et al., 1991). The metal is responsible for inducing oxidative stress which may also result in the cellular damage (Orino et al., 2001, Sevcikova et al., 2011).

Li et al. (2006) observed lipid peroxidation (LPO) and alterations in antioxidant enzyme activity in embryonic and adult medaka, *Oryzias latipes*, exposed to nano-iron. High concentration of Cu was recorded in the blood ($315.5 \text{ Mgkg}^{-1} \text{ dw}$) of *C. gariepinus* whereas it was least in integument ($15.6 \text{ Mgkg}^{-1} \text{ dw}$). Jefferies and Firestone (1984) also found low (0.965 mg) Cu content in the muscle of chub. Copper is one of the essential trace nutrients that is required in small amounts (5-20 micrograms per gram ($\mu\text{g/g}$) by humans, other mammals, fish and shellfish for carbohydrate metabolism, proper functioning of many important enzymes, and for the formation of oxygen-transporting pigments in the blood of vertebrates. However, exceeding levels have been reported to be toxic ($>20 \mu\text{g/g}$) (Bradl, 2005; Wright and Welbourn, 2002). The most bioavailable/toxic form is cupric ion (Cu^{+2}), and fishes and crustaceans are 10 to 100 times more sensitive to the deleterious effects of copper than mammals (Forstner and Witmann (1979); Hodson et al (1979); Wright and Welbourn (2002)). This metal has an impact on hematological parameters of fishes, causing a significant decrease in haemoglobin (Hb) content from 10.73 to 6.60%, and also red blood cells (RBC) (2.86 to $1.84 \times 10^6/\text{mm}^3$) (Singh et al., 2008). Zinc concentrations followed a similar pattern. Liver was found to be the most targeted organ of *C. gariepinus* (Coetzee et al., 2002). Murugan (2008) working on *Channa punctatus* observed highest accumulation in liver followed by kidney, intestine, gill and muscle. The reason for high accumulation of Zn in the liver could be due to metallothionein (MT). Low molecular weight proteins called metallothionein are responsible for zinc homeostasis and poisoning; and zinc on the other hand is a potent inducer of metallothionein. Its interaction with many chemicals produces altered patterns of accumulation, metabolism, and toxicity. Corroborating to the present study, Ayed (2011) reported higher concentration of Zn in the gonad. Lesions in testes are suspected to be caused by exceeding zinc load. Zinc is one of the most ubiquitous and mobile of elements. Mance et al (1984) reported its existence in natural waters both in dissolved forms, and associated with suspended particles. In river water, however, Zn is predominantly present in the dissolved form. In the present study, high Ni concentrations were recorded, being least in integument ($7.55 \text{ Mgkg}^{-1} \text{ dw}$) and maximum in blood ($210.15 \text{ Mgkg}^{-1} \text{ dw}$). According to Neilson (1977) Ni after absorption in epidermis, combines with body protein and also penetrates other sites. *Cyprinus carpio* fingerlings exposed to Ni exhibited decreased blood parameters including hematocrit and haemoglobin count, lowered values of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (Athikesavan et al., 2006). Its capacity of accumulation has been reported to be negligible under laboratory conditions ranging between 0.02 and 2 mg kg^{-1} in whole fish (wet weight), being almost 10 times lower than concentrations found in fish procured from contaminated waters. Aquatic organisms bioaccumulate Pb more efficiently from contaminated water than diet (Creti et al., 2010). Its accumulation has been noticed in liver, kidney, spleen, digestive tract and gills leading to disorders in the fish body

(Jeziarska and Witeska, 2006). Eboh et al. (2006) reported low Pb (0.001 - $0.002 \mu\text{g g}^{-1}$) accumulation in the muscle, gills and liver tissue of five common commercially available fishes (catfish, mudskipper, *tilapia*, *hilsa* and bonga). Laboratory studies on *Clarius batrachus* have shown 5 ppm Pb (during 150 days trial) accumulation resulting in marked inhibition of gonadal growth, along with decrease in cholesterol and lipid levels in brain, testes and ovary (Castro-González, 2008). Similar to the findings in the present study, hepatocyte vacuolization, nuclear pyknosis necrosis, hepatic cirrhosis, shrinkage, parenchyma degeneration and increase of sinusoidal spaces were the distinct changes observed in the liver of lead-exposed fish by Iger and Abraham (1997). Manganese poses toxic effect for the immune system of fish. Like other metals Mn concentration was highest in blood ($293.01 \text{ Mgkg}^{-1} \text{ dw}$). This clearly indicates the affinity of the metal towards blood proteins and its tendency to cause damage as reported by Sharma and Langer (2014). Its deficiency influences skeletal and reproductive health of fishes (Akan et al., 2012). Highest concentrations of Cr ($177.95 \text{ Mgkg}^{-1} \text{ dw}$) and Cd ($98.25 \text{ Mgkg}^{-1} \text{ dw}$) were found in testes of *C. gariepinus*. Both Cr (III) or (Cr^{3+}). Hexavalent Cr(VI) or (Cr^{6+}) are stable forms of chromium and induce oxidative stress, but the ability of chromium (Cr^{6+}) to cross cell membranes makes it more toxic (i.e. carcinogenic) (Pacheco et al., 2013; WHO, 1990, Eisler, 2000). Presence of Cr (3.7 - $26.9 \mu\text{g g}^{-1}$) has been reported in the tissue of *H. fossilis* obtained from the Yamuna River water (Ajmal and Razi-ud-din, 1988). Toxicity induced by Cr in fish influences hematology, immune functions, growth, histology and morphology along with production of reactive oxygen species (ROS) (Ahmed et al., 2013; Reid 2011). Similarly there are reports of high concentrations of Cd destroying erythrocytes, reducing hematocrite value and haemoglobin concentration leading to anemia (Omer et al., 2012). Cadmium was also found to disturb hematopoietic system in carp (Witeska, M et al 2009)). Cadmium- inducing hematotoxicity, anemia and immunosuppression has been reported by several workers (Gill and Epple, 1993; Seong-Gil et al., 2004; Ates et al., 2008; Witeska et al., 2009, 2010).

Heavy metals generally follow three possible routes to enter fish body: through the body surface, gills and digestive tract (Pourang, 1995). Javed and Hayat (1996) suggested that food is also an important source of heavy metal accumulation in fish. Considering this aspect, during present execution of study, their concentrations in stomach content were also estimated.

Bioindices studies

Study of bioindices can offer information on the general health condition of the organisms. Pollutants like heavy metals affect organisms by reducing their growth and reproduction, and can gradually cause mortality. Growth and reproduction can serve as an important indicator of the general well-being of the organism. Besides the heavy metal concentration low values for GaSI obtained were also due to low feeding particularly during colder seasons as October and November (period during which the fish were collected

for the present study) were also observed by Bhererai S. et al (2015) (2015). Liver is the primary detoxification organ, and undergoes biochemical alterations to meet the metabolism of xenobiotic compounds. Hepatosomatic index is an organ-level biomarker for exposure to pollutants which can lead to an increase in liver size from hypertrophy and hyperplasia of hepatocyte. HSI value can, therefore, provide information regarding fish health as well as water quality. In male *Cirrhinus mrigala*, the HSI was highest in March (1.184) and declined in July (Behererai et al 2015). HSI decreases with maturation of gonads in many teleosts (Aida, 1930; Haug T., 1998). Higher HSI values are related to good aquatic environment with healthy fish. Lower values suggest poor growth and unhealthy environment. HSI values give indication of development pattern of fishes. Study of GSI is also important and essential. At maturity stage fish has maximum GSI value and after spawning GSI value declines. The value obtained in our study was recorded to be 0.425 suggesting October to be the non-breeding season. The fact that gonads were not in mature/maturing phase is evident from the absence of mature sperms in the histological preparations. Structural changes marked by disappearance of spermatocytes pointed to the impact of heavy metal load in the water. GSI values reported by Ashwini et al. (2012) for testes were higher (18.4317) in the month of June and lower (3.2995) in the month of January. Amtyaz et al. (2013) determined the highest gonadosomatic index value in males (5.792). This was maturing phase, with massive volume, whitish color, visible blood capillaries. The lowest GSI value was 1.020 (Testes shrunk with wrinkles, flaccid, grayish, no milt expression). Present study recorded GSI values for testes only as all the fish procured randomly from pond happened to be male.

Bioaccumulation factor (BAF)

High BAF values were obtained during the study. It was maximum in blood (24191.92) and minimum in integument (13.01). The values followed the order: Blood > stomach content > liver > testes > GIT > stomach > integument. BAF for stomach content clearly indicated that the food procured by the fish contained substantial amounts of heavy metals. *C. gariepinus* is a piscivorous fish but it has also been observed to consume other food items at its feeding time. Perhaps the feeding habit is such that food materials unknowingly taken up by the fish were contaminated with the heavy metals. Liver also accumulated metals in large amount. This can be attributed to its function of detoxifying pollutants and not letting them reach other vital organs. Studies by Conceição et al. (2013) have reported that the use of fertilizers can increase concentration of heavy metals. The pond from where the fish were caught was located near a plantation area and fertilizers used there could have entered the water body through runoff.

Histopathological studies in different tissues of *C. gariepinus* (liver, stomach and testes)

Water contamination due to pollutants not only has an effect on the morphology of fish but also the histology of its tissues, leading to metabolic disruptions. This has serious

consequences for the survival and health of the fish. The detoxification function of the liver makes it such a vital organ for the proper functioning of the whole body. Liver tissue showed increased areas of inflammation due to necrosis. Pyknosis was distinctly visible as cells were found to be shrunken and were stained darker than the normal cells. Dilation of vessels was clearly observed in liver. Similar results have been reported from different sites and fish species like *Oreochromis mossambicus* (Van Dyk et al., 2007), *Channa punctatus* (Mishra, 2008), *C. batrachus* (Joshi, 2011) and *C. carpio* (Parvathi et al., 2011; Swaleh and Usmani, 2016). Chronic copper accumulation in the liver of fish causes hepatocyte lysis, cirrhosis and eventually death (Pourahamad and O'Brien, 2000; Varanka et al., 2001). Any contaminant in the food passes through the walls of the stomach. Histological examination of the organ leaves no doubt on the damage caused. Microvilli disruption was clearly visible in the mucosal layer instead of a smooth running gastric fold along with the degenerative changes in vessels and vacuolization, and macrophage infestation in the base lining of gastric folds and beginning of the inner circular muscle layer. Very few studies have been reported on stomach histopathological changes due to heavy metals. Heavy metal contamination also has an impact on the reproductive health of fishes. Healthy gonads are required to maintain the fish populations. Damage to the gonad unequivocally reflects on the implications for population recruitment. Vergilio (2013) working on male reproductive systems of fish exposed to Hg reported 36.8% reduction in sperm, along with congestion of blood vessels, proliferation of interstitial tissue and reduction in germ cells. Unfortunately, there are no standards or guidelines for metal load of organs in the fish.

Conclusion

A northern Indian pond was selected for evaluation of heavy metals in water and fish since the fish from the area are considered safe for consumption. These man-made ponds are maintained by local personnel and they assume no undesirable substance can make its way into them. Presence of heavy metals is an alarm regarding pollution status of the ponds and also of the fish inhabiting these water bodies. Nearby locality and the plantation were identified to be the source of contamination. People who consume fish are at risk of adverse health conditions. The data collected by researchers and government organizations should be analyzed and some regulations should be laid down, to protect such small water bodies as they contribute to the economy of the local communities, and to protect public health.

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