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Assessment of Heavy Metal Contamination and Ecotoxicological Implications in Freshwater Aquatic Food Webs

[1*] Hiroshi Nakamura, Japan Institute for Advanced Digital Futures, Tokyo, Japan.

[2] Yuki Tanaka, Japan Institute for Advanced Digital Futures, Tokyo, Japan.

Abstract: Metal pollution is a source of threats in freshwater ecosystems; hence the need for assessment methods linking environmental exposure to biological effects. This paper describes an approach for assessment of metal pollution and possible ecotoxicological effects in freshwater food webs. The suggested approach includes sampling from six sampling stations characterized by reference conditions, discharge conditions, and downstream conditions during two hydrological seasons. Water, sediments, and aquatic life forms are sampled for analysis of cadmium, lead, chromium, copper, and zinc using reliable laboratory analytical methods. Benthic richness, abundance, Shannon diversity, and evenness are combined with chemical variables. Mixed-effects models have been recommended for handling repeated samples. Numerical examples illustrate the reporting approach rather than actual results of studies. The concentrations of dissolved cadmium range from 0.4 to 2.8 µg/L, dissolved lead from 2.1 to 14.6 µg/L, and dissolved zinc from 24.5 to 112.3 µg/L. Benthic richness decreases from 24 to 12 taxa, which is a decrease of 50.0%, whereas Shannon diversity ranges from 2.65 to 1.58, a decrease of approximately 40.4%. The downstream richness and diversity levels are 17 taxa and 2.12, respectively. This is an example of spatial patterns that can be observed without any proof of toxicity, biomagnification, or statistical significance. This model can facilitate integrated monitoring and ecologically based evaluation; however, the empirical support will be achieved only through replicating measurements, quantifying the uncertainty, and adjusting to environmental conditions.

Keywords: Heavy Metals; Freshwater Ecosystems; Aquatic Food Webs; Bioaccumulation; Ecotoxicology; Benthic Communities; Environmental Monitoring.

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I. Introduction

Freshwater habitats support biodiversity, fishery resources, nutrient cycling, and human sustenance, but their ecological health faces increasing risk due to contamination by heavy metals. Industrial discharges, mining operations, fertilizer use, and urban sewage discharge introduce contaminants that could be toxic into freshwater systems. Weathering processes also release metals, but human interventions may significantly elevate metal concentrations in the environment. Metals are resistant to degradation and accumulate in the environment due to these properties. Reviews have discussed the connection between environmental pollution, toxicity to aquatic species, and biomagnification of metals (Sonone et al., 2020; Sharma et al., 2025; Edo et al., 2024).

Aquatic food webs offer a necessary perspective through which contamination transfer among compartments and organisms can be understood. The primary producers, zooplankton, benthic invertebrates, and fish will be exposed to metals via water, sediment, and dietary means. These interactions form exposure routes via which accumulated contaminants can enter consumers. Contaminant research in freshwater ecosystems reveals the significance of examining matrices in the environment and more than one biological group (Griboff et al., 2018; Yi et al., 2017). Polluted aquatic food chains research at large indicates that the examination of exposure paths is preferable rather than studying single organisms (González et al., 2021).

Contaminant accumulation does not imply biomagnification. Bioaccumulation implies the uptake and accumulation in organisms, whereas biomagnification implies concentration increase in trophic

connections. Metal transfer varies according to metal, chemical forms, organism physiology, diet, and environmental factors. Research that focuses on estuarine and marine food webs provides comparative insights into metal occurrence and trophic magnification, but should be cautiously interpreted prior to being applied to freshwater ecosystems (Mendoza-Carranza et al., 2016; Liu et al., 2022).

Therefore, the freshwater study must consider the relationship of metals on a species-specific basis instead of assuming an upward increase of pollutants at the higher level of the trophic scale.

However, ecotoxicological consequences not only include high levels of metals but also affect the growth, reproduction, respiration, feeding, and behavior of an organism, which might have some effect on its interaction within the ecosystem (Sharma et al., 2025). Another impact of pollution includes nutrient pathways, as has been shown through the study of fatty acids' transfer in freshwater (Mahboob et al., 2019). Moreover, heavy metal pollution combined with eutrophication reveals the significance of responses of zooplankton communities (Gagneten, 2010). The stage-structured food web approach further extends the ecological view by including both predators and contamination effects along with terrestrial-aquatic linkages (Wesner, 2019).

However, in spite of significant studies, it is still necessary to develop approaches for the integration of contamination data, trophic transfers, and their ecotoxicological impacts. Reviews on aquatic food webs and food safety reveal deficiencies in terms of understanding exposure through several trophic levels and transferring knowledge about contamination into useful management strategies (Rakib et al., 2024; Sarker et al., 2022). Therefore, the objective of this paper is to evaluate the problem of heavy metal contamination in freshwater compartments, investigate accumulation and transfer within aquatic food webs, and study the ecotoxicological implications of such contamination. Such an approach will enable the identification of susceptible organisms and exposure routes, improve ecotoxicological interpretation, and contribute to monitoring and protection tasks.

Organization of the paper: Section 2 discusses relevant literature on the subject. Section 3 explains the sampling method, metal analysis, ecological evaluation, and statistics used. Section 4 shows examples of contamination and benthic community patterns. Section 5 interprets the patterns discussed and identifies their limitations. Section 6 is the conclusion of the paper.

II. Literature Review

Studies on heavy metal contamination of aquatic life consider the environmental persistence, biological accumulation, trophic transfer, and ecological toxicity of such contaminants. In reviews, the contamination process is discussed, along with the consequences of exposure in terms of food chains for both aquatic life and humans (Sonone et al., 2020; Edo et al., 2024). The current review on aquatic ecosystems and their toxicity adds the consideration of effects on growth, reproduction, respiration, and behavior, thus confirming the significance of understanding contamination through biological impacts (Sharma et al., 2025).

Reviews that focus on Bangladesh analyze the issue of heavy metal contamination of edible fish, aquatic food webs, and the general problem of food safety (Rakib et al., 2024; Sarker et al., 2022). In these works, limitations of studies on exposure and toxicity related to multiple trophic levels are considered. The attention given to contamination sources, ecological interactions, and management confirms the necessity to link the problem of fish consumption with environmental processes. This broad approach to pollution in aquatic food chains proves the interconnection (González et al., 2021).

The studies on freshwater present direct examples of compartment-based assessments. The research conducted in Córdoba, Argentina, assessed the bioaccumulation and trophic transfer of metals, arsenic, and selenium in a food web that was affected by anthropogenic activity (Griboff et al., 2018). The study on the upper Yangtze River involved sediment, zoobenthos, and fish sampling with ecological network analysis and risk assessment (Yi et al., 2017). The methods reveal the necessity to pay attention to benthic organisms

and transfer pathways in addition to fish and warn about the inapplicability of trophic position as an indication of pollution and vulnerability.

Comparative research expands the environmental framework. Metal distribution and bioconcentration were studied in a Mexican tropical estuarine lagoon, while metal concentration, trophic magnification, and health risks were assessed in a marine food web of Laizhou Bay (Liu et al., 2022). These studies can be taken into consideration because of the methodological perspective they provide; however, the differences in salinity, species, and exposure do not allow drawing conclusions about freshwater contamination.

The ecological evaluations also examine indirect impacts and reactions of ecological communities. Studies of fatty acid transfer in freshwaters relate pollution to nutritional interactions between elements of the food web (Mahboob et al., 2019). Studies related to pollution caused by heavy metals and eutrophication assess the zooplankton reaction to them and the relevance of the reaction to freshwater food webs (Gagneten, 2010). Analysis of stage-structured food webs additionally takes into account the impact of contaminants and predators on the aquatic-terrestrial connections (Wesner, 2019). Altogether, these studies encourage conducting assessments that would incorporate concentrations, biological accumulation, trophic transfers, and ecological responses. Such assessment needs to differentiate the evidence of freshwaters from comparative evidence and distinguish the adverse effects from contamination risks.

III. Materials and Methods

Study Design and Sampling Stations

A total of six stations in rivers correspond to two upriver reference locations, two discharge-related locations, and two downriver locations. The reference stations are of similar habitat and minimal pollution. Sampling is done during two hydrological seasons, with sampling conducted three times per season at four-week intervals. Three water and three sediment samples are taken per station in every sampling period, resulting in a total of 108 samples for every matrix. The coordinates, rain, depth, temperature, pH level, dissolved oxygen, and conductivity are noted.

Water and Sediment Collection

Water samples (1 litre) are obtained using acid washed polyethylene bottles. Aliquots containing dissolved metals are then filtered at 0.45 μm and acidified to pH < 2, while total aliquots that contain recoverable metals are not filtered. Three replicate sediment grabs for metal, moisture content, grain size, and organic matter analysis are taken from the top 0-5 cm depth of sediment, each sample weighing about 200 g. Samples are then labeled, shipped, and stored following standard laboratory procedure.

Biological Sampling

Primary producers, benthic invertebrates, and resident fish serve as the documented prey groups. In the first step, the goal is to collect five fish for each selected species, station, and season; thus, three species for six stations yield 180 fish. Collection will be based on availability, permits, and ethics clearance. Fish length, mass, and the location where fish are caught are recorded, and the dorsal muscle is analyzed. Small animals can be pooled, with each pooled sample being considered as one sample.

Metal Analysis and Quality Assurance

The metals of interest for analysis are cadmium, lead, chromium, copper, and zinc; mercury is included only when there are valid methods for doing so. Water samples are analyzed using a validated procedure based on EPA Method 200.8 by ICP-MS. Sediment and tissue samples are analyzed by matrix-specific digestion using microwave digestion based on EPA Method 3052. Quality controls used are blanks, certified reference material, matrix spike, duplicates, and calibration verification. Detection limits, recovery, and precision are provided.

Accumulation and Trophic Transfer

Concentrations of metals are compared among different stations, seasons, matrices, and biological taxa using similar tissues and concentration units. Stable isotope analysis is an optional procedure that is useful for determination of the trophic position of organisms and trophic magnification factor calculation. In the absence of stable isotope analysis, the comparison is only possible within known feeding groups and does not prove biomagnification.

$$\mathrm{TMF} = 10^{b} \qquad (1)$$

Equation (1). Here, b is the slope resulting from the regression of $\log_{10}$ metal concentration on estimated trophic position. In the absence of trophic position data based on isotope measurements, remove this formula.

Ecological Assessment

Three independent benthic samples per station visit are taken at an equal sampling effort. Community metrics used include richness, abundance, Shannon's diversity index, and evenness. Metal concentration is evaluated against the benchmarks set for freshwater and sediment. Exceedance is an indication of concern, and ecological associations are determined along with habitat and water/sediment conditions.

Shannon diversity index

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i) \qquad (2)$$

Equation (2). Here, H'represents species diversity, $S$is the number of species, and $p_i = n_i/N$is the proportion of individuals belonging to species i.

Pielou's evenness index

$$J' = \frac{H\prime}{\ln(S)} \qquad (3)$$

Equation (3). Here, J'measures the uniformity of species abundance and ranges from 0 to 1. Higher values indicate more even abundance. This index applies when $S > 1$.

Statistical Analysis

R is used for analysis, with versions of software and packages reported. Analysis of mixed effects examines exposure category and seasonal differences, controlling for nested or repeated sampling. Analysis of fish considers species and size where appropriate. Effect estimates, 95% confidence intervals, sample size, and p-values are given, using a 0.05 threshold of significance with correction for false discovery rate. Values below detection, missing values, exclusions, and sampling anomalies are noted.

Figure 1 provides an overview of the methodological workflow that starts with the choice of stations and the sampling of water, sediments, and aquatic organisms during different seasons. Analysis of metals and characterization of the benthic communities are performed in order to evaluate exposure.

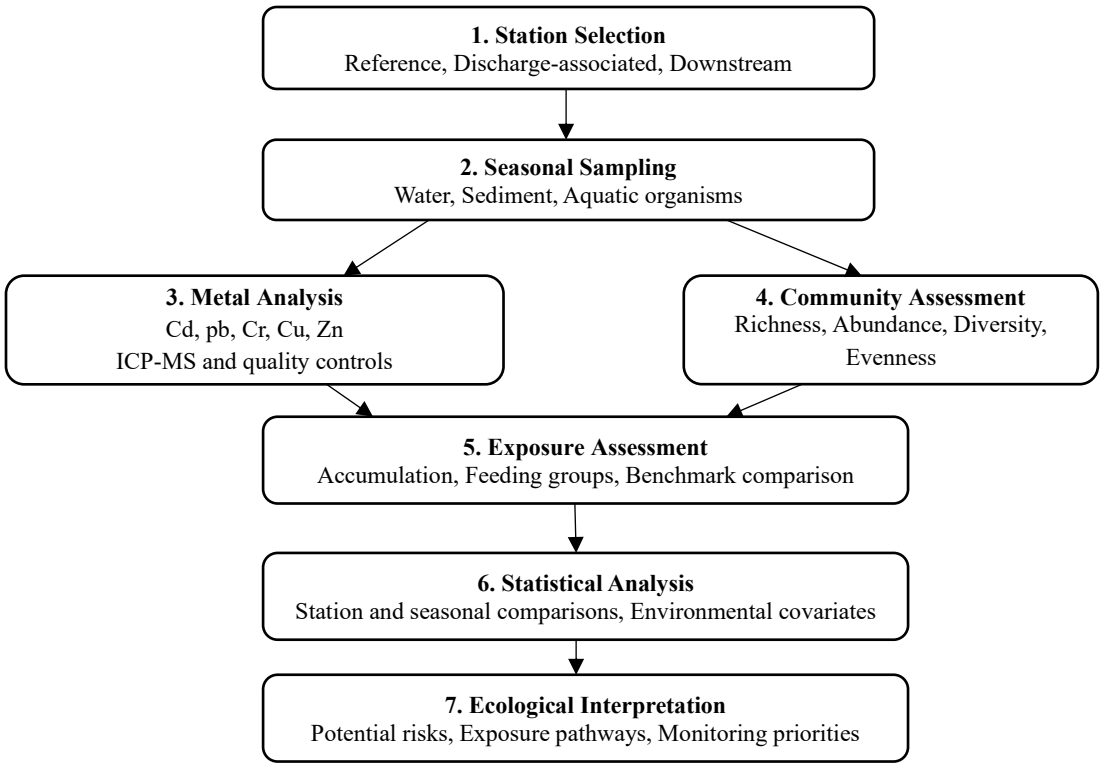


Figure 1: Methodological Workflow for Assessing Heavy Metal Contamination in Freshwater Food Webs

IV. Results

Metal Distribution in Water and Sediment

In this case study, the discharge-associated stations have the highest concentrations for all five metals, while the downstream and reference stations have lower amounts, but higher than background stations. Concentrations of dissolved cadmium increase from 0.4 to 2.8 µg/L, lead from 2.1 to 14.6 µg/L, and zinc from 24.5 to 112.3 µg/L.

Cadmium in sediments shows a similar distribution, with 0.3 mg/kg dry weight concentration in reference stations, 2.4 mg/kg dry weight in discharge-associated stations, and 1.5 mg/kg dry weight in downstream stations.

Lead shows similar concentrations, with 12.8 mg/kg dry weight in reference stations, 76.5 mg/kg dry weight in discharge-associated stations, and 48.2 mg/kg dry weight in downstream stations.

Table 1: Illustrative Metal Concentrations in Water and Sediment

Metal	Reference water (µg/L)	Discharge water (µg/L)	Downstream water (µg/L)	Reference sediment (mg/kg DW)	Discharge sediment (mg/kg DW)	Downstream sediment (mg/kg DW)
Cadmium	0.4	2.8	1.6	0.3	2.4	1.5
Lead	2.1	14.6	8.3	12.8	76.5	48.2
Chromium	3.2	18.4	11.2	21.4	94.8	63.7
Copper	5.8	28.7	17.5	18.6	82.3	54.1
Zinc	24.5	112.3	68.4	65.2	248.6	167.4

DW: dry weight. Water values represent dissolved concentrations. All entries are illustrative category means; variability and statistical significance *have not been estimated.*

Table 1 is a depiction of a spatial concentration gradient of the five metals at reference sites, discharge-related sites, and downstream sites. The five metals attain their highest example concentrations closest to the discharge areas. The downstream concentrations still exceed the reference concentrations in both matrices. These are the hypothetical means used for reporting purposes only.

Benthic Community Structure

In the case of taxon richness, there is a decline of 50.0% from 24 taxa/s to 12 taxa at the source of discharge, while downstream richness reaches 17 taxa/s. Abundance declines from 860 individuals/m² to 420 individuals/m² at the source of discharge but rises to 610 individuals/m² downstream.

Shannon diversity declines from 2.65 at reference stations to 1.58 at the source of discharge stations, or a decrease of 40.4%. Evenness declines from 0.83 to 0.64, while downstream diversity and evenness reach 2.12 and 0.75, respectively.

Table 2: Illustrative Benthic Community Indicators Across Exposure Categories

Indicator	Reference stations	Discharge-associated stations	Downstream stations
Taxon richness per sample	24	12	17
Abundance (individuals/m²)	860	420	610
Shannon diversity, H'	2.65	1.58	2.12
Pielou's evenness, J'	0.83	0.64	0.75

The data shown in table 2 show low benthic richness, abundance, diversity, and evenness close to the discharge areas, after which there is partial recovery downstream. Reference areas maintain the highest values for all of the above parameters. This serves as an example of reporting on ecology and does not indicate metal toxicity.

Interpretation of Chemical and Ecological Patterns

The concentration gradient correlates with low benthic diversity close to the discharge points. Downstream sites have intermediate levels of chemical concentrations and biological indices. The actual analysis needs to confirm the presence of these trends after taking into account factors such as seasonality, dissolved oxygen content, habitat, and sediment characteristics. Bioaccumulation, food chain effects, benchmarks, and statistical significance need actual measurements and not the illustrative tables.

Metal Concentration Plot

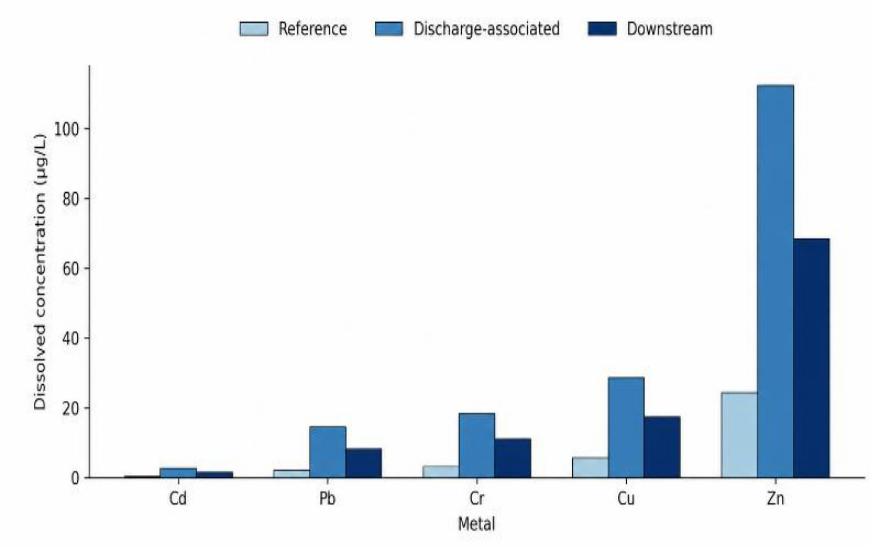


Figure 2: Illustrative Dissolved Metal Concentrations Across Exposure Categories

Figure 2 gives the dissolved concentrations of cadmium, lead, chromium, copper, and zinc in three different exposure groups. Discharge-related stations provide the maximum dissolved concentration values, whereas the intermediate levels are recorded further downstream. Figure 2 displays water values from table 1.

V. Discussion

The hypothetical findings reveal an increase in metal levels at discharge-associated stations compared to reference stations. The dissolved level of cadmium rises from 0.4 µg/L to 2.8 µg/L, lead rises from 2.1 µg/L to 14.6 µg/L, and zinc from 24.5 µg/L to 112.3 µg/L. There is a decline in benthic richness from 24 to 12 species, as well as in Shannon's diversity from 2.65 to 1.58.

The approach points out that both chemical and biological analyses are necessary in order to assess monitoring priorities and potentially sensitive areas. Still, those are just examples of numbers and cannot prove any real contamination, toxicity, biomagnification, or statistically significant results. Habitat differences, seasonality, oxygen concentration, and sediments can also affect community structure. Future studies should include replicates of field measurements, data validation, and use of mixed-effects models. Trophic transfer could be evaluated by stable-isotope measurements and standardization of tissue analysis.

VI. Conclusion

Metal pollution is an environmental challenge that needs an approach to assess environmental concentration, biological accumulation, and ecological effects. In this paper, such an approach is presented based on a seasonal sampling scheme that includes lab metal analysis, benthic community assessment, and statistical analysis for reference, associated, and downstream stations. An example of this scenario shows how reporting should be done. The dissolved cadmium level rises from 0.4 µg/L at reference stations to 2.8 µg/L at discharge-associated stations, the lead level rises from 2.1 to 14.6 µg/L, and the zinc level rises from 24.5 to 112.3 µg/L. The benthic richness drops from 24 to 12 species, thus making up a 50.0% decrease, while Shannon diversity drops from 2.65 to 1.58, which accounts for 40.4%. The downstream richness attains 17 species, while the downstream diversity equals 2.12, thus proving the intermediary state of the community. The main conclusion is that the data on chemical analysis and biological factors have to be considered together with the habitat and seasonality. However, these numbers do not prove any contamination or its effects. This is rather a framework for future research, not the results. The next step in the study will be obtaining replicated data, calculating uncertainties, analyzing the analytical performance, and using stable isotopes when studying biomagnification.

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