Annals of Plant and Soil Research 26(2): 302-310 (2024)
https://doi.org/10.47815/apsr.2024.10364

A comparative study of acquisition of heavy metals and its toxicological effects in fish species: *Siluriformes* and *Channa punctata*

BHARTI GUPTA[1*] AND RAMAKANT MAURYA

Maharishi University of information technology, Department of Zoology, Dubagga Lucknow 226013

Received, January, 2024; Revised accepted, May, 2024

ABSTRACT

The heavy metals damage fish's physiologic and chemical composition, causing structural damage and hindering their activities. Lucknow's fisheries sector, accounting for 1% of GDP, faces contamination from heavy metals, pesticides, and fertilizers. This study examines heavy metal levels in Siluriformes and Channa punctata fish and their buildup in the Gomati River water reservoir. Fish samples from the Gomati River near Lucknow, India, were collected and dissected into pieces with stainless steel blades. The samples were then dried at 100°C for 24 hours, revealing the anatomical features of Siluriformes and Channa punctata species. The Pb, a heavy metal, was found to have the highest concentration in the scales of Siluriformes, with values in various regions, while Cd had the lowest concentration, ranging from 0.016 to 0.112 mg/kg. Excessive consumption of fish can be dangerous due to toxic metallic elements accumulation. Siluriformes and Channa punctata fish species have high levels of Lead and Cadmium, respectively, indicating potential health risks. Channa punctata and Siluriformes fish have higher heavy metal concentrations than Siluriformes, with lead, iron, and nickel levels beyond WHO limits, while chromium, zinc, cadmium, and copper levels are below WHO limits.

Keywords: Heavy metals toxic effects, *Siluriformes, Channa punctata,* Gomati River

INTRODUCTION

Heavy metals damage fish's physiologic and chemical composition, leading to organ dysfunction and the accumulation of high-atomic weight metallic elements over time. Heavy metals in fish cause structural damage, alter condition markers, and cause genetic material damage, impacting their biodiversity, despite their high number of species. Lucknow's fisheries sector, a subsidiary of agriculture, contributes 1% to the GDP. Utter Pradesh's freshwater resources yielded 701,726 metric tons in 2020 **(**Shahjahan *et al.,* 2022**)**. The growing population demands increased food production, making the fish industry a vital source of protein and lipid-rich sustenance (Ali *et al.,* 2014). Fish lipids, rich in polyunsaturated n-3PUFA, are essential for biological processes and nutritional value, reducing cholesterol levels and reducing heart disease risk (Zeitoun *et al.,* 2014). Following this study, fish are considered a viable treatment option for individuals with cardiovascular conditions (Rashed *et al.,* 2001). The farming industry is causing water supplies to be contaminated with hazardous contaminants like heavy metals, pesticides, and artificial fertilizers, posing significant health risks to fish consumption (Burger *et al.,* 2005; Castro-González *et al.,* 2008; Witeska *et al.,* 2005 and Gupta *et al.,* 2015). Fish bioaccumulation accumulates heavy metals in tissues, serving as a bioindicator for high atomic weight elements in water bodies, which are transferred to predators via the food chain (Tiwari *et al.,* 2014 Kumari *et al.,*2018). Pollution in fish populations reduces fertility and affects their biological characteristics. Elevated metals interfere with biochemistry and accumulate in aquatic organisms, damaging animal and plant tissues (Trivedi *et al.,* 2016; Singh *et al.,* 2009; Ali *et al.,* 2018 Singh *et al.,* 2016). Aquatic species experience sublethal illness in their reproductive, kidney, liver, respiration, and nervous systems as a result of heavy metal exposure (Tiwari *et al.,* 2014). Polyunsaturated fatty acids undergo membrane lipid peroxidation, causing lipid-free radicals that harm lipids, proteins, and carbohydrates. Heavy ions like Cadmium and lead in wastewater pose health risks and environmental issues (Mishra *et al.,* 2008). For intensity, Cd (II) is categorized as a class I human carcinogen, whereas Pb (II) poses a greater risk to children compared to adults since it is ingested at a higher rate (Mehmood *et al.,* 2019 and Maurya *et al.,* 2016).

*Corresponding Email: bg6568053@gmail.com

MATERIALS AND METHODS

The samples of *Siluriformes* and *Channa punctata* species of fish, along with samples of water, were collected from the Gomati River near Lucknow, India. The water and sample sediments were separately gathered in transparent cleaned plastic bottles. The sediments underwent desiccation in an oven at a temperature of 100°C for 24 hours. The fish sample was separately stored in vials filled with a 5 % strength solution of formalin. To prevent deterioration, the samples were safeguarded using cleaned polythene bags and kept at a temperature of -20°C in a deep refrigerator until they were prepared for subsequent examination. The fish samples were dissected into separate pieces, including the anatomical features of a fish including the head, tails, lower abdomen, scales, fins, as well as gills, using stainless steel blades that are resistant to corrosion. Every single sample was set aside in a separate porcelain dish and thereafter transferred to the oven for drying at an oven temperature of 100°C for 24 hours.

Sample preparation

The desiccated specimens were pulverized into tiny fragments. Every specimen underwent treatment with a volume of 10 milliliters of highly concentrated (HNO_3) and 2 milliliters of (H_2O_2), with approximately 2.0 grams of each sample undergoing this treatment. The specimens underwent digestion utilizing a Janeway Model-1000 hot plate, and 6.0 g of each sample was extracted for total lipids following the standard protocol (Siddiqui *et al.,* 2019).

Acidic decomposition of fish and samples of sediment

Each fish sample, weighing 2.0 g, was dried, crushed, and then deposited into a 50 mL conical flask. Subsequently, 10 milliliters of concentrated HNO_3 (70%) and 2 milliliters of H_2O_2 were introduced. The flask underwent gradual heating, first at 50°C and gradually reaching 120°C within 30 minutes, utilizing a hot plate. Afterward, the process was carried out by repeatedly introducing HNO_3 and H_2O_2, with each subsequent addition raising the temperature by 12°C. The process of digestion was interrupted when a clear solution appeared. Once the samples were completely digested, the resulting solution was moved to an open container for cooling. The sample was thereafter strained through Whatman Filter Paper 42 into sterile plastic bottles with airtight stoppers, each with a volume of 50 mL. The solution's volume was augmented to 7 mL by adding 2 mL of saturated HNO_3, and a total of 25 mL of filtered water was introduced to the solution to achieve further dilution. All the specimens were accurately labeled and utilized for the examination of heavy metal concentrations. The entire process was repeated until all the specimens were disintegrated and made ready for examination using the atomic absorption spectrometer. The calibration value for every metal element was established by employing the standard solution and consistently examined to evaluate the instrument's efficacy (Zeitoun *et al.,* 2014).

Lipid extraction procedure

After the fish organs were dried and crushed, each one was subjected to individual treatment with 200 mL of acetone. The specimen has a mass of 6.0 grams. Lipid extraction was performed using acetone as the solvent in a continuous extractor for a maximum period of 12 hours. The acetone was distilled utilizing a rotary evaporator until only 10-15 mL of acetone was left in the flask. Afterward, the leftover acetone was transferred to the beaker. Ultimately, to completely remove any remnants of oils, the flask underwent a meticulous cleansing process using recently acquired acetone. The beaker was heated to speed up the evaporation of water and fatty acetone while ensuring a constant temperature through the use of a water bath. The beaker was subjected to a temperature of 80°C in the oven for 1 hour to guarantee thorough evaporation. Afterward, it was transferred to a cooler environment and measured using desiccators. According to the official analytical procedure 948 (Arisekar *et al.,* 2020 and Kumari *et al.,* 2021) of the AOAC, the total weight of the lipids is equivalent to the aggregated weight of the recovered lipids.

RESULTS

This study aimed to assess the levels of heavy metals levels in several anatomical locations of *Siluriformes* and *Channa punctata*.

Figures 1 and 2 present the quantities of the heavy metals Pb, Cr, Cd, Ni, Zn, Cu, and Fe in six distinct anatomical regions of the fish species *Siluriformes* and *Channa punctata*. The element Pb had the highest concentration (22.49 mg kg^{-1}) out of all the heavy metals identified in the scales of *Siluriformes*. Measurements were taken to determine the metal concentrations in various regions of the *Siluriformes*. Pb exhibited the highest concentration, with values of 15.60, 17.15, 20.20, 14.32, 20.10, and 22.50 mg/kg in the skull, the gills stomach, tail, fins, and scale, respectively. In contrast, Cd exhibited the lowest concentration, ranging from 0.016 to 0.112 mg/kg in the corresponding sections. The tail of *Channa punctata* exhibited the highest concentration of the heavy metal Pb (32.9 mg/kg) among its body parts. The skull, gills stomach, tail, fins, and scale of *Channa puncta* contained several heavy metals. The concentration of Pb reached its highest levels at 21.2, 30.0, 22.24, 32.2, 25.6, and 30.8 mg kg^{-1}, respectively. In contrast, Cd exhibited the lowest concentration at 0.176, 0.144, 0.016, 0.08, 0.096, and 0.144 mg kg^{-1}, respectively.

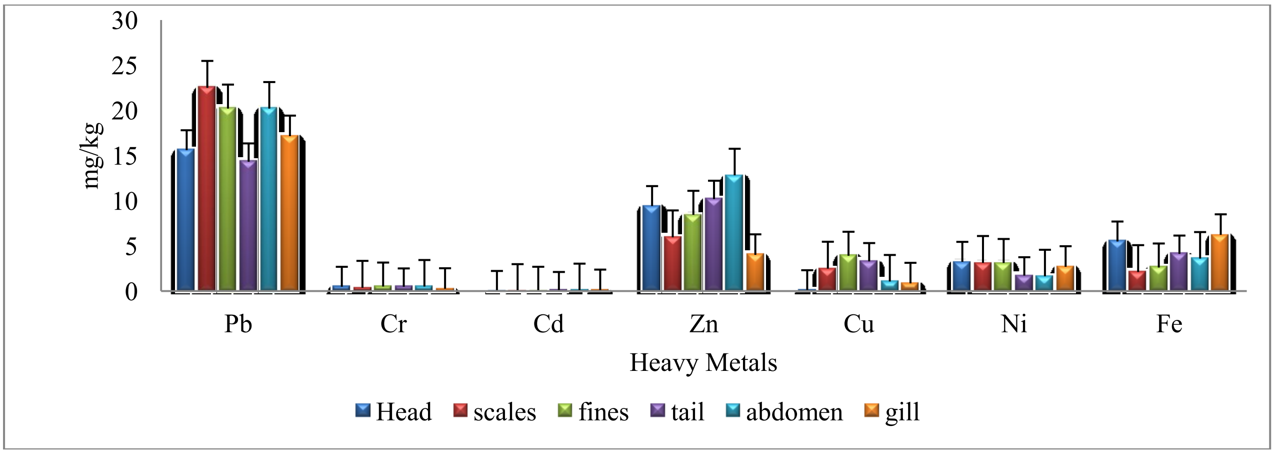

Figure 1: Quantification of heavy metal levels in various organs of Siluriformes

Figure 3 presents the level of several heavy metals detected in samples of water obtained from the Daliganj, Saheed smarak, and Hanumansetu regions along the Gomati river bank in Lucknow. The effluent water sample displayed the most elevated levels of Pb compared to other heavy metals, measuring 19.802 mgL^{-1}. The recorded Pb levels in the Daliganj, Saheedsmarak, and Hanumansetu portions were 15.40±0.05, 18.10±0.08, and 19.802±0.03 mg/L, correspondingly. Cadmium (Cd) had the most minimal quantity compared to other metals, with levels of 0.144±0.05, 0.16±0.01, and 0.128±0.09 mg/L at the Daliganj, Saheedsmarak, and Hanumansetu sections, respectively. The sediment sample retrieved from the river is represented in Figure 3, which displays the levels of heavy metals detected. The element Pb had the greatest concentration, measuring 12.5 mg kg^{-1}, while Cd demonstrated the lowest amount, measuring 0.16 mg/kg.

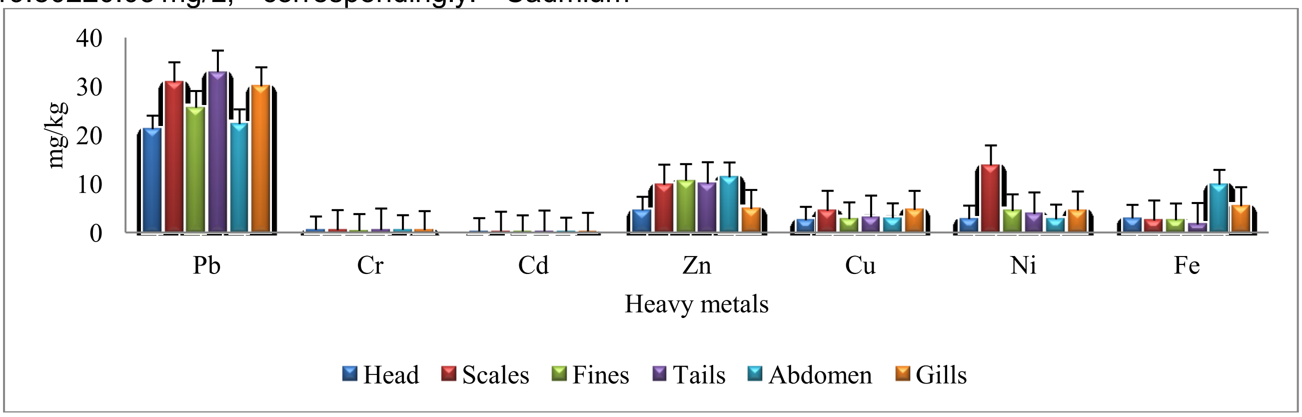

Figure 2: Quantification of heavy metal levels in various organs of Channa *punctata.*

Figure 4 displays a comparative analysis of the heavy metal concentrations for both species. The abdominal region of *Channa punctata* exhibited the highest concentration of iron across all organs in both species. The amount of iron (Fe) in the anatomical features of a fish including the skull, tails, fins, and gill of *Siluriformes* was higher compared to that of *Channa punctata*. Conversely, the iron levels in the scales and belly of *Channa punctata* were more than that in *Siluriformes*. The scales of *Channa punctata* had the most elevated content of nickel (Ni). *Channa punctata* exhibited elevated levels of nickel present in the tail, the gills the fins, along with scales when compared to *Siluriformes*. The nickel concentration in the skull of *Siluriformes* exceeded that in *Channa punctata*. Elevated concentrations of copper (Cu) were seen in the scales, the head, stomach, and gill of *Channa punctata* in comparison to *Siluriformes*. However, the appendages located at the back of the body, specifically the fins as well as the tail, of the*Siluriformes* exhibited a much higher copper concentration in comparison to that of the *Channa punctata*. *Siluriformes* possess a higher concentration of zinc in their cranial, caudal, and abdominal regions in comparison to *Channa punctata*. However, the *Channa punctata* species displays elevated concentrations of zinc in its scales, gills, and fins in comparison to the *Siluriformes*.

Upon analyzing the cadmium content, it was observed that *Channa punctata* had higher amounts in the head, scales, gills, and fins in comparison to *Siluriformes*. In contrast, the tail and abdomen of *Siluriformes* exhibited elevated cadmium levels compared to *Channa punctata*. Chromium concentrations in the head, abdomen, and gills of *Channa punctata* were found to be elevated in comparison to *Siluriformes*. In contrast, the concentrations of chromium in the fins and tail of *Channa punctata* were comparatively lower than those found in *Siluriformes.* Nevertheless, both species displayed comparable quantities of chromium in their scales. The concentration of lead was higher in all six organs of Siluriformes when compared to *Channa punctata. Channa punctata* had a higher quantity of heavy metals, specifically lead, in comparison to *Siluriformes*. Figure 5 displays a comparative analysis of the lipid proportions in both species. The *Siluriformes* species had the largest lipid proportion, measuring 39.34 percent, while the *Channa punctata* species had the smallest lipid proportion, detecting 8.56 percent. The documented lipid content in all six body segments of both species is as follows: scales< fins<tail<abdomen<gills< head. The comprehensive research indicated that the total amount the lipid content in each of the six tissues was higher in *Siluriformes* compared to Channa *punctata*.

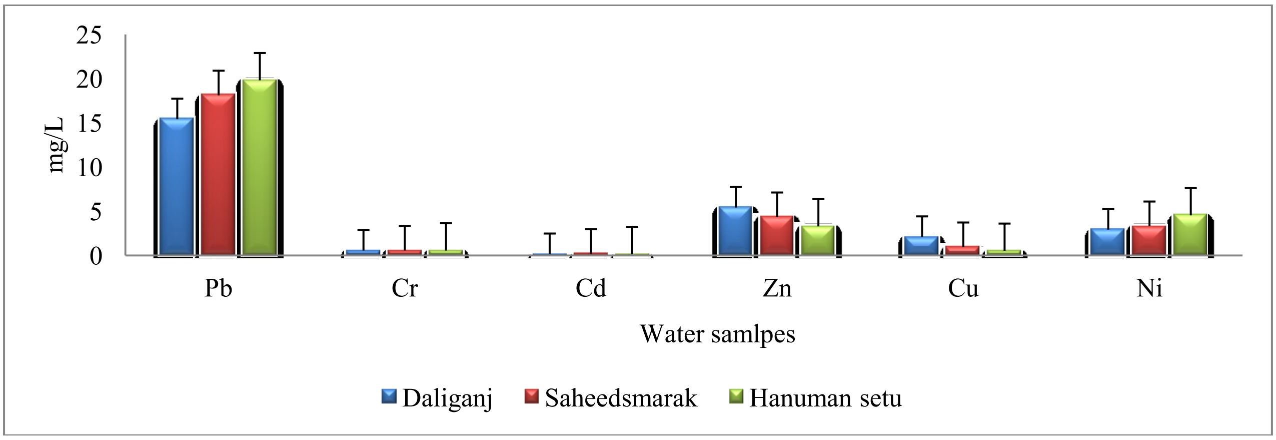

Figure 3: Water samples were taken from three separate locations along the Gomati River to measure the levels of heavy metals

DISCUSSION

This investigation focused on two types of fish, specifically *Siluriformes* and *Channa punctata*, which were found in the Gomati River in Lucknow. Scientific research suggests that consuming an excessive number of fish can be dangerous because of the accumulation of toxic metallic elements. Therefore, it was imperative to evaluate the existence of toxic metals in the fish species *Siluriformes* and *Channa punctata.* The concentrations of metallic elements and the amount of lipids in both of these species were examined and displayed in Figure 1-5. The figure presents the concentrations of several heavy metals detected in different anatomical regions of S*iluriformes* and *Channa punctata* fish species collected from the Gomati River in Lucknow. The element Lead (Pb) exhibits the most elevated level among *Siluriformes* scales, specifically measuring 22.5 mg/kg. On the other hand, cadmium (Cd) has the smallest amount, measuring 0.016 mg/kg, among all the heavy metals that are present. The *Siluriformes* had the highest levels of Pb concentration in several body parts, specifically the head (15.60 $mgkg^{-1}$), gills (17.15 $mgkg^{-1}$), belly (20.20 $mgkg^{-1}$), tails (14.32 $mgkg^{-1}$), fins (20.10 $mgkg^{-1}$), and scales (22.50 $mgkg^{-1}$). Conversely, Cd exhibited the lowest level (0.032, 0.112, 0.112, 0.096, 0.064, and 0.016 $mgkg^{-1}$) in the corresponding anatomical regions. The levels of lead, nickel, and iron, as specified by the WHO, were above the permissible limits, whereas the quantities of Cr, Cu, Zn, and Cd were within the acceptable range (Kumari *et al.,*2019; Kumar *et al.,* 2020; Siraj *et al.,* 2014 and Ahmed *et al.,* 2019). Figure 1 presents the amounts of heavy metal content in different anatomical regions of the *Channa punctat.* Among all the elements examined, the tail of *Channa punctata* contained the highest concentration of lead (Pb) at 32.9$mgkg^{-1}$, while the stomach had the lowest level of cadmium (Cd) at 0.016 $mgkg^{-1}$.

An examination of heavy metal concentrations in several body sections of *Channa punctata* demonstrated that the scales, fins, tails, abdomen, and head exhibited elevated levels of lead, with respective values of 30.8, 25.6, 32.2, 22.24, 30.0, and 21.2 mg kg^{-1}. In contrast, these components exhibited the most minimal levels of Cd, at 0.176, 0.144, 0.016, 0.08, 0.096, and 0.144 mg/kg, respectively. The concentrations of lead in different anatomical areas of *Channa punctata* ranged from 21.2 to 32.9 mg/kg. The levels of lead (Pb), iron (Fe), and nickel (Ni) were above the permissible thresholds, but Cd, Zn, and Cr stayed within the permissible limits. Conversely, Cu remained within the allowable limits threshold as per the WHO criterion. The current investigation unveiled distinct concentrations of metals in the interior tissues of both animals. Kalay *et al.,* 1999, noted that various piscine species display diverse degrees of metal concentration within their bodily tissues. Moreover, Canli *et al.,* 2003, have recorded that the levels of heavy metal content in fish display fluctuations depending on the species and the specific aquatic habitat they reside. Kamaruzzaman *et al.,* 2010, noted a significant increase in the concentrations of Pb and Cd in tissues of *Cyprinus carpio* for all heavy metals. Figure 2 presents the levels of heavy metals in three water samples (Daliganj, Saheedsmarak, and Hanumansetu) obtained from the Gomati River Lucknow. The exit water sample exhibits the most elevated level of lead (Pb) content compared to all other metals, particularly measuring 19.802 mg/kg.

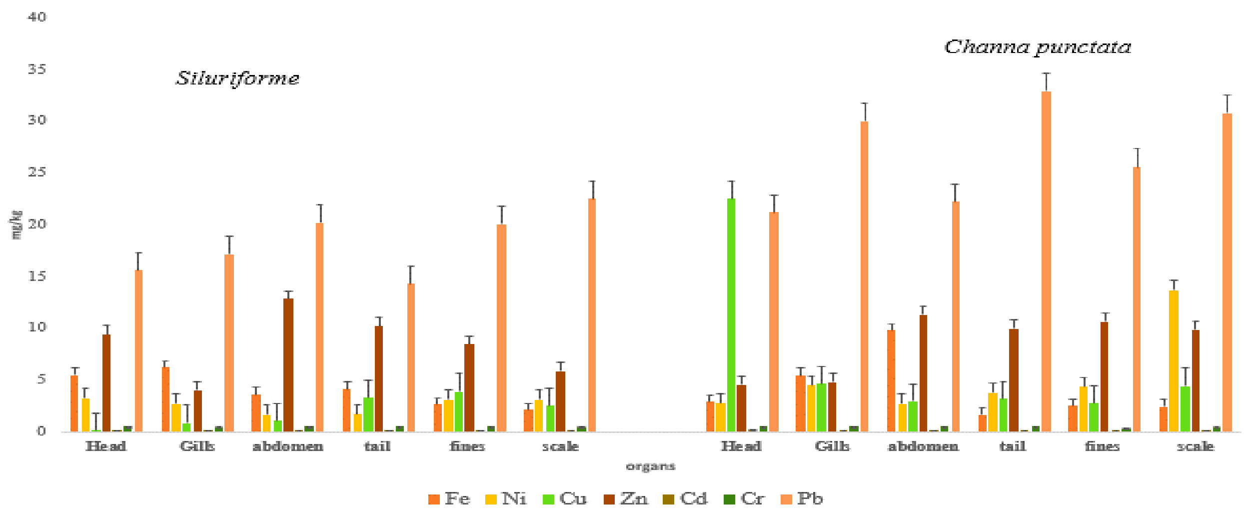

Figure 4: A comparative analysis of the heavy metal concentrations for both species

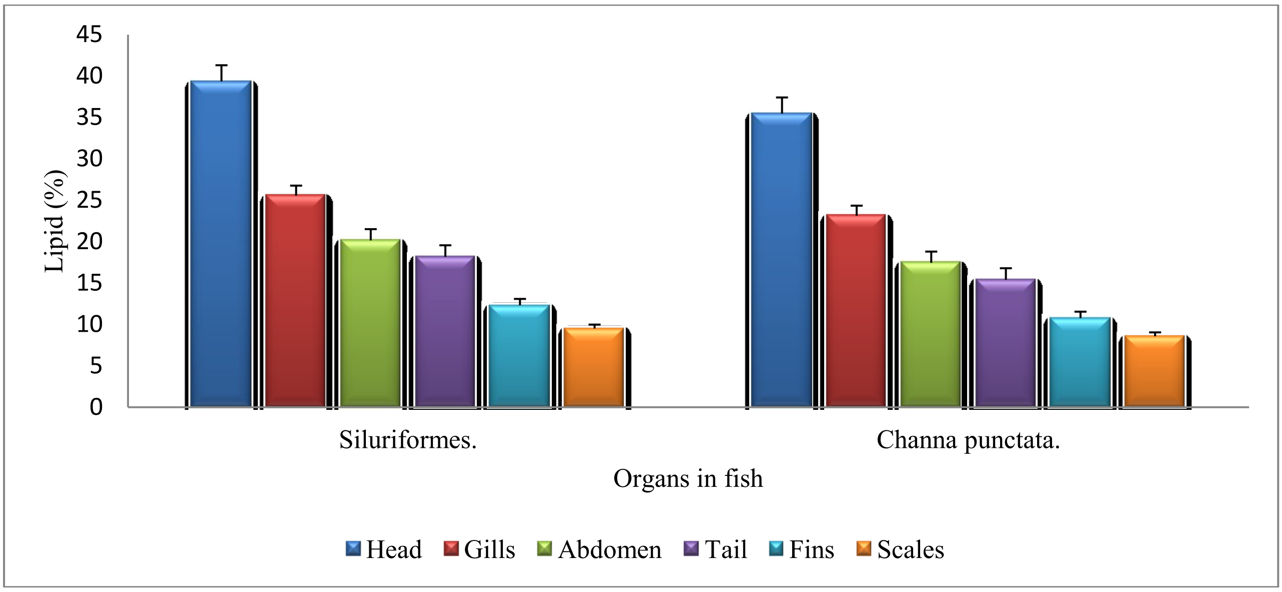

Figure 5: Comparative analysis of lipid % in different body parts of fish

The recorded levels of metallic elements with high atomic weights in the primary Hanumansetu, Saheedsmarak, and Daliganj, and regions were as stated: The element Pb had the highest quantity, measuring 19.802±0.10, 18.10±0.14, and 15.40±0.18 mg/L, correspondingly. In contrast, Cd exhibited the most minimal quantity compared to the other metals, with measurements of 0.128±0.05, 0.16±0.17, and0.144±0.14mg/L, respectively. The metals in the Hanumansetu, water region were found to have the following concentration sequence: Cd < Cr < Cu < Ni < Fe< Zn < Pb. The concentration order in the Saheedsmarak water zone was as follows: Cd < Cr < Cu< Fe < Ni < Zn < Pb. The Daliganj, water zone was found to have heavy metal concentrations in the following order: Cd< Cu < Cr< Fe < Zn < Ni < Pb. All metals were present in the water above the standard threshold established by the World Health Organization, except for Cu.

Figure 3 displays the levels of levels of Fe, Ni, Cd, Zn, Cu Cr, and Pb found in the sediment sample taken from Gomati River Lucknow. Lead has the largest concentration among these elements, measuring 12.5 mg/kg, whilst Cd has the lowest level, measuring 0.16 mg/kg. Analyzed sediment samples revealed metal levels, ranked in decreasing order of intensity (mg/kg), which were as follows: Pb, Ni, Zn, Cu, Fe, Cr, and Cd. The levels of iron, cadmium, lead, and copper amounts surpassed the permissible barrier, whereas the Zn, Cr, and Ni levels persisted within the limit established by the World Health Organization. Establishing an association between the levels of heavy metals is a significant challenge, particularly when comparing the same organs among different species. These variations are determined by factors such as dietary habits, the fish's ecosystem being located in extremely deep locations feeding behavior, and age. Kamaruzzaman *et al.,* 2010, discovered the relationship between the concentration of metals and other important characteristics of fish, such as their size, age, and genetic composition, which is being examined for association. Figure 4 displays a level comparison of (Fe), (Ni), (Cu), (Zn), (Cd), (Cr), and (Pb) in the two species. The abdominal region of *Channa punctata* displayed the highest iron concentration compared to all other organs, at precisely 9.82 mg/kg. The *Siluriformes* exhibited a higher concentration than Channa punctata in the head, neck, tail, and wings. The *Channa punctata* species exhibited higher levels of abdomen and scale Fe content compared to the *Siluriformes* species. The scales of *Channa punctata* showed the highest concentration among all the organs, with a precise measurement of 13.74 mg/kg. The *Channa punctata* had higher nickel levels in its fins, tail, scales, and gills in comparison to the *Siluriformes*. The nickel concentration in the skull of *Siluriformes* exceeded that in *Channa punctata*. *Channa punctata* had elevated copper levels in the head, abdomen, gills, and scales in comparison to *Siluriformes*. In contrast, *Siluriformes* exhibited greater copper concentrations in their fins and tails compared to *Channa punctata*. The zinc concentration in the head, abdomen, and tail of *Siluriformes* was greater in comparison to *Channa punctata*. *Channa punctata* had a greater zinc concentration in the gills, scales, and fins compared to *Siluriformes.*

The *Channa punctata* had higher concentrations of cadmium (Cd) in the skull, scales, fins, and gills in comparison to *Siluriformes*. In contrast, the tail and abdomen of *Siluriformes* exhibited greater levels of cadmium compared to *Channa punctata. Channa punctata* exhibited higher concentrations of Cr in the head, abdomen, and gills compared to *Siluriformes*. Nevertheless, the fins and tail of *Siluriformes* exhibited a greater chromium concentration in comparison to *Channa punctata*. Both species possess scales with the same concentration of chromium. *The Channa punctata* showed higher levels of lead in all 6 organs compared to the *Siluriformes*. *Channa punctata* had a higher overall concentration of heavy metals, specifically lead, in comparison to *Siluriformes*. The occurrence can be ascribed to the inflow of water from the catchment area of District Lucknow, lead he inundation of the Gomati River with excessive amounts of water. The heightened level of Pb is ascribed to the erosion caused by unidentified mountains and valleys.

CONCLUSIONS

Studies have shown that the accumulation of heavy metals causes a decrease in the lipid composition of fish. The heavy metal concentration in *Channa punctata* exceeded that in *Siluriformes,* despite *Channa punctata* possessing a lower total lipid % in comparison to *Siluriformes.* Both fish species exhibited lead, iron, and nickel concentrations that were beyond the permissible range set by WHO. In contrast, chromium (Cr), zinc (Zn), cadmium (Cd), and copper (Cu) were found to be below the allowable limit. The metal concentrations exhibited variation among several anatomical locations of both fish species, as evidenced in figure 1 and 2. This fact demonstrates the magnitude of heavy metal accumulation in the tissues of many fish species.

ACKNOWLEDGMENT

The author would like to thank, the Department of Zoology, Maharishi University of Information Technology, Dubagga, Lucknow (Uttar Pradesh) India. For their guidance and support in completing this article.

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