# SEASONAL VARIATION OF HEAVY METAL BIOACCUMULATION IN ZOOPLANKTON AND FISH LIVER IN THE GIRI RIVERINE ECOSYSTEM

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**KEYWORDS**

Heavy metal concentrations; Giri River; Seasonal variation; Zooplankton; Fish liver; Bioaccumulation.

**ABSTRACT**

Water is the fundamental basis of the life support system, yet anthropogenic activities have significantly degraded water quality, adversely affecting aquatic life and depleting certain zooplankton and fish species. This research analyzed seasonal variations in heavy metal concentrations [chromium (Cr), nickel (Ni), arsenic (As), cadmium (Cd), and lead (Pb)] and their bioaccumulation in zooplankton and the liver of *Tor putitora* fish across different sites in the Giri River ecosystem in Himachal Pradesh, India, using an Inductively Coupled Plasma Emission Spectrometer (ICAP-6000 Series). The selected sites, characterized by distinct topographical features, pollution sources, and exposure to anthropogenic activities, were sampled during both the pre-monsoon and post-monsoon seasons to assess seasonal fluctuations in heavy metal concentrations and their bioaccumulation in aquatic organisms. The findings revealed significant site-specific and seasonal variations in metal concentrations, with downstream sites such as Bangran Giri Bridge generally showing higher pollutant levels compared to upstream locations like Giripul Bridge. These differences are attributed to anthropogenic factors such as wastewater discharge, vehicular emissions, mining activities, agricultural runoff, and urban pollution, which increase metal bioavailability in the river system. Seasonal trends indicated elevated metal concentrations during the pre-monsoon season, likely due to lower water flow, increased evaporation, sediment resuspension, and limited rainfall, leading to greater bioaccumulation. Post-monsoon concentrations were reduced, likely due to rainfall-induced dilution, pollutant removal, and sedimentation. Although concentrations remained within FAO's permissible limits, indicating minimal immediate toxicological risk, the findings emphasize the need for continuous monitoring to track long-term trends and mitigate potential ecological impacts. This study underscores the influence of human activities and natural cycles on heavy metal bioaccumulation, highlighting the importance of targeted strategies to manage contamination and protect the Giri River ecosystem.

## 1. Introduction

Water is the most precious gift of nature and a fundamental ingredient for life's existence and continuation. Its vital role in human settlements has determined the growth of civilizations and cultural development. Water covers around 80% of the Earth's surface, with more than 95% of this being oceans, and the remainder in lakes, rivers, and subsoil reserves (Saha and Paul, 2019; Mohanta *et al*., 2019). Freshwater ecosystems, though occupying a small portion of the Earth's surface compared to marine and terrestrial environments, are crucial for humanity as they provide the most accessible and affordable water for domestic and agricultural use. In these ecosystems, zooplankton and fish are essential components of aquatic food webs, contributing significantly to ecological balance and biogeochemical processes. Rivers and lakes are primary sources for drinking, irrigation, and other needs (Kumari *et al*., 2018; Ali and Khan, 2018; Thorpe, 2024). Despite water covering about 80% of the Earth's surface, inland freshwater availability accounts for less than 1%. The availability of clean water is a critical crisis of the 21st century, as drinking water is vital for survival and development (Kumari *et al*., 2021).

An aquatic environment is a water-based ecosystem where species interact within water bodies, classified as static or flowing and divided into freshwater, brackish, and marine ecosystems based on salinity. These include oceans, rivers, estuaries, lakes, streams, and wetlands. Water, sediment, plankton, and fish (which are at the top of the food chain and are mostly consumed as human food) are all intertwined in an aquatic ecosystem's healthy functioning (Bhutiani *et al*., 2016; Pandiyan *et al*., 2021; Rahman *et al*., 2021). Water is necessary for life, supporting biochemical reactions and the circulation of body fluids in both plants and animals. It is essential for reproduction and various cyclic processes that sustain life (Matta *et al*., 2018). Additionally, water plays a crucial role in global ecosystem processes, connecting the atmosphere, lithosphere, and biosphere by transporting chemicals and facilitating chemical reactions. However, the addition of contaminants, including metals, alters water quality and negatively affects water resources (Skipin *et al*., 2016; Bhardwaj *et al*., 2020).

Water pollution occurs when industrial effluents, fertilizers, pesticides, detergents, and heavy metals contaminate water bodies, introducing hazardous compounds into aquatic systems (Achary *et al*., 2017). Rapid industrialization, urbanization, and the green revolution have intensified water contamination (Rahman *et al*., 2014). Domestic and industrial wastes are often discharged untreated into freshwater sources due to inadequate attention to wastewater management (Mohiuddin *et al*., 2011; Yuan *et al*., 2021). These pollutants alter the ecology, chemistry, and biological quality of water, directly impacting its use by humans and aquatic life (Saravi and Shokrzadeh, 2013). Heavy metals enter aquatic systems through natural processes like atmospheric deposition, water-rock interactions, and water-soil interactions, and anthropogenic activities such as industrial and urban discharges, as well as irrigation runoff containing pesticides and fertilizers (Wang *et al*., 2015; Ekere *et al*., 2018). These metals, found in various wastes, pose a significant threat to ecosystems, raising global concerns over their toxic impacts (Mdegela *et al*., 2009). The term "heavy metals" refers to metals and metalloids with an atomic number greater than 20 and an atomic density greater than 5 g $cm^{-3}$, being toxic or poisonous at low concentrations, including elements such as Pb, Zn, Cd, Hg, Ag, Cu, Fe, As, Cr, Ni, and platinum group elements (Zhang *et al*., 2013; Ali and Khan, 2018; Ali and Khan, 2019). Trace metal contamination in aquatic ecosystems has increased due to runoff from fly ash dumps and industrial waste, with anthropogenic sources now surpassing natural ones (Gupta *et al*., 2017; Nyamete *et al*., 2020). Toxic metals like mercury, cadmium, and lead accumulate in aquatic species, including fish, crustaceans, and plankton, through sediments, soil erosion, air deposition, and wastewater, posing serious ecological and health risks as they bio-magnify through the food chain (Rahman *et al*., 2014; Zafarzadeh *et al*., 2018; Sibal and Espino, 2018; Rigo *et al*., 2020; Ngoc *et al*., 2020). While trace metals like Fe, Cu, Zn, and Mg are essential in small amounts, others, such as As, Pb, Cd, and methylated mercury, are toxic even in trace concentrations, making them persistent pollutants with long-term impacts (Rai, 2009; Gupta *et al*., 2009; Tabari *et al*., 2010; Malik and Maurya, 2014; Islam *et al*., 2015).

Zooplankton play a crucial role in the biogeochemical cycling of metals in aquatic systems, especially when particle-reactive metals are present in the water column (Rejomon *et al*., 2008). Due to their widespread presence, significant role in food webs, and considerable biomass, macro- and mesozooplankton are commonly used as biomonitors to assess the bioavailability of elements across spatial and temporal scales (Achary *et al*., 2020; Basu *et al*., 2021). Plankton derivatives, including phytoplankton and zooplankton, interact with dissolved and particulate trace metals through processes like adsorption, desorption, biological uptake, and microbial decomposition (Annabi-Trabelsi *et al*., 2021). As primary consumers, zooplankton are vital intermediates between producers (phytoplankton) and consumers in food chains, and their widespread distribution, rapid turnover, large biomass, and high metal accumulation capacity make them effective biomonitors for trace metals in aquatic environments, while also serving as an important food source for higher organisms, including fish (Zauke and Schmalenbach, 2006; Srichandan *et al*., 2016; Dobaradaran *et al*., 2018; Ju *et al*., 2019). In the entire food web, fish perform a crucial ecological role and are an important resource providing food, recreation, and economic value to our environment (Maurya and Malik, 2019). Fish cannot survive in isolation since they are part of an ecosystem and interact with their aquatic system's physical, chemical, and biochemical environment. They are completely reliant on the ecosystem for development, reproduction, and survival. Changes in fish community structure, such as species abundance and variety, can reflect the effects of diverse pressures on the biotic integrity of the water body as a whole (Derrag and Youcef, 2014; Pilehvarian *et al*., 2015). Fish are important biomonitors for estimating metal pollution levels in aquatic systems. Fish occupy a higher trophic level in an aquatic ecosystem. Metal toxicity significantly affects fish's physical and physiological behavior, and they are an important part of human nutrition worldwide (Töre *et al*., 2021).

Fish can absorb and concentrate heavy metals directly from the surrounding water or indirectly through other organisms like small fish, zooplankton, phytoplankton, and aquatic vegetation, leading to bioaccumulation in their tissues and organs over time, often resulting in excessive buildup compared to organisms not exposed to pollutants (Vinodhini and Narayanan, 2008; Maurya and Malik, 2019; Mutlu, 2021). This accumulation varies with factors such as the concentration and duration of exposure, water characteristics like pH, temperature, salinity, and hardness, and fish metabolism. Heavy metals, once absorbed, particularly in muscle and liver tissues, can be transferred through food links, resulting in biomagnification across the food chain (El-Moselhy *et al*., 2014; Rajeshkumar and Li, 2018). As top predators in aquatic ecosystems, fish can concentrate on significant amounts of heavy metals, posing a risk to human health when consumed, given their role as a critical dietary component (Giri and Singh, 2014; Dizman *et al*., 2017).

The study aimed to evaluate heavy metal concentrations in zooplankton and their bioaccumulation in fish liver, emphasizing the impact of pollution on the aquatic ecosystem of the Giri River Basin. The Giri River, a vital tributary of the Yamuna River in Himachal Pradesh, India, sustains diverse aquatic life and supports local communities but is increasingly threatened by anthropogenic activities such as agricultural runoff, mining, vehicular emissions, industrial discharge, and urbanization. However, there is limited research on the seasonal variations in heavy metal concentrations and their bioaccumulation in zooplankton and fish liver in the Giri River. Heavy metals pose a significant concern due to their toxic, persistent, and bioaccumulative properties, and once released into aquatic environments, they can adversely impact the health of aquatic organisms. The present study seeks to address these gaps by investigating the seasonal variations in heavy metal concentrations and their bioaccumulation in zooplankton and fish liver at different sites of the Giri River Basin. The liver, as the primary detoxifying organ, was selected for the study because its accumulation levels reflect environmental pollution and provide insights into pollutant transfer across trophic levels and their impact on higher organisms, including humans. This knowledge is crucial for assessing potential ecological impacts and guiding conservation efforts. Based on the research findings, the study will provide recommendations for regulatory measures and management strategies to reduce heavy.

## 2. Materials and methods

### 2.1 Study area

Himachal Pradesh, located in northern India within the western Himalayas, is renowned for its mountainous terrain, high-altitude peaks, and extensive river systems. Spanning latitudes 30°22'40" to 33°12'40" North and longitudes 75°45'55" to 79°04'20" East, the state ranges from 350 to 6,975 meters above sea level. Known as Dev Bhoomi (Land of Gods) and Veer Bhoomi (Land of the Brave), it borders Jammu and Kashmir, Ladakh, Punjab, Haryana, Uttarakhand, and Tibet. The study focuses on the Giri River, the largest in the southern Sirmaur district, covering 2,825 sq km with hilly terrains and valleys like Paonta Sahib. Sirmaur, bordered by Shimla and Solan, is traversed by rivers such as the Giri, Yamuna, Bata, Tons, Ghagghar, and Markanda, crucial for irrigation and as Yamuna tributaries. The Giri River, also called "Giri Ganga," originates from the Jubbal hills, flows through Kot-Khai, Tatesh, and Shimla before entering Sirmaur, dividing it into Cis-Giri and Trans-Giri regions, and joins the Yamuna near Paonta Sahib. The catchment area, rich in fir, deodar, blue pine, and oak, attracts tourists in summer. The Giri River supports fishing, timber floating, and irrigation. Major tributaries include Ashani, Jalal, and streams like Nait, Palar, Bajhethy, Pervi, Khal, and Joggar. Sirmaur's climate ranges from sub-tropical to temperate, influenced by elevation, with four seasons: summer (March-June), monsoon season (July-September), autumn (October-November), and winter (December-February). Most annual rainfall, averaging 1405 mm, occurs during the monsoon. Higher elevations above 1500 m receive winter snowfall, with Choordhar peaks snow-covered. Lower hills and valleys experience occasional winter rainfall. Temperatures range from a mean maximum of 30°C to a minimum of 0°C.

### 2.2 Experimental details

A preliminary survey along the Giri River was conducted to select study sites and sampling locations, as the river's water is contaminated by domestic sewage, agricultural runoff, urbanization, and industrial activities. Six sites were selected between Giripul Bridge and Shivpur (near Govt. School) Paonta Sahib in the Sirmaur district, each offering unique topographical characteristics and varying pollution sources. The sites are: Site 1: Giripul Bridge (30.881793º N, 77.210479º E), Site 2: Khairi Bridge (30.774508º N, 77.297364º E), Site 3: Dadahu (30.60113º N, 77.439024º E), Site 4: Ambaun (30.568825º N, 77.549915º E), Site 5: Sataun (30.558016º N, 77.63201º E), and Site 6: Bangran Giri Bridge (30.497487º N, 77.678717º E) for year 2022-23 and 2023-24. To assess heavy metal bioaccumulation in zooplankton and fish liver, a 150 km stretch from Giripul to Paonta Sahib was selected. The six sites (S1-S6) were divided into three equal stretches of 8 km, serving as replications. Samples were randomly collected during the pre-monsoon and post-monsoon seasons. Zooplankton and fish liver samples were analyzed for heavy metals (Cr, Ni, As, Cd, and Pb) during both seasons.

**2.3 Assessment of zooplankton**

Zooplankton samples were collected by filtering 100 liters of water through a 50 µm plankton net (mouth diameter: 75 cm) at the sampling sites. A 100 ml filtrate was collected and stored in pre-cleaned high-density polyethylene (HDPE) plankton tubes. Three such filtrates were combined into a single composite sample, with three composite samples taken from each station. A subset of zooplankton samples for heavy metal analysis was transported to the laboratory in ice-cold conditions (~4°C) on the same day, as per the American Public Health Association (APHA, 2017). In the lab, zooplankton samples were rinsed thoroughly with distilled water to remove contaminants. Samples for metal estimation were dried in an oven at 60-70°C to a constant weight for 24 hours, then homogenized. One gram of the sample was digested with a mixture of perchloric and nitric acids until a clear solution was obtained, cooled to room temperature, and filtered using Whatman filter paper (No.1). The filtrate was diluted with HCl to a total volume of 50 ml. A blank correction was applied by subjecting the acid digestion mixture to the same sequence of treatments (APHA, 2017). Heavy metals were estimated using an Inductively Coupled Plasma Emission Spectrometer (iCAP-6000 Series, Model No. 6300) and expressed as mg/L.

**2.4 Assessment of fishes**

For the present study, the selection of fish species was based on specific criteria to ensure relevance and suitability. The criteria included the edible status of the species, its presence across all selected locations along the river, and its popularity among the local population of Himachal Pradesh. Considering these factors, the widely consumed and locally favored fish species, *Tor putitora* (Hamilton), commonly known as Mahseer chiniaru, was chosen for the study. Fish samples were procured from fishermen in the local market of each selected site in the morning. After measurements and identification, the fish were washed with deionized water and sealed in polyethylene bags. Collected fish samples were transported to the laboratory in an icebox on the same day and kept in a freezer until chemical analysis, as per APHA (2017).

**2.5 Heavy metals analysis (Cr, Ni, As, Cd, and Pb)**

The fish samples were weighed, and their total length was recorded in the laboratory. Later, distilled water was used to wash them. The samples were then dried with filter paper, and a stainless-steel knife was used to remove the skin of the fish to avoid false readings due to the use of a metallic knife. The liver was removed and kept in a crucible, which will be prewashed with 10% nitric acid. Sample tissues were blended separately, and their weights were recorded. Homogenized tissue samples were oven-dried at $70^0$C for 48 hours until a constant weight was recorded (APHA, 2017). Later, these dry tissue samples were powdered using a porcelain mortar and pestle. The powdered tissue sample of 1 gm was taken in a conical flask of 250 ml. Then, a mixture was prepared by mixing 10 ml of concentrated nitric acid (AR grade) into it. The mixture was boiled to remove the oxidizable matter for 45 minutes. After cooling the mixture, 5 ml of prechloric acid was added. The mixture was again heated until it boiled, releasing white fumes. 20 ml of double-distilled water was added to it, and further boiling was continued to release any gas. Lastly, the mixture was cooled and filtered using Whatman filter paper (No.1). The filtrate was packed into air-tight polyethylene labeled bottles for analysis. The above procedure was completed according to the official method recommended by the Association of Analytical Communities (AOAC) and the American Public Health Association (APHA, 2017). The heavy metals were estimated using an Inductively Coupled Plasma Emission Spectrometer (iCAP-6000 Series, Model No. 6300) duo of thermo make and expressed as mg $l^{-1}$.

### 2.6 Statistical analysis

The data obtained from the present investigation was analyzed using the methodology described by Gomez and Gomez (1984). The data collected for various parameters were statistically analyzed using ANOVA (Analysis of Variance) with a two-factor factorial Randomized Block Design for the assessment of zooplankton and fish in R-Studio (v4.3.0). The statistical significance was assessed using an 'F' test, and the differences in means across the treatments were examined using the least significant difference (LSD) method at a 5% significance level.

## 3. Results and discussion

### 3.1 Zooplankton

#### 3.1.1 Chromium (Cr)

The results of the study revealed that chromium (Cr) concentrations in zooplankton exhibited significant variations across sites and seasons (Table 1). Bangran Giri Bridge recorded the highest pooled mean concentration of Cr (0.0267 mg $l^{-1}$), followed closely by Khairi Bridge (0.0265 mg $l^{-1}$), Sataun (0.0264 mg $l^{-1}$), and Dadahu (0.0261 mg $l^{-1}$), which all have similar concentrations. The elevated concentration of Cr at Bangran Giri Bridge and other sites might result from wastewater discharge, mining activities, agricultural runoff, and industrial effluents, which are then incorporated into the zooplankton. However, Giripul Bridge consistently recorded the lowest Cr concentration, with a pooled mean of 0.0247 mg $l^{-1}$. The Cr concentration in zooplankton was generally higher in the pre-monsoon season at all sites, with a pooled mean of 0.0289 mg $l^{-1}$ compared with 0.0232 mg $l^{-1}$ in the post-monsoon season. These results align with the findings of Goswami *et al*. (2014), Bhuvaneswari & Serfoji (2016), Achary *et al.* (2017), and Achary *et al.* (2020), who reported the highest Cr concentration in zooplankton during the pre-monsoon season and the lowest Cr concentration in the post-monsoon months. Moreover, Singaram *et al.* (2023) reported that the Cr concentration in zooplankton averaged 2.3 ± 0.3 µg/g in the western Bay of Bengal during the pre-monsoon season. The elevated Cr concentrations during the pre-monsoon season can be attributed to increased rates of evaporation, sediment resuspension, and decreased water flow, which concentrate the dissolved metals and lead to greater bioaccumulation in zooplankton. In contrast, the post-monsoon season consistently had lower Cr concentrations, which might be the result of dilution from monsoon rainfall and runoff, contaminant removal, and the sedimentation of chromium, leading to reduced bioaccumulation. The interaction effects of site and season were also significant, with the highest Cr concentrations recorded at Bangran Giri Bridge, Sataun, and Khairi Bridge during the pre-monsoon season (0.0295 mg $l^{-1}$) (Figure 1). The lowest concentration was observed at Giripul Bridge in the post-monsoon season (0.0218 mg $l^{-1}$). These site-specific and seasonal variations were evident in the Cr concentrations in zooplankton, highlighting the influence of anthropogenic activities and hydrological patterns on metal bioaccumulation.

#### 3.1.2 Nickel (Ni)

The current research revealed that nickel (Ni) concentrations in zooplankton significantly varied across different sites (Table 1). Among the sites, Giripul Bridge consistently recorded the lowest Ni concentrations in zooplankton, with a pooled mean of 0.0162 mg $l^{-1}$, whereas the highest concentrations were observed at Sataun and Bangran Giri Bridge, both with pooled means of 0.0226 mg $l^{-1}$. The increase in the Ni concentration from upstream to downstream could result from cumulative anthropogenic inputs such as agricultural runoff, industrial discharges, and soil leaching, along with natural processes that increase the Ni concentration in zooplankton as the water flows further from the origin. A pronounced seasonal trend was evident. Ni concentrations in zooplankton were consistently higher during the pre-monsoon season than during the post-monsoon season across both years and in the pooled data (Figure 1). The pooled seasonal mean Ni concentration was 0.0180 mg $l^{-1}$ in the post-monsoon season and 0.0242 mg $l^{-1}$ in the pre-monsoon season. These results agree with the findings of Kontas (2008), Goswami *et al*. (2014), Achary *et al.* (2017), and Achary *et al.* (2020), who reported similar trends in the Ni concentration under the influence of seasons. Additionally, Singaram *et al*. (2023) reported an average Ni concentration of 58.7 ± 5.5 µg/g in zooplankton from the western Bay of Bengal during the pre-monsoon season. The higher concentrations during the pre-monsoon season may be due to elevated temperatures and intense sunlight, which increase evaporation, reduce water volume, concentrate contaminants, and lead to their bioaccumulation in zooplankton. On the other hand, the lower Ni concentration in zooplankton during the post-monsoon months could be ascribed to the sedimentation of nickel-bound particles, increased water flow, and dilution from monsoon rainfall, which resulted in significantly lower Ni concentrations. These results suggest that both anthropogenic activities and natural seasonal dynamics significantly impact Ni bioaccumulation in zooplankton.

**3.1.3 Arsenic (As)**

The results of the present research indicated that the arsenic (As) concentrations in zooplankton varied both significantly and non-significantly by site from 2022-23 and 2023-24 (Table 1). The highest As concentrations in zooplankton were generally found at Khairi Bridge and Dadahu, with pooled mean concentrations of 0.0086 mg $l^{-1}$ and 0.0092 mg $l^{-1}$, respectively. These relatively high concentrations may be due to local pollution sources, such as agricultural runoff or other anthropogenic factors. Sites such as Ambaun and Giripul Bridge presented relatively low As concentrations in zooplankton, with pooled means of 0.0052 mg $l^{-1}$, indicating non-significant differences (Figure 1). However, significant seasonal variation was observed in As concentrations in zooplankton, with notably higher levels during the pre-monsoon season than during the post-monsoon season. The pooled mean concentration of As during the pre-monsoon season was 0.0097 mg $l^{-1}$, whereas in the post-monsoon season, it was 0.0044 mg $l^{-1}$. The higher As concentrations in the pre-monsoon season could be due to the reduced water volume, slower flow rates, sediment resuspension, and increased evaporation, which concentrate dissolved substances in the water and enhance their bioavailability for uptake by zooplankton. In contrast, during the post-monsoon period, As concentrations were significantly lower, which may be attributed to rainfall-induced dilution, removal of pollutants, and sedimentation, leading to reduced bioavailability. These seasonal variations underscore the impact of environmental dynamics on the bioaccumulation of As in aquatic organisms. These results contrast with the findings of Srichandan *et al.* (2016) and Battuello *et al.* (2016), who reported that the post-monsoon season had the maximum As concentration, whereas the pre-monsoon months had the lowest As concentration.

**3.1.4 Cadmium (Cd)**

The present investigation revealed a significant variation in cadmium (Cd) concentrations in zooplankton among the six sites, with Ambaun consistently showing the highest concentrations with a pooled mean of 0.0039 mg $l^{-1}$, followed by Dadahu (0.0034 mg $l^{-1}$), Khairi Bridge (0.0032 mg $l^{-1}$), and Sataun (0.0031 mg $l^{-1}$) (Table 1). On the other hand, Giripul Bridge and Bangran Giri Bridge had the lowest Cd concentrations in zooplankton, with pooled means of 0.0024 mg $l^{-1}$ and 0.0025 mg $l^{-1}$, respectively. The variation in Cd concentrations across sites may also reflect differences in environmental factors such as water flow, sediment types, and surrounding land use. This increase in Cd concentration may be due to cumulative inputs from agricultural runoff, mining activities, or other sources further along the river. The Cd concentration in zooplankton consistently peaked in the pre-monsoon season across all sites, with a pooled mean of 0.0042 mg $l^{-1}$, compared with 0.0019 mg $l^{-1}$ in the post-monsoon period (Figure 1). This seasonal pattern may be attributed to factors such as anthropogenic inputs, high temperatures, and increased evaporation during the pre-monsoon period, which reduce the water volume in surface water bodies, leading to a concentration of Cd in the water column and increased bioavailability for uptake by zooplankton. Conversely, the lower Cd concentration during the post-monsoon months could be ascribed to the deposition of cadmium-bound particles, increased water volume, and dilution from heavy rainfall, which decreased Cd bioavailability in the water, making it less accessible to zooplankton. The interaction between site and season also had a significant influence on the Cd concentration. Notably, the highest recorded Cd concentration was 0.0056 mg $l^{-1}$ at Ambaun during the pre-monsoon season, whereas the lowest was 0.0011 mg $l^{-1}$ at Giripul Bridge during the post-monsoon season. This finding highlights the combined effects of localized environmental factors and seasonal variations in Cd bioaccumulation in zooplankton. These results are in line with the findings of Rath & Panigrahy (2017), Dobaradaran *et al*. (2018), Achary *et al.* (2020), and Rath *et al.* (2021), who also reported similar trends in Cd concentrations influenced by seasonal variations. Similarly, Singaram *et al*. (2023) reported an average Cd concentration of 16.4 ± 0.9 µg/g in zooplankton from the western Bay of Bengal during the pre-monsoon season.

**3.1.5 Lead (Pb)**

The present study demonstrated significant variations in lead (Pb) concentrations in zooplankton across different sites in the Giri River Basin. Khairi Bridge consistently recorded the highest Pb concentrations, with a pooled mean of 0.0661 mg $l^{-1}$, whereas Sataun had a pooled mean of 0.0497 mg $l^{-1}$ (Table 1). These significantly higher concentrations at Khairi Bridge could be attributed to anthropogenic factors such as vehicular emissions and runoff from agricultural fields near this site. In contrast, Ambaun presented the lowest Pb concentrations, with a pooled mean of 0.0270 mg $l^{-1}$, indicating significantly lower levels of Pb pollution sources in the region (Figure 1). The Pb concentrations in zooplankton were significantly higher in the pre-monsoon season than in the post-monsoon season at all the sites. The pooled mean Pb concentration during the pre-monsoon season was 0.0547 mg $l^{-1}$, significantly exceeding the post-monsoon mean of 0.0296 mg $l^{-1}$. The present results are in confirmation with the findings of Malik *et al*. (2013), Goswami *et al*. (2014), Rath & Panigrahy (2017), Dobaradaran *et al*. (2018), and Rath *et al*. (2021), who also reported maximum Pb concentrations during the pre-monsoon season and minimum concentrations during the post-monsoon months. Similarly, Singaram *et al*. (2023) reported Pb concentrations in zooplankton averaging 25.6 ± 4.7 µg/g in the western Bay of Bengal during the pre-monsoon season. The significantly higher concentrations of Pb during the pre-monsoon months may have been due to reduced water flow and dilution, an increase in temperature, and a high rate of evaporation in the water sources, which might have concentrated the Pb in the aquatic environment, leading to greater bioaccumulation in zooplankton. In contrast, the lower Pb concentration in the post-monsoon months might be due to dilution from rainfall, sedimentation, and removal of contaminants, which reduced the bioavailability of Pb. These seasonal patterns reflect the dynamic relationship between environmental conditions and metal bioaccumulation in aquatic organisms.

### 3.2 Fish liver

#### 3.2.1 Chromium (Cr)

The results of the present study revealed a significant variation in the chromium (Cr) concentration in the fish liver, which increased noticeably as the river moved downstream (Table 2). The Giripul Bridge, located near the river's origin, had a pooled mean chromium concentration of 0.0276 mg $l^{-1}$, whereas the Bangran Giri Bridge, the site farthest downstream, had the highest pooled mean concentration of 0.0310 mg $l^{-1}$, which was normal and within the permissible limits suggested by the FAO. The elevated Cr concentration observed downstream can be attributed to various factors, such as agricultural runoff, mining activities, wastewater discharge, and urban or industrial runoff, which introduce Cr pollutants into surface water, increasing its bioavailability to aquatic organisms, including fish. Seasonally, the Cr concentrations in the fish liver were significantly greater during the pre-monsoon season compared to the post-monsoon season. The pooled mean Cr concentration in the pre-monsoon season was 0.0326 mg $l^{-1}$, whereas that in the post-monsoon season pooled mean Cr concentration was 0.0263 mg $l^{-1}$ (Figure 2). These results align with the findings of Batvari *et al*. (2008), Gummadavelli *et al*. (2013), Batvari *et al*. (2015), Bhuyan & Islam (2017), Tiwari *et al*. (2020), Maruf *et al*. (2021), and Inayat *et al*. (2024), who reported the highest Cr concentration in fish liver during the pre-monsoon season and the lowest Cr concentration in the post-monsoon months. The higher concentration of Cr during the pre-monsoon season could be attributed to several interrelated factors, including increased rates of evaporation, sediment resuspension, reduced dilution due to limited rainfall, and decreased river flow, which raised Cr bioavailability in the water and can subsequently bioaccumulate in fish. The minimum Cr concentration during the post-monsoon season may be due to rainfall-induced dilution, pollutant flushing, sedimentation, and improved water quality, which reduce chromium bioavailability. Moreover, the interaction between site and season was significant, with Bangran Giri Bridge recording the highest Cr concentration (0.0344 mg $l^{-1}$) during the pre-monsoon period, whereas the lowest Cr concentration (0.0254 mg $l^{-1}$) was observed at Giripul Bridge during the post-monsoon season. These observations reinforce the critical role of seasonal hydrological changes and site-specific factors in influencing heavy metal accumulation in aquatic organisms. Overall, Cr concentrations remained within the permissible limits set by FAO, suggesting that, despite elevated levels at certain sites and during specific seasons, they do not pose an immediate toxicological threat. However, the significant seasonal and spatial variations underscore the need for continuous monitoring to assess long-term trends and potential ecological impacts.

#### 3.2.2 Nickel (Ni)

The current research revealed that the concentration of nickel (Ni) in fish liver increased significantly as the river flowed downstream (Table 2). Among all the sites, Giripul Bridge, located upstream, consistently had the lowest Ni concentrations in the fish liver, with a pooled mean of 0.0195 mg $l^{-1}$ across both years. However, the Bangran Giri Bridge, located downstream, exhibited the highest Ni concentrations, with a pooled mean of 0.0260 mg $l^{-1}$, which was within the permissible guidelines recommended by the FAO. The elevated concentration of Ni at Bangran Giri Bridge and other sites can be attributed to natural and anthropogenic sources, such as industrial effluents, urban runoff, and agricultural practices, which contributed to higher Ni concentrations in aquatic systems during this period. This study revealed a discernible and significant seasonal variation in the Ni concentration in the fish liver. Across all the sites and years, pre-monsoon Ni concentrations were significantly higher than post-monsoon concentrations were, with pooled means of 0.0270 mg $l^{-1}$ for the pre-monsoon season and 0.0208 mg $l^{-1}$ for the post-monsoon season (Figure 2). The higher Ni concentrations in the pre-monsoon season may be due to stagnant or low-flow water, reduced dilution, increased water temperatures, and intensified anthropogenic inputs, which can concentrate Ni in the aquatic environment and lead to greater accumulation in the fish liver. The liver, as the primary organ for detoxification and heavy metal storage in fish, accumulates Ni during the pre-monsoon season because of increased exposure and its role in metabolizing and sequestering toxins. Conversely, the lower concentration in the post-monsoon months could be the result of heavy rainfall, strong water currents, sedimentation of nickel-bound particles, improved water quality, and reduced Ni exposure and bioaccumulation. These seasonal variations highlight the influence of environmental and human factors on Ni dynamics in aquatic ecosystems. These results are in agreement with the findings of George *et al.* (2012), Gummadavelli *et al*. (2013), Negi & Maurya (2015), Maruf *et al.* (2021), and George *et al.* (2022), who reported similar trends in the Ni concentration under the influence of seasons.

### 3.2.3 Arsenic (As)

The results of the present research revealed non-significant variations in arsenic (As) concentrations in fish liver across sites and years (Table 2). The Giripul Bridge had the lowest As concentration in the fish liver, with a pooled mean of 0.0061 mg $l^{-1}$, whereas the Khairi Bridge and Dadahu had the highest As concentrations among all the sites, with pooled means of 0.0104 mg $l^{-1}$ and 0.0100 mg $l^{-1}$, respectively. These levels remained within the acceptable limits prescribed by the FAO. The elevated concentrations of As near Dadahu and Khairi Bridge may result from anthropogenic or environmental factors, including runoff from agricultural areas or localized contamination sources, but they did not show significant variations across the study sites. Similarly, the lack of significant variation across sites during the years 2022-23 and 2023-24 suggested that As concentrations in fish liver remained relatively constant over time, implying consistent levels of As pollution in the river system. Furthermore, seasonal analysis demonstrated that As concentrations in fish liver were significantly higher in the pre-monsoon season, with a pooled mean of 0.0108 mg $l^{-1}$, compared to 0.0057 mg $l^{-1}$ in the post-monsoon season. The pre-monsoon season may amplify bioaccumulation due to significant evaporation, reduced water volume, increased pollutant concentration, and anthropogenic inputs, coupled with a potential increase in dietary As exposure in fish during this period (Figure 2). In contrast, lower As concentrations during the post-monsoon season could be attributed to dilution by rainfall and sedimentation processes, which might reduce contaminant levels in the water, along with their bioavailability and subsequent bioaccumulation in aquatic organisms, including fish. These results contrast with the findings of Iqbal *et al.* (2017), Kinare & Shingadia (2019), and Bhattacharya & Talapatra (2023), who reported that the post-monsoon season had the maximum As concentration, whereas the pre-monsoon season had the lowest As concentration.

### 3.2.4 Cadmium (Cd)

The findings of the present study revealed both significant and non-significant variations in cadmium (Cd) concentrations in fish liver across different sites and years in the Giri River Basin (Table 2). The non-significant variations across sites suggested that the Cd concentrations remained relatively consistent throughout the study area, with only slight differences noted at specific locations. The Giripul Bridge and Bangran Giri Bridge had the lowest Cd concentrations, with pooled means of 0.0032 mg $l^{-1}$ and 0.0034 mg $l^{-1}$, respectively. However, the highest Cd concentrations in the fish liver were observed at Ambaun with a pooled mean of 0.0045 mg $l^{-1}$, which was within the permissible limits as prescribed by the FAO. The relatively high Cd concentrations at Ambaun may be due to localized environmental, natural, or anthropogenic factors, such as sediment dynamics, mining activities, or runoff from agricultural areas. Moreover, the pre-monsoon season had significantly higher Cd concentrations (pooled mean 0.0050 mg $l^{-1}$) in the fish liver than the post-monsoon season (pooled mean 0.0026 mg $l^{-1}$) at all the sites (Figure 2). These results are in line with the findings of George *et al.* (2012), Gummadavelli *et al*. (2013), Batvari *et al*. (2015), Bhuyan & Islam (2017), Tiwari *et al.* (2020), Batvari & Saravanan (2020), George *et al.* (2022), Inayat *et al.* (2024), and Shashank & Velayudhannair (2024), who also reported a decrease in the Cd concentration during the post-monsoon months and an increase in the Cd concentration during the pre-monsoon months. The Cd concentrations were higher during the pre-monsoon period, possibly due to increased anthropogenic inputs, reduced water flow, sediment resuspension, and evaporation-induced concentrations, which can concentrate Cd and other contaminants in water and sediment, leading to greater bioaccumulation in the fish liver. In contrast, the minimum Cd levels observed during the post-monsoon season could be attributed to rainfall-induced dilution, pollutant flushing, sedimentation, and natural detoxification in fish. These seasonal dynamics explain the observed variation in Cd accumulation in the fish liver.

**3.2.5 Lead (Pb)**

The present study demonstrated that lead (Pb) concentrations in fish liver varied significantly by site (Table 2). Ambaun presented the lowest Pb concentrations in the fish liver, with a pooled mean of 0.0353 mg $l^{-1}$. Moreover, Khairi Bridge presented the highest Pb concentrations among all the sites, with a pooled mean of 0.0727 mg $l^{-1}$, which was within the FAO-recommended acceptable limits. The relatively high Pb concentrations at Khairi Bridge may be attributed to nearby wastewater discharge, vehicular emissions, runoff from agricultural fields, or other human activities that can increase Pb levels in aquatic ecosystems. The pooled seasonal means confirmed the seasonal effect on Pb concentrations, with significantly higher levels recorded in the pre-monsoon period compared to the post-monsoon season. The mean Pb concentration during the pre-monsoon season was 0.0609 mg $l^{-1}$, whereas it decreased to 0.0375 mg $l^{-1}$ during the post-monsoon season (Figure 2). The present results are in confirmation with the findings of George *et al.* (2012), Ahmed *et al.* (2014), Batvari & Saravanan (2020), Tiwari *et al.* (2020), Maruf *et al.* (2021), George *et al.* (2022), Chordiya & Chandanshive (2023), and Inayat *et al.* (2024), who also reported maximum Pb concentrations during the pre-monsoon season and minimum concentrations during the post-monsoon months. The elevated Pb concentration in the fish liver during the pre-monsoon season can be attributed to environmental factors such as higher temperatures, low water flow or stagnant conditions, and sediment resuspension, which may increase the concentration of Pb in the water and sediment, subsequently leading to greater bioaccumulation in the fish liver. Conversely, the lower Pb concentration in the fish liver during the post-monsoon season may be due to the deposition of lead-bound particles, detoxification processes in the fish, and monsoon-induced water flow, which effectively removes accumulated Pb and other contaminants from the surface water column. Overall, the findings highlight the significant influence of site-specific and seasonal factors on Pb concentrations in fish liver, emphasizing the need for targeted mitigation strategies to manage Pb contamination in the Giri River Basin.

## 4. Conclusion

The assessment of heavy metals in zooplankton and fish liver from the Giri River Basin provides valuable insights into bioaccumulation patterns and their ecological implications. Zooplankton reflected seasonal and spatial variations in heavy metal concentrations, with higher bioaccumulation observed during the pre-monsoon season due to reduced water volume and increased evaporation, which concentrates pollutants. This highlights their potential role as effective bioindicators for monitoring water quality. The detected heavy metal levels in zooplankton remained within permissible limits, suggesting minimal immediate ecological risk. Similarly, fish liver samples showed site- and season-specific variations in heavy metal accumulation, with levels being more pronounced during the pre-monsoon season. Despite these variations, heavy metal concentrations in fish liver remained within FAO-recommended limits, ensuring no immediate threat to aquatic life and the ecosystem. Overall, while the bioaccumulation of heavy metals in aquatic organisms indicates potential risks linked to anthropogenic activities and seasonal factors, the levels do not currently pose significant threats to the aquatic ecosystem or its biodiversity. However, the findings underscore the necessity for ongoing monitoring and management to address seasonal peaks in contamination and maintain the ecological health of the Giri River Basin. Sustainable practices and mitigation strategies are essential to prevent long-term ecological impacts and ensure the river's resilience to anthropogenic and natural pressures.

## The way forward

The assessment of heavy metal bioaccumulation in zooplankton and fish liver from the Giri River Basin suggests crucial areas for future action and research.

- To gain a more comprehensive understanding of ecosystem health, it is important to further investigate the impact of water quality and bioaccumulated heavy metals on aquatic organisms, especially zooplankton and fish, by expanding studies on bioaccumulation in other species, while also ensuring continuous monitoring of heavy metal concentrations, particularly during the pre-monsoon season when bioaccumulation is most pronounced, to effectively manage the ecological health of the basin.

▪ Expanding studies to include additional species will provide a more comprehensive understanding of ecosystem dynamics and potential risks.
▪ Incorporating advanced technologies, such as remote sensing and GIS-based models, could enhance monitoring capabilities and offer real-time data for more effective decision-making.
▪ Sustainable management practices, including the reduction of industrial effluents and adoption of eco-friendly agricultural methods, must be prioritized to mitigate heavy metal contamination and prevent future ecological degradation.
▪ Collaborative efforts involving local communities, government agencies, and environmental organizations are key to implementing mitigation strategies and ensuring the long-term health of the Giri River Basin.
These combined efforts will help to protect the Giri River Basin's ecosystem from future pollution threats, ensuring its ecological resilience and the health of its aquatic life.

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**Table: 1. Seasonal variations in heavy metals in zooplankton at different sites in the Giri River Basin.**

| Treatment | Chromium (Cr) (mg $l^{-1}$) | | | Nickel (Ni) (mg $l^{-1}$) | | | Arsenic (As) (mg $l^{-1}$) | | | Cadmium (Cd) (mg $l^{-1}$) | | | Lead (Pb) (mg $l^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean |
| **Season** | | | | | | | | | | | | | | | |
| **Post-Monsoon** | 0.0232$^{b}$ | 0.0231b | 0.0232$^{b}$ | 0.0181$^{b}$ | 0.0178$^{b}$ | 0.0180$^{b}$ | 0.0044$^{b}$ | 0.0044$^{b}$ | 0.0044$^{b}$ | 0.0019$^{b}$ | 0.0019$^{b}$ | 0.0019$^{b}$ | 0.0296$^{b}$ | 0.0296$^{b}$ | 0.0296$^{b}$ |
| **Pre-Monsoon** | 0.0289$^{a}$ | 0.0289$^{a}$ | 0.0289$^{a}$ | 0.0240$^{a}$ | 0.0244$^{a}$ | 0.0242$^{a}$ | 0.0097$^{a}$ | 0.0096$^{a}$ | 0.0097$^{a}$ | 0.0042$^{a}$ | 0.0042$^{a}$ | 0.0042$^{a}$ | 0.0549$^{a}$ | 0.0544$^{a}$ | 0.0547$^{a}$ |
| **$LSD_{0.05}$ (Season)** | 0.0005 | 0.0005 | 0.0004 | 0.0008 | 0.0007 | 0.0007 | 0.0025 | 0.0023 | 0.0024 | 0.0008 | 0.0007 | 0.0008 | 0.0081 | 0.0078 | 0.0079 |
| **Sites** | | | | | | | | | | | | | | | |
| **Giripul Bridge** | 0.0250$^{b}$ | 0.0245$^{c}$ | 0.0247$^{d}$ | 0.0165$^{b}$ | 0.0159$^{c}$ | 0.0162$^{c}$ | 0.0050 | 0.0053$^{ab}$ | 0.0052 | 0.0024$^{b}$ | 0.0024$^{b}$ | 0.0024$^{b}$ | 0.0393$^{bc}$ | 0.0391$^{bc}$ | 0.0392$^{bc}$ |
| **Khairi Bridge** | 0.0263$^{a}$ | 0.0267$^{a}$ | 0.0265$^{ab}$ | 0.0212$^{a}$ | 0.0211$^{b}$ | 0.0211$^{b}$ | 0.0085 | 0.0085$^{ab}$ | 0.0086 | 0.0033$^{ab}$ | 0.0031$^{ab}$ | 0.0032$^{ab}$ | 0.0663$^{a}$ | 0.0658$^{a}$ | 0.0661$^{a}$ |
| **Dadahu** | 0.0261$^{a}$ | 0.0261$^{ab}$ | 0.0261$^{bc}$ | 0.0219$^{a}$ | 0.0218$^{ab}$ | 0.0219$^{ab}$ | 0.0093 | 0.0091$^{a}$ | 0.0092 | 0.0033$^{ab}$ | 0.0034$^{ab}$ | 0.0034$^{ab}$ | 0.0350$^{c}$ | 0.0348$^{c}$ | 0.0350$^{c}$ |
| **Ambaun** | 0.0262$^{a}$ | 0.0253$^{bc}$ | 0.0258$^{c}$ | 0.0222$^{a}$ | 0.0224$^{a}$ | 0.0223$^{ab}$ | 0.0054 | 0.0049$^{b}$ | 0.0052 | 0.0039$^{a}$ | 0.0038$^{a}$ | 0.0039$^{a}$ | 0.0272$^{c}$ | 0.0268$^{c}$ | 0.0270$^{c}$ |
| **Sataun** | 0.0262$^{a}$ | 0.0265$^{a}$ | 0.0264$^{abc}$ | 0.0225$^{a}$ | 0.0227$^{a}$ | 0.0226$^{a}$ | 0.0073 | 0.0072$^{ab}$ | 0.0073 | 0.0031$^{ab}$ | 0.0030$^{ab}$ | 0.0031$^{ab}$ | 0.0501$^{b}$ | 0.0494$^{b}$ | 0.0497$^{b}$ |
| **Bangran Giri Bridge** | 0.0266$^{a}$ | 0.0269$^{a}$ | 0.0267$^{a}$ | 0.0223$^{a}$ | 0.0229$^{a}$ | 0.0226$^{a}$ | 0.0069 | 0.0070$^{ab}$ | 0.0069 | 0.0024$^{b}$ | 0.0026$^{ab}$ | 0.0025$^{b}$ | 0.0359$^{c}$ | 0.0361$^{bc}$ | 0.0360$^{bc}$ |
| **$LSD_{0.05}$ (Site)** | 0.0010 | 0.0009 | 0.0007 | 0.0014 | 0.0011 | 0.0012 | NS | 0.0040 | NS | 0.0014 | 0.0012 | 0.0013 | 0.0140 | 0.0136 | 0.0138 |
| **$LSD_{0.05}$ (Site×Season)** | 0.0014 | 0.0012 | 0.0009 | 0.0020 | 0.0016 | 0.0017 | 0.0061 | 0.0056 | 0.0058 | 0.0020 | 0.0017 | 0.0019 | 0.0198 | 0.0192 | 0.0195 |

**Table: 2. Seasonal variations in heavy metals in fish liver at different sites in the Giri River Basin.**

| Treatment | Chromium (Cr) (mg $l^{-1}$) | | | Nickel (Ni) (mg $l^{-1}$) | | | Arsenic (As) (mg $l^{-1}$) | | | Cadmium (Cd) (mg $l^{-1}$) | | | Lead (Pb) (mg $l^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean | 2022-2023 | 2023-2024 | Mean |
| **Season** | | | | | | | | | | | | | | | |
| **Post-Monsoon** | $0.0263^{b}$ | $0.0264^{b}$ | $0.0263^{b}$ | $0.0207^{b}$ | $0.0207^{b}$ | $0.0208^{b}$ | $0.0057^{b}$ | $0.0057^{b}$ | $0.0057^{b}$ | $0.0025^{b}$ | $0.0026^{b}$ | $0.0026^{b}$ | $0.0376^{b}$ | $0.0373^{b}$ | $0.0375^{b}$ |
| **Pre-Monsoon** | $0.0326^{a}$ | $0.0325^{a}$ | $0.0326^{a}$ | $0.0269^{a}$ | $0.0270^{a}$ | $0.0270^{a}$ | $0.0110^{a}$ | $0.0105^{a}$ | $0.0108^{a}$ | $0.0049^{a}$ | $0.0051^{a}$ | $0.0050^{a}$ | $0.0612^{a}$ | $0.0606^{a}$ | $0.0609^{a}$ |
| **$LSD_{0.05}$ (Season)** | 0.0006 | 0.0005 | 0.0005 | 0.0007 | 0.0005 | 0.0005 | 0.0028 | 0.0026 | 0.0027 | 0.0008 | 0.0006 | 0.0007 | 0.0078 | 0.0077 | 0.0077 |
| **Sites** | | | | | | | | | | | | | | | |
| **Giripul Bridge** | $0.0276^{c}$ | $0.0276^{c}$ | $0.0276^{d}$ | $0.0198^{d}$ | $0.0192^{c}$ | $0.0195^{e}$ | 0.0061 | 0.0060 | 0.0061 | 0.0031 | $0.0031^{b}$ | $0.0032^{b}$ | $0.0460^{bc}$ | $0.0460^{bc}$ | $0.0460^{bc}$ |
| **Khairi Bridge** | $0.0293^{b}$ | $0.0295^{b}$ | $0.0294^{c}$ | $0.0231^{c}$ | $0.0237^{b}$ | $0.0234^{d}$ | 0.0105 | 0.0103 | 0.0104 | 0.0039 | $0.0040^{ab}$ | $0.0039^{ab}$ | $0.0730^{a}$ | $0.0723^{a}$ | $0.0727^{a}$ |
| **Dadahu** | $0.0290^{b}$ | $0.0286^{bc}$ | $0.0288^{c}$ | $0.0245^{b}$ | $0.0240^{b}$ | $0.0243^{cd}$ | 0.0104 | 0.0096 | 0.0100 | 0.0038 | $0.0041^{ab}$ | $0.0040^{ab}$ | $0.0406^{c}$ | $0.0404^{c}$ | $0.0406^{c}$ |
| **Ambaun** | $0.0298^{b}$ | $0.0294^{b}$ | $0.0296^{bc}$ | $0.0250^{ab}$ | $0.0256^{a}$ | $0.0253^{ab}$ | 0.0065 | 0.0067 | 0.0066 | 0.0045 | $0.0044^{a}$ | $0.0045^{a}$ | $0.0354^{c}$ | $0.0351^{c}$ | $0.0353^{c}$ |
| **Sataun** | $0.0300^{ab}$ | $0.0305^{a}$ | $0.0302^{ab}$ | $0.0248^{ab}$ | $0.0246^{b}$ | $0.0247^{bc}$ | 0.0085 | 0.0081 | 0.0083 | 0.0039 | $0.0039^{ab}$ | $0.0039^{ab}$ | $0.0570^{b}$ | $0.0561^{b}$ | $0.0566^{b}$ |
| **Bangran Giri Bridge** | $0.0309^{a}$ | $0.0311^{a}$ | $0.0310^{a}$ | $0.0257^{a}$ | $0.0262^{a}$ | $0.0260^{a}$ | 0.0084 | 0.0080 | 0.0082 | 0.0033 | $0.0035^{ab}$ | $0.0034^{ab}$ | $0.0442^{bc}$ | $0.0439^{bc}$ | $0.0441^{bc}$ |
| **$LSD_{0.05}$ (Site)** | 0.0010 | 0.0009 | 0.0008 | 0.0011 | 0.0009 | 0.0009 | NS | NS | NS | NS | 0.0011 | 0.0012 | 0.0135 | 0.0133 | 0.0134 |
| **$LSD_{0.05}$ (Site×Season)** | 0.0014 | 0.0013 | 0.0012 | 0.0016 | 0.0013 | 0.0013 | 0.0069 | 0.0063 | 0.0066 | 0.0020 | 0.0015 | 0.0017 | 0.0191 | 0.0188 | 0.0190 |

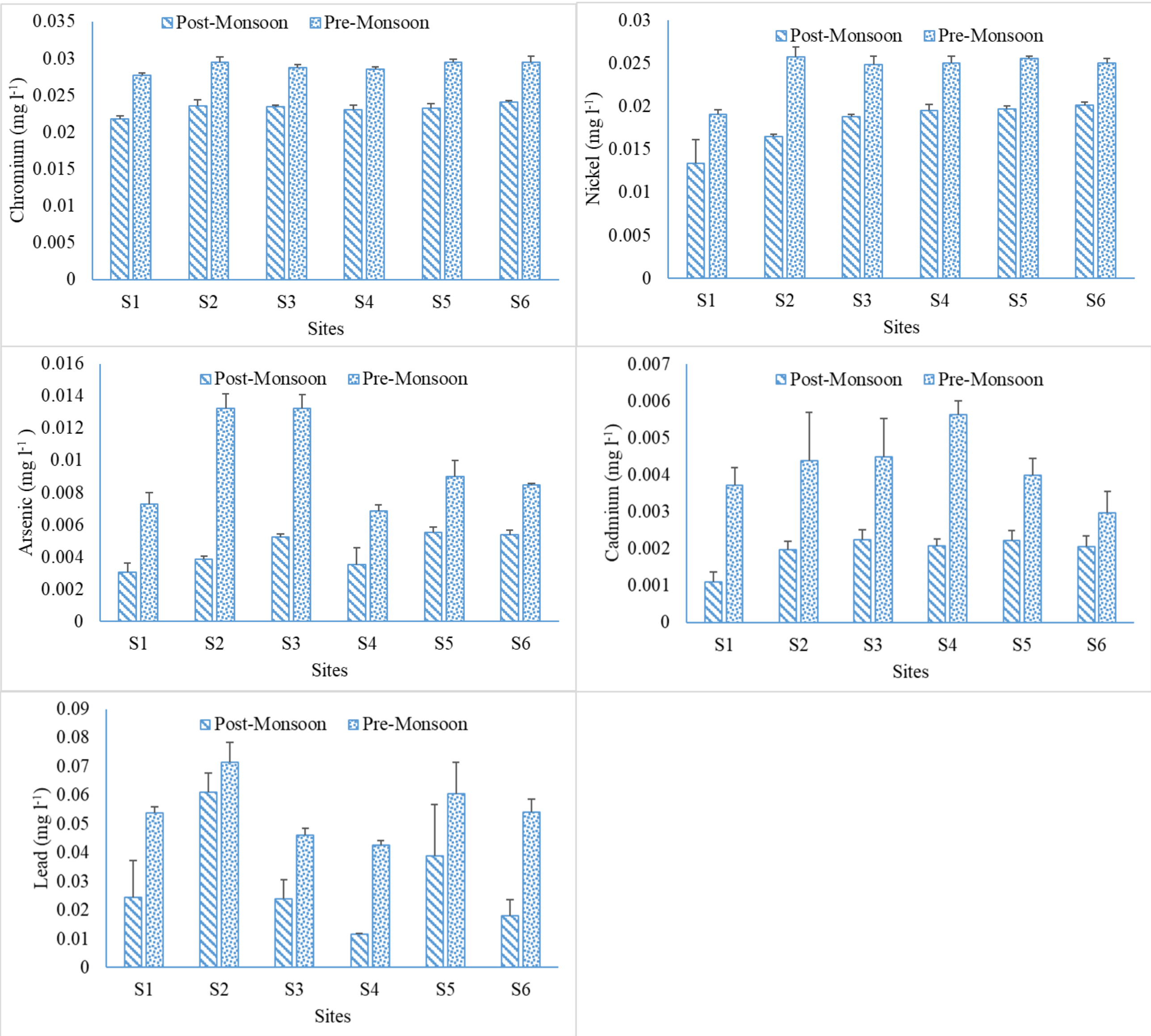


**Figure 1.** Seasonal variations in heavy metal concentrations in zooplankton across different sites in the Giri River Basin during two distinct seasons.

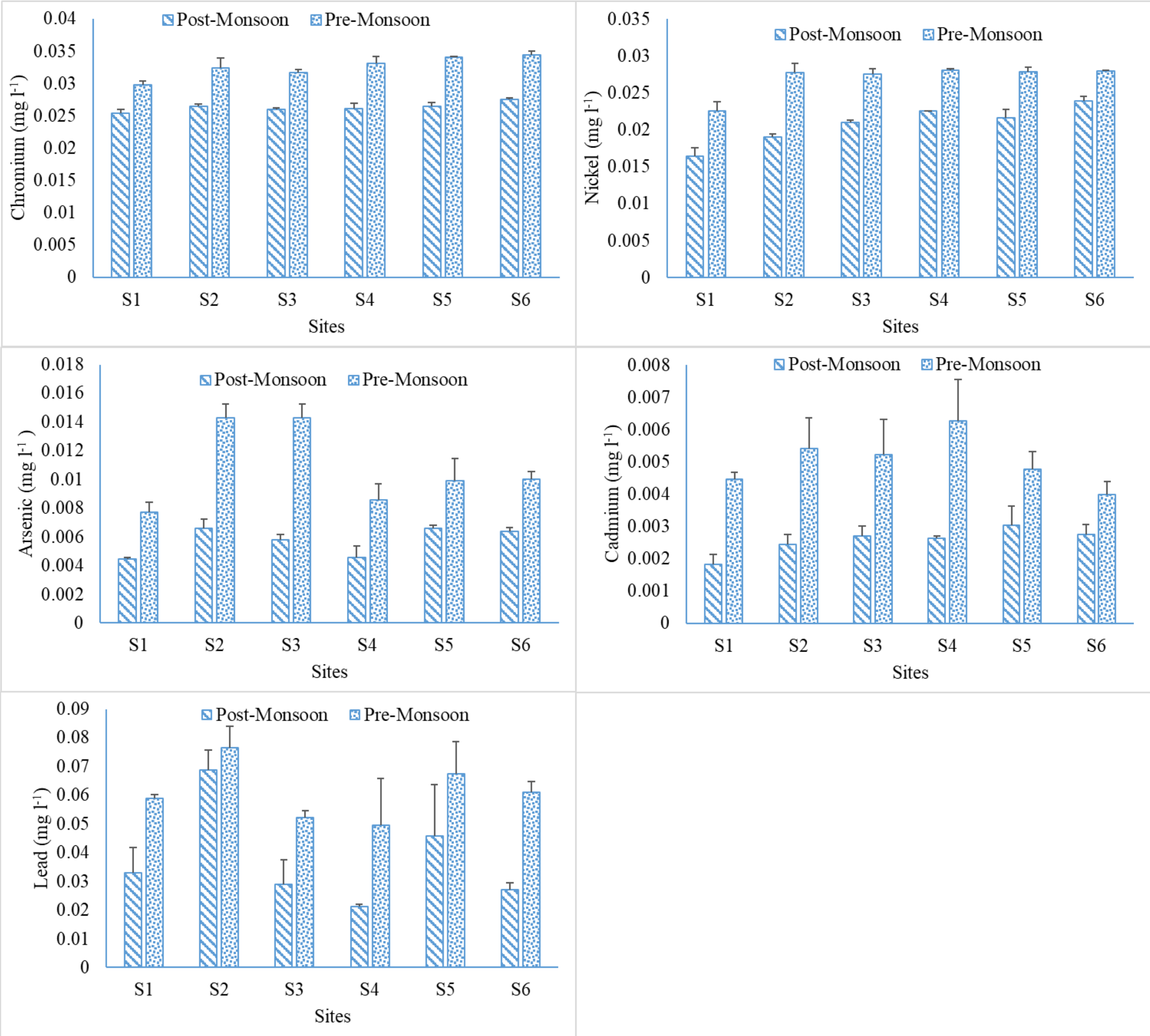


**Figure 2.** Seasonal variations in heavy metal concentrations in fish liver across different sites in the Giri River Basin during two distinct seasons.