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Original Research Article

Assessment of Heavy Metals Accumulation in Freshwater Snails (*Physella acuta*) as Potential Bioindicators of Aquatic Pollution

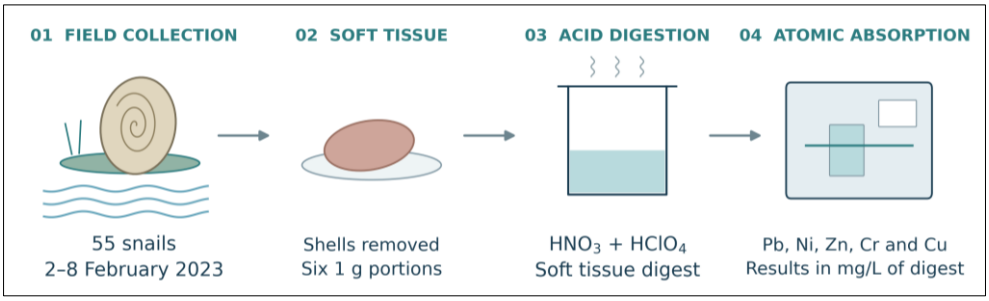
Haider Ali[1], Muqaddas Khalid[2]*, Mubashir Khattak[2]*, Ayesha Khalid[2], Iqra Hakim[2], Zaib Un Nisa[2], Malaika Tajamul[3], Masood Shah[4]

[1]Institute of Zoological Sciences, University of Peshawar, Peshawar 52120, Pakistan/Department of Zoology, Quaid-i-Azam University, Islamabad
[2]Department of Zoology, Quaid-i-Azam University, 45320, Islamabad, Pakistan
[3]Department of Zoology, Institute of Zoological Sciences, University of Peshawar, Peshawar 52120, Pakistan
[4]Shaheed Benazir Bhutto University Dir Upper Khyber Pakhtunkhwa Pakistan 18050

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***Corresponding author:** Muqaddas Khalid and Mubashir Khattak
Department of Zoology, Quaid-i-Azam University, 45320, Islamabad, Pakistan

1. Abstract



Graphical Abstract

The presence of heavy metals in freshwater represents a global health problem. Freshwater gastropods can integrate exposure to trace metals over time and therefore have potential value in aquatic biomonitoring, but interpretation depends critically on sample identity, tissue basis digestion volume, quality-control records and the environmental matrices measured alongside the organisms. This study evaluated the concentrations of lead (Pb), nickel (Ni), zinc (Zn), chromium (Cr), and copper (Cu) in acid-digested soft tissue samples of the freshwater snail *Physella acuta* collected from the Shah Alam River, Peshawar District, Khyber Pakhtunkhwa, Pakistan. The fresh snails were collected from an approximately 100 m^2 area of river, in a period from 2-8 February 2023. After removal of shells, soft tissues were separated and six samples were prepared from them each weighing 1g. Two strong acids, nitric and perchloric acids were used for tissue digestion. And the heavy metals concentrations were found out by using a technique called atomic absorption spectrophotometry (AAS). The analytical tables contained six labels, including sample 1, sample 2, sample 3, sample 4, sample 5 and sample 6. For Samples 1–6, reported digest concentrations ranged from 1.53 to 5.73 mg/L for Pb, 0.146 to 0.295 mg/L for Zn, 0.089 to 0.110 mg/L for Cr, 0.044 to 0.090 mg/L for Ni and 0.041 to 0.096 mg/L for Cu. The results represent that, the concentration of 3 heavy metals out of 5 are exceeded from the acceptable level. The results of this study showed high concentration of lead (Pb) followed by Zinc (Zn), Chromium (Cr), Nickel (Ni) and Copper (Cu) in tissues sample, out of which Pb had the highest nominal digest concentrations among the metals reported, Cr and Ni also exceeded from the normal level. However, the concentration of Zn and Cu are within the permissible levels provided by world health organization (WHO) of the United Nation. The findings provide a preliminary analytical record of heavy metals for snail-derived digests from the Shah Alam River and identify specific requirements for a stronger follow-up study.

Keywords: *Physella acuta*, trace metals, soft tissue, acid digestion, atomic absorption spectrophotometry, Shah Alam River, freshwater biomonitoring, Pakistan.

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2. INTRODUCTION

Freshwater contamination by potentially toxic elements is a persistent environmental problem because metals are not degraded in the same way as many organic contaminants. Instead, they are redistributed among dissolved phases, suspended particles, sediments and living organisms. Their ecological significance is controlled not only by total concentration but also by chemical speciation, pH, hardness, dissolved organic matter, redox conditions and the biological traits of exposed species. Consequently, a single concentration measured in one environmental compartment rarely provides a complete picture of exposure. Monitoring programmes increasingly combine water, sediment and biota so that short-term dissolved exposure, longer-term sediment storage and biological uptake can be interpreted together [Elder & Collins, 1991; Baroudi *et al.,* 2020; Li *et al.,* 2018; Qasim *et al.,* 2025].

Biological monitoring is valuable because organisms integrate exposure through multiple pathways, including direct uptake from water, ingestion of food and sediment particles, and contact with contaminated surfaces. Tissue measurements may therefore reveal biologically available fractions that are not obvious from a single water sample. At the same time, bioaccumulation is not a simple passive reflection of ambient concentration. Uptake rates, elimination, growth dilution, age, reproductive status, feeding mode, tissue composition and metal-specific regulation can all alter the relationship between environmental concentrations and internal burdens [Spyra *et al.,* 2019; Mullen *et al.,* 2025; Chukaeva & Petrov, 2023]. This is why a tissue result must be interpreted using a clearly defined mass basis and, ideally, with matched environmental measurements.

Freshwater gastropods are frequently considered useful sentinel organisms because they are relatively sedentary, abundant in many habitats, closely associated with sediments and periphyton, and able to accumulate a range of inorganic contaminants. Reviews of snail biomonitoring show that both soft tissues and shells have been used, but the two compartments can behave differently and should not be combined conceptually [Baroudi *et al.,* 2020; Elder & Collins, 1991]. Soft tissues are metabolically active and can reflect recent and accumulated exposure, whereas shells may record a different temporal and chemical history. Studies that explicitly compare tissues, shells, water and sediment are therefore more informative than studies that report only one compartment [Li *et al.,* 2018; Mullen *et al.,* 2025].

Physella acuta (often historically reported as *Physa acuta*) is a globally distributed freshwater pulmonate snail with ecological characteristics that make it practical for field monitoring and laboratory ecotoxicology. Its tolerance of disturbed habitats can be advantageous for monitoring polluted waters because the species may remain present where more sensitive taxa decline. However, tolerance should not be confused with absence of biological effect. Field and experimental work has shown that *P. acuta* can respond to environmental stressors, and its abundance can be influenced by pollution-related variables [Camargo & Alonso, 2017]. The species has also been investigated directly for metal accumulation, making it especially relevant to the present dataset [Spyra *et al.,* 2019; Al-Warid *et al.,* 2020].

Spyra *et al.,* [2019] demonstrated that *P. acuta* can accumulate metals and that measured concentrations differ between soft body and shell and among size or age classes. Their results are especially important for study design because they show that the biological unit cannot be treated as homogeneous. A pooled sample dominated by small individuals may not be directly comparable with a pool dominated by larger individuals, and a whole-body result cannot be assumed equivalent to a soft-tissue result. Al-Warid *et al.,* [2020] likewise reported accumulation of Cd, Ni, Pb and Cu in *P. acuta* and another aquatic snail from the Tigris River, supporting the broader use of this species in metal biomonitoring while also illustrating the need for environmental context.

Metal handling in gastropods is also shaped by physiological detoxification. Metallothioneins and related metal-binding systems can sequester essential metals such as Cu and Zn as well as non-essential metals such as Cd, and their binding selectivity differs among gastropod lineages [Dallinger, 2024; Dallinger *et al.,* 2020]. Such mechanisms help explain why tissue concentrations can be high without producing an immediate lethal response, and why different metals show different accumulation patterns. Experimental studies have also shown oxidative and cellular stress responses during metal exposure in freshwater snails [Gnatyshyna *et al.,* 2023]. Thus, a chemical concentration is an exposure metric, not automatically a toxicity endpoint.

The five metals considered here have different biological roles. Pb is non-essential and is generally interpreted as a contaminant when present above background exposure. Ni has essential functions in some microorganisms but is not generally treated as an essential trace nutrient for freshwater gastropods; its interpretation is particularly sensitive to analytical detection and reporting quality. Zn and Cu are essential elements that participate in numerous biochemical processes but become toxic when regulation is overwhelmed. Chromium requires additional caution because total elemental analysis does not determine oxidation state; Cr (III) and Cr (VI) differ substantially in environmental behaviour and toxicity. These differences make metal-by-metal interpretation preferable to a single combined 'heavy metal' narrative.

Laboratory exposure studies confirm that freshwater snails differ in sensitivity to individual metals. In *Melanoides tuberculata*, Cu produced greater acute toxicity than several other metals tested, while Pb and Ni were less acutely toxic under the specific experimental conditions [Shuhaimi-Othman *et al.,* 2012]. In *Pomacea insularum*, metal toxicity varied by metal, exposure duration and life stage [Yap *et al.,* 2023]. These experiments are useful for biological context but are not direct thresholds for field-collected *P. acuta*. Species, water chemistry, life stage, exposure pathway and exposure duration differ too greatly for such values to be transferred directly.

Pakistan's freshwater systems receive metal inputs from a combination of municipal wastewater, industrial discharge, traffic-related deposition, agricultural runoff and natural geological sources. A national review concluded that several major river systems, including the Kabul River, have documented contamination in water, sediments and fish [Afzaal *et al.,* 2022; Qasim *et al.,* 2026]. Regional studies from the Kabul River system have reported metals in sediments and fish and have associated elevated concentrations at some sites with sewage and industrial inputs [Khan *et al.,* 2016; Ali & Khan, 2018; Ahmad *et al.,* 2015; Ather *et al.,* 2026]. These studies establish the plausibility of metal exposure in connected river systems but do not provide a numerical baseline that can be substituted for measurements from the exact Shah Alam River site and sampling date.

The Shah Alam River is a tributary within this regional drainage network and has previously been investigated for metal contamination. Khan *et al.,* [2011] measured selected metals in surface water, while Ali *et al.,* [2020] examined water, sediments and freshwater fish. The latter study is particularly relevant because it illustrates a matrix-integrated design in which environmental compartments and biota are measured together. Nevertheless, comparisons across studies must account for year, season, station, analytical method and concentration basis. A water concentration reported in mg/L cannot be compared as an 'exceedance' with an acid digest concentration expressed in the same unit, because the digest concentration is determined by laboratory dilution as well as by the tissue metal burden.

Against this background, the present dataset is best regarded as an exploratory analytical record rather than a complete bioaccumulation study. The available information permits description of the reported digest values and identification of analytical inconsistencies, but it does not permit calculation of tissue burdens, environmental accumulation factors, trophic transfer or ecological risk indices. The scientific value of the work therefore lies in transparent reporting of what the data do and do not support and in using those limitations to define a stronger follow-up monitoring framework.

The objectives of the present study were to: (i) document the reported Pb, Ni, Zn, Cr and Cu concentrations in soft-tissue digests of *P. acuta* collected from the Shah Alam River; (ii) compare the descriptive patterns among the six samples without treating them as statistically independent site replicates; (iii) evaluate internal consistency of the laboratory summaries; (iv) place the findings in the context of freshwater-snail biomonitoring and regional metal studies; and (v) identify the analytical and field information required before claims about bioaccumulation, toxicity, guideline exceedance or pollution trends can be justified.

3. MATERIALS AND METHODS

3.1 Sample collection and preservation

The snails *(Physella acuta)* were collected from February 2 to February 8 2023, from Shah Alam River (Latitude; 34 4’ 35” N. Longitude; 71 42’ 39”), Peshawar District, Khyber Pakhtunkhwa. Through the simple hand capturing method. The collection was done across the area of about 100m^2. Each collection was put in plastic bottles for bringing to lab. After collection the snails were preserved in 70% alcohol in a separate bottle.

3.2 Snails Identification

The snails were identified with the help of identification key, named “*Land and Freshwater Mollusks key*” by Horst Janus. There were some other species of the snails in the collection but the major specie was *Physella acuta*. So, we have restricted our heavy metals detection to the species of *Physella acuta.*

3.3 Sample preparation and tissues digestion

We have separated 55 number of snails (*P. acuta)* for tissue digestion and weighed them, with the help of digital balance which was about 11.65g (Along with shell). After that, we have removed hard body parts (Shells) from the snails, with a dissecting pin. And weighed again snail’s soft body parts which was 6.34g. After that, we have taken the soft body parts, poured into beakers for tissue digestion and make 6 samples from them each one carrying 1g of soft body parts. For tissues digestion the method was followed as described by Murtala, Abdul, and Akinyemi (2012). Briefly, two strong acids were required for tissues digestion, nitric acid (HNO_3) having concentration 55%. (Diluted from 60%) and perchloric acid ($HClO_4$) having concentration 70%. On day first, 5ml of HNO_3 and 1ml $HCLO_4$ were added and kept them as, in open container for 1 day. On day second, we have followed the same acid addition with a little increase of $HCLO_4$ volume that is, 4ml of $HCLO_4$ were added and kept the samples for one night in open container. On day third the digestion was almost done, but for further digestion the samples were kept on heating plates for about 2 hours. Each sample was then poured into blue bottles (Heat resistant) and placed on electric heating stirrer. The white fumes were ejecting from the samples while placed on the stirrer until the color of the contents becomes transparent which indicates the complete digestion of the tissues. After the

transparent solution formation, the samples were kept on room temperature to cool down for about 30 minutes. Next to cooling, all the samples were filtered through Whatman filter paper so the cellular debris remains attached with filter paper which was discarded along with filter paper. After getting the transparent liquid sample, 50ml of distilled water were added to each sample for dilution. Now all the samples were completely ready for heavy metals detection so, the diluted samples were poured into plastic bottles, covered with aluminium foil and stored for further analysis away from the direct contact of sunlight and other such type of lights in order not to react heavy metals with something else.

3.4 Metal Analysis and Reporting Basis

Atomic absorption spectrophotometry (AAS) remains a widely used technique for elemental analysis when appropriate calibration, blanks, sensitivity and matrix controls are applied. All the prepared samples were submitted to centralized resources laboratory (CRL), University of Peshawar, KPK for atomic absorption spectrophotometric determination of Pb, Ni, Zn, Cr and Cu. The analytical labels included Pb 283.3, Ni 232.0, Zn 213.9, Cr 357.9 and Cu 324.8, interpreted as element-specific wavelengths in nanometres. Results were reported in mg/L, with a mean, standard deviation (SD) and percentage relative standard deviation (RSD) for each analytical label. These concentrations are treated as digest-solution values rather than tissue concentrations.

3.5 Data Presentation and Interpretive Scope

No contamination index, hazard quotient, bioaccumulation factor (BAF), bioconcentration factor (BCF) or biota–sediment accumulation factor (BSAF) was calculated. These indices require compatible numerator and denominator data, typically tissue concentrations expressed per mass and matched environmental concentrations with clearly defined units and fractions. Applying such equations to digest mg/L values would produce dimensionally inconsistent or biologically misleading results.

4. RESULTS AND DISCUSSION

4.1 Overall Pattern of Reported Digest Concentrations

Lead was by far the most abundant of the five metals in the snail tissue digests. Mean Pb concentrations in the six samples ranged from 1.53 to 5.73 mg/L (overall mean 3.67 mg/L), which is more than an order of magnitude higher than any other metal measured. The remaining metals followed the order Zn > Cr > Ni > Cu, with overall means of 0.195, 0.100, 0.070 and 0.064 mg/L, respectively (Tables 1–5; Figures 1–5).

Zn ranged from 0.146 to 0.295 mg/L, Cr from 0.089 to 0.110 mg/L, Ni from 0.044 to 0.090 mg/L and Cu from 0.041 to 0.096 mg/L. When compared with the WHO (2022) drinking-water guideline values (Table 7), Pb, Cr and Ni were above the guideline in all six samples, whereas Zn and Cu were well below their reference values. The WHO values are used here as a screening reference to identify the metals of greatest concern (Section 4.8). All six samples were prepared from the same pooled collection of snails, so the similarity among them mainly reflects their common origin rather than uniform conditions along the river.

4.2 Lead (Pb) Concentration in Snail Soft Tissue Digests

Table 1: Reported Pb results for soft tissue digests of Physella acuta at the analytical label 283.3.

Sample label	Mean (mg/L)	Reported SD (mg/L)	Reported RSD (%)
Sample 1	1.53	0.288	18.86
Sample 2	3.96	0.686	17.32
Sample 3	1.92	1.092	56.89
Sample 4	4.45	1.11	24.96
Sample 5	5.73	3.126	54.53
Sample 6	4.44	0.101	2.28

The results are presented as the mean lead (Pb) concentration (mg/L) along with the standard deviation (SD) and relative standard deviation (RSD) for each sample of soft tissues digests of Physella acuta.

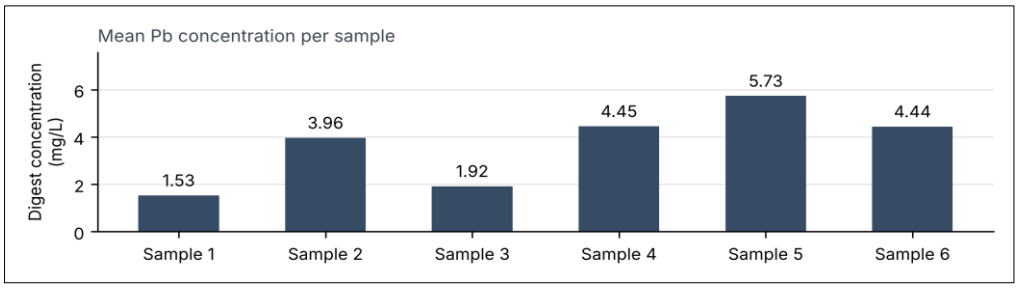


Figure 1: Mean Pb concentrations (mg/L) in soft-tissue digests of Physella acuta (Samples 1–6)

Pb concentrations ranged from 1.53 mg/L in Sample 1 to 5.73 mg/L in Sample 5, and four of the six samples (Samples 2, 4, 5 and 6) were above 3.9 mg/L (Table 1; Figure 1). Every sample was more than 150 times higher than the WHO drinking-water guideline of 0.010 mg/L. Although a digest concentration is not directly equivalent to a water concentration, the size of this difference makes Pb the clearest signal in the dataset. Variation between replicate readings was high in Sample 3 (RSD 56.89%) and Sample 5 (54.53%), whereas Sample 6 was very consistent (2.28%).

Accumulation of Pb in freshwater snails is well documented. Mahmoud and Abu Taleb [2013] found Pb in snail tissues collected together with field water, and Mullen *et al.,* [2025] related Pb in snail tissues and shells to the concentrations in water and sediment. Spyra *et al.,* [2019] showed that Pb levels in *P. acuta* differ between the soft body and the shell and between size classes. Pb has no known biological function and can disturb several physiological systems in aquatic invertebrates, so its presence at these levels in a grazing snail is a concern. In the present study it is not possible to say whether the Pb came mainly from the water, from ingested sediment and periphyton, or partly from material remaining in the gut, because water and sediment were not analysed at the same time.

The high Pb levels are consistent with earlier work in the region. Studies along the Kabul River system have reported Pb in water, sediments and fish, particularly near sewage and industrial discharges [Khan *et al.,* 2016; Ali & Khan, 2018; Ahmad *et al.,* 2015], and Ali *et al.,* [2020] found Pb in water, sediment and fish of the Shah Alam River itself. Freshwater snails have also recently been used as Pb biomonitors elsewhere in Pakistan [Tariq *et al.,* 2026]. The present results add snail tissue to this picture and indicate that Pb should be the first priority in future monitoring of the river.

The high RSDs in Samples 3 and 5 may reflect an uneven distribution of Pb within the tissue portions, incomplete homogenization before digestion, or instrumental variation between readings. Stating whether the SD is based on repeated instrument readings or on replicate digestions would make this variation easier to interpret in future studies.

4.3 Nickel (Ni) Concentration in Snail Soft Tissue Digests

Table 2: Reported Ni results for soft tissue digests of Physella acuta at the analytical label 232.0

Sample label	Mean (mg/L)	Reported SD (mg/L)	Reported RSD (%)
Sample 1	0.044	0.1017	541.78
Sample 2	0.077	0.0476	62.15
Sample 3	0.056	0.0978	1373.9
Sample 4	0.064	0.0499	78.09
Sample 5	0.09	0.0412	434.7
Sample 6	0.087	0.1268	488.03

The results are presented as the mean Nickel (Ni) concentration (mg/L) along with the standard deviation (SD) and relative standard deviation (RSD) for each sample of soft tissues digests of Physella acuta.

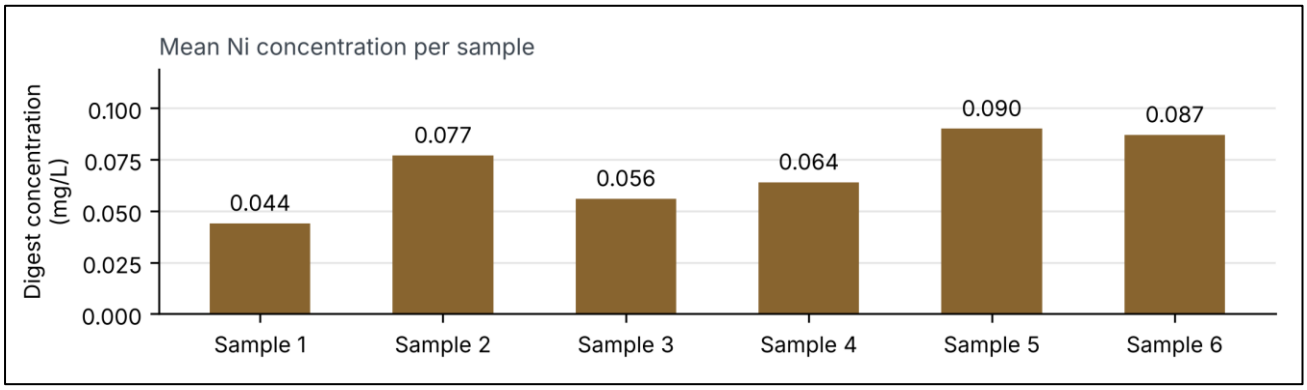


Figure 2: Mean Ni concentrations (mg/L) in soft-tissue digests of Physella acuta (Samples 1–6)

Nickel was detected in all six samples, with mean concentrations between 0.044 mg/L (Sample 1) and 0.090 mg/L (Sample 5) (Table 2; Figure 2). Samples 2 (0.077 mg/L), 5 (0.090 mg/L) and 6 (0.087 mg/L) were above the WHO drinking-water guideline of 0.070 mg/L, Sample 4 (0.064 mg/L) was close to it, and the mean of all six samples (0.070 mg/L) was equal to the guideline value. Together with Pb and Cr, Ni is therefore one of the three metals in this study whose concentrations reached or exceeded the WHO reference value, and on average it was present at higher concentrations than Cu.

Nickel accumulation has been reported in several freshwater gastropods. Al-Warid *et al.,* [2020] measured Ni together with Cd, Pb and Cu in *P. acuta* and *Bellamya bengalensis* from the Tigris River and

concluded that both species are useful bioindicators, and Mullen *et al.,* [2025] found Ni accumulation in the soft tissues and shells of invasive mystery snails. Orabi and Khalifa [2020] showed that Ni accumulation in *Melanoides tuberculata* differed from that of other metals, which means that each metal has to be assessed on its own rather than inferred from the behaviour of the others.

Ni is not regarded as an essential nutrient for freshwater gastropods, and exposure experiments have shown that it can be toxic to snails, with effects depending on concentration, exposure time, species and life stage [Shuhaimi-Othman *et al.,* 2012; Yap *et al.,* 2023]. Those experiments used known waterborne concentrations and cannot be compared directly with tissue digests. Nevertheless, the presence of Ni in every sample, at or above the WHO reference value in half of them, shows that the snails at this site are exposed to a measurable Ni load. Industrial effluent, municipal wastewater and the weathering of Ni-bearing rocks are possible sources in the catchment, but they could not be distinguished in this study.

4.4 Zinc (Zn) Concentration in Snail Soft Tissue Digests

Table 3: Reported Zn results for soft tissue digests of Physella acuta at the analytical label 213.9

Sample label	Mean (mg/L)	Reported SD (mg/L)	Reported RSD (%)
Sample 1	0.184	0.0048	2.58
Sample 2	0.161	0.0273	16.96
Sample 3	0.146	0.0033	2.24
Sample 4	0.295	0.0096	3.24
Sample 5	0.222	0.0046	2.08
Sample 6	0.161	0.0191	11.85

The results are presented as the mean Zinc (Zn) concentration (mg/L) along with the standard deviation (SD) and relative standard deviation (RSD) for each sample of soft tissues digests of Physella acuta.

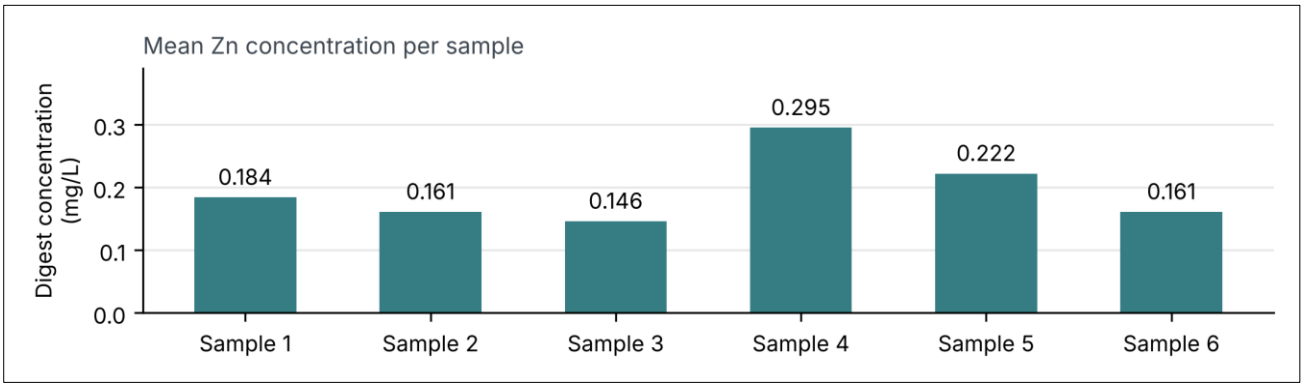


Figure 3: Mean Zn concentrations (mg/L) in soft-tissue digests of Physella acuta (Samples 1–6)

Zinc ranged from 0.146 mg/L (Sample 3) to 0.295 mg/L (Sample 4), with Samples 2 and 6 both at 0.161 mg/L (Table 3; Figure 3). It was the second most abundant metal after Pb, but all values were far below the WHO acceptability level of about 3 mg/L. Replicate readings were consistent in Samples 1, 3, 4 and 5 (RSD 2.08–3.24%) and more variable in Sample 2 (16.96%) and Sample 6 (11.85%).

Zinc is an essential trace element involved in enzyme activity, protein structure and cellular metabolism, so its presence in snail tissue is expected. Snails regulate Zn more actively than non-essential metals such as Pb, but high exposure can still be stressful: Moolman *et al.,* [2007] showed that Zn exposure altered cellular energy allocation in freshwater gastropods.

Part of the Zn in gastropod tissue is bound to metallothioneins, which take part both in normal metal homeostasis and in detoxification [Dallinger, 2024]. The Zn concentrations measured here therefore reflect the snail's own regulation as well as environmental exposure, and without a reference site it is not possible to say whether they are above normal background levels for *P. acuta*. The higher value in Sample 4 may simply reflect natural variation between tissue portions.

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4.5 Chromium (Cr) Concentration in Snail Soft Tissue Digests

Table 4: Reported Cr results for soft tissue digests of Physella acuta at the analytical label 357.9

Sample label	Mean (mg/L)	Reported SD (mg/L)	Reported RSD (%)
Sample 1	0.096	0.0164	17.05
Sample 2	0.096	0.0115	11.98
Sample 3	0.089	0.0115	12.95
Sample 4	0.101	0.0232	25.01
Sample 5	0.106	0.0167	15.78
Sample 6	0.110	0.0408	37.19

The results are presented as the mean Chromium (Cr) concentration (mg/L) along with the standard deviation (SD) and relative standard deviation (RSD) for each sample of soft tissues digests of Physella acuta.

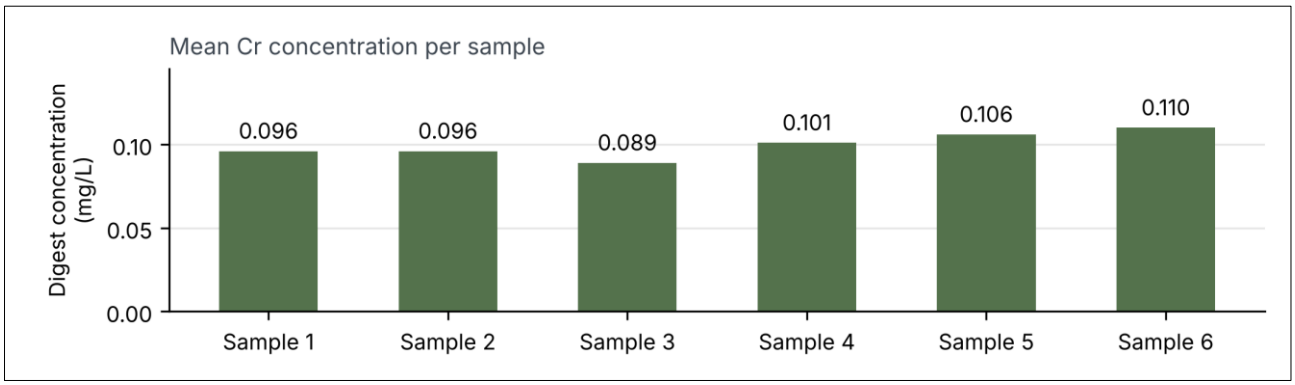


Figure 4: Mean Cr concentrations (mg/L) in soft-tissue digests of Physella acuta (Samples 1–6)

Chromium showed the narrowest range of all the metals, from 0.089 mg/L (Sample 3) to 0.110 mg/L (Sample 6), and Samples 1 and 2 had identical means of 0.096 mg/L (Table 4; Figure 4). All six samples were above the WHO guideline of 0.050 mg/L for total chromium, by a factor of about 1.8 to 2.2. RSDs ranged from 11.98% to 37.19%. For Sample 4, the reported mean and SD correspond to an RSD of about 22.97% rather than the 25.01% shown in the table, so this entry should be checked against the original readings.

The AAS method used here measures total chromium and does not distinguish between Cr(III) and Cr(VI), which differ greatly in toxicity and environmental behaviour. The WHO guideline also refers to total chromium [World Health Organization, 2022]. The results therefore show that chromium is elevated in the snail tissue, but not which form is present; speciation analysis would be needed to assess the risk from hexavalent chromium specifically.

Chromium has previously been reported in surface water of the Shah Alam River [Khan *et al.,* 2011] and in water, sediments and fish of the Kabul River system [Khan *et al.,* 2016; Ali & Khan, 2018; Ali *et al.,* 2020]. Because these studies measured different matrices in different years, they cannot show whether Cr levels have changed over time, but they do indicate that Cr is a persistent part of metal contamination in this river network. Identifying its sources would require sampling upstream and downstream of known discharge points.

4.6 Copper (Cu) Concentration in Snail Soft Tissue Digests

Table 5: Reported Cu results for soft tissue digests of Physella acuta at the analytical label 324.8

Sample label	Mean (mg/L)	Reported SD (mg/L)	Reported RSD (%)
Sample 1	0.076	0.0035	4.61
Sample 2	0.041	0.0109	26.84
Sample 3	0.048	0.005	10.42
Sample 4	0.076	0.0055	7.2
Sample 5	0.096	0.0032	3.38
Sample 6	0.045	0.0052	11.48

The results are presented as the mean Copper (Cu) concentration (mg/L) along with the standard deviation (SD) and relative standard deviation (RSD) for each sample of soft tissues digests of Physella acuta.

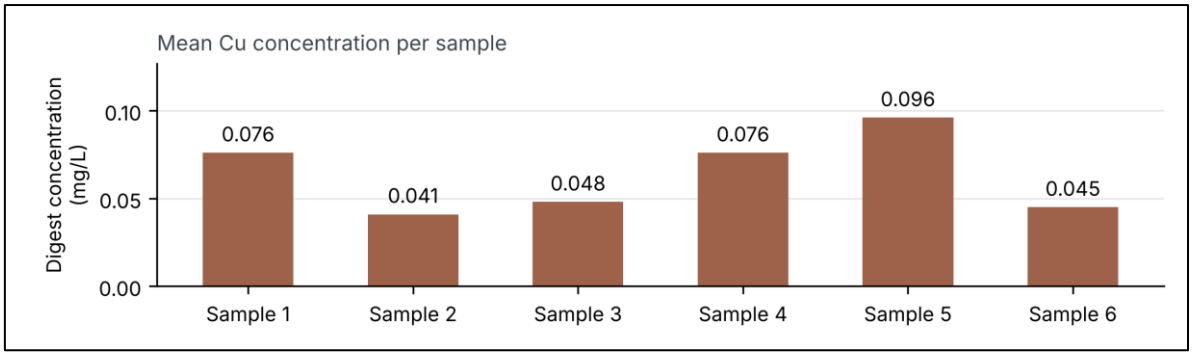


Figure 5: Mean Cu concentrations (mg/L) in soft-tissue digests of Physella acuta (Samples 1–6)

Copper had the lowest overall mean of the five metals (0.064 mg/L). Concentrations ranged from 0.041 mg/L (Sample 2) to 0.096 mg/L (Sample 5), with Samples 1 and 4 both at 0.076 mg/L and Sample 6 at 0.045 mg/L (Table 5; Figure 5). All values were far below the WHO guideline of 2.0 mg/L. Replicate readings were consistent in most samples (RSD 3.38–11.48%), the exception being Sample 2 (26.84%).

Copper is an essential element for molluscs, notably as part of the oxygen-carrying pigment haemocyanin, and gastropods regulate it closely, partly through metallothioneins [Dallinger, 2024]. This regulation probably helps to keep tissue Cu within a narrow range. Low tissue Cu should not, however, be read as evidence that Cu poses no risk: in exposure experiments Cu was among the most toxic metals to *Melanoides tuberculata* and *Pomacea insularum* [Shuhaimi-Othman *et al.,* 2012; Yap *et al.,* 2023], and its bioavailability in water depends strongly on hardness and dissolved organic matter.

The low variability of most Cu results also suggests that the digestion and AAS procedure produced reproducible measurements, which makes Cu a useful reference analyte for checking analytical performance in future surveys.

4.7 Freshwater-Snail Biomonitoring: Lessons from the Literature

Studies from different regions agree that freshwater snails accumulate metals and can be useful biomonitors, but they also show that results depend on species, body size, tissue type and the environmental matrix sampled (Table 6). Spyra *et al.,* [2019] found that metal levels in *P. acuta* differed between soft tissue and shell and between size classes. Li *et al.,* [2018] combined gastropod, water and sediment measurements to evaluate river remediation, Orabi and Khalifa [2020] calculated both bioconcentration and biota–sediment accumulation factors, and Mullen *et al.,* [2025] compared tissues, shells, water and sediment and examined possible trophic transfer.

These studies also show that metals do not all behave in the same way. Essential metals such as Zn and Cu are regulated, non-essential metals such as Pb can be stored, and the relationship between environmental and tissue concentrations varies from one element to another [Chukaeva & Petrov, 2023; Li *et al.,* 2018]. *P. acuta* is therefore most useful as one part of a monitoring programme that also includes water and sediment, rather than as a replacement for them.

Table 6: Selected freshwater-gastropod studies relevant to metal biomonitoring. Concentrations are not directly comparable between studies

Study	Snail taxon	Matrices / design	Metals	Relevance to present study
Mahmoud & Abu Taleb, 2013	Multiple freshwater snails including *P. acuta*	Water + whole snail tissues	Cd, Cu, Pb	Demonstrated accumulation and value of matched water/biota measurements.
Spyra *et al.,* 2019	*Physella acuta*	Body + shell + environmental variables	Cd, Pb, Cu, Zn	Showed compartment- and size-class-dependent accumulation.
Al-Warid *et al.,* 2020	*P. acuta*; *Bellamya bengalensis*	Soft tissue	Cd, Ni, Pb, Cu	Supported use of aquatic snails as metal bioindicators.
Orabi & Khalifa, 2020	*Melanoides tuberculata*	Soft tissue + water + sediment	Mn, Fe, Ni, Cu, Zn, Cd, Pb	Applied BCF and BSAF; demonstrated metal-specific behaviour.
Li *et al.,* 2018	*Bellamya aeruginosa*	Soft tissue + shell + water + sediment	Multiple trace metals	Integrated biota with environmental matrices during remediation assessment.

Study	Snail taxon	Matrices / design	Metals	Relevance to present study
Chukaeva & Petrov, 2023	*Viviparus* spp.	Shell + water + sediment	Multiple metals	Showed hydrology- and metal-dependent accumulation in urban rivers.
Yap *et al.,* 2023	*Pomacea insularum*	Controlled exposure	Cd, Cu, Ni, Pb, Zn	Demonstrated metal-, duration- and life-stage-dependent toxicity.
Mullen *et al.,* 2025	Invasive mystery snails	Soft tissue + shell + water + sediment	Cd, Cu, Ni, Pb, Zn	Evaluated accumulation factors and maternal/trophic-transfer implications.
Tariq *et al.,* 2026	*Indoplanorbis exustus*; *Lymnaea acuminata*	Soft tissue + water	Pb	Recent Pakistan evidence supporting snail-based Pb biomonitoring.

4.8 Guideline Context and Matrix Compatibility

Table 7 lists the WHO (2022) drinking-water guideline values used as reference values in this study. These values were developed for drinking water, not for animal tissue or acid digests, and no tissue-specific guideline exists for *P. acuta*. They are therefore used here as a screening reference to identify the metals of greatest concern. On this basis, Pb, Cr and Ni were the metals of concern, whereas Zn and Cu were well within their reference values.

Table 7: WHO (2022) drinking-water guideline values used as reference values in this study

Metal	WHO 2022 values (mg/L)	Meaning and applicability
Pb	0.010	Provisional drinking-water guideline; not a tissue or digest limit.
Ni	0.070	Drinking-water guideline; not a tissue or digest limit.
Zn	No health-based value	Above approximately 3 mg/L may be unacceptable to consumers; not a tissue limit.
Cr	0.050	Guideline for total chromium in drinking-water; not a tissue or digest limit.
Cu	2.000	Drinking-water guideline; not a tissue or digest limit.

Source: *World Health Organization [2022]. Values apply to drinking water and are used here only as screening references.*

A stricter comparison would require tissue concentrations expressed per unit tissue mass (mg/kg wet or dry weight) compared with tissue data for similar species, or river-water samples compared with water guidelines. The digest concentrations reported here depend both on the metal content of the tissue and on the volume of acid and water used in preparation. Converting them to mg/kg tissue concentrations, and measuring metals in the river water and sediment at the same sites, would allow a more direct assessment of risk in future work.

4.9 Regional Context and Ecological Interpretation

Previous work on the Shah Alam River has measured metals in surface water [Khan *et al.,* 2011] and in water, sediment and fish muscle [Ali *et al.,* 2020], and studies across the wider Kabul River system have linked elevated metal levels to sewage and industrial discharges [Khan *et al.,* 2016; Ali & Khan, 2018; Ahmad *et al.,* 2015; Siraj *et al.,* 2018]. A national review also identified metal contamination in the water, sediments and fish of several major Pakistani rivers [Afzaal *et al.,* 2022]. The elevated Pb, Cr and Ni found in *P. acuta* in the present study are consistent with this regional pattern.

The sources of these metals were not investigated directly. Industrial effluent, untreated household wastewater, road runoff and natural weathering of rocks are all plausible contributors. Separating them would require sampling at an upstream reference site and at stations below identified outfalls, together with measurements of sediment grain size and organic matter, which strongly influence metal retention [Khan *et al.,* 2016].

Because all snails were collected within a single week in February, the results represent winter conditions only. Seasonal changes in river flow can dilute or concentrate dissolved metals and resuspend contaminated sediment, so sampling in different seasons would be needed to judge whether the levels observed here are typical of the river.

4.10 Ecological Meaning of Bioaccumulation and Metal Regulation

Bioaccumulation and toxicity are related but not the same. A snail can store large amounts of a metal in bound, relatively inert forms and still survive, whereas another metal may cause harm at much lower tissue levels if it interferes with sensitive physiological processes. Metallothionein binding in gastropods is a good example of this difference [Dallinger, 2024].

For essential metals such as Zn and Cu, regulation means that tissue concentrations may change little until exposure becomes high enough to overwhelm the snail's control mechanisms. Non-essential metals such as Pb and Ni are not regulated in the same way and

tend to reflect exposure more directly, which is one reason why they are particularly informative in biomonitoring. Comparisons between the concentrations of different metals should be made with these differences in mind.

4.11 Potential Value of *Physella acuta* as a Local Sentinel

P. acuta has several features that make it practical for local monitoring: it is abundant, easy to collect, much less mobile than fish, and has an established record in metal accumulation studies [Spyra *et al.,* 2019; Baroudi *et al.,* 2020]. It was also the dominant snail at the study site, which made it possible to collect enough tissue in a short time. The present results show that it accumulates measurable amounts of all five metals studied.

To confirm its value as a sentinel, future work should test whether tissue concentrations in *P. acuta* follow water and sediment concentrations across sites and seasons, whether different size classes accumulate metals differently, and how much variation occurs within a site. Comparison with another common local snail would show whether *P. acuta* is unusually tolerant or accumulates metals more readily than other species.

Biological effect measures such as oxidative-stress markers, metallothionein levels or condition indices could be added to the chemical analysis, although they also respond to stressors other than metals and need proper controls [Gnatyshyna *et al.,* 2023].

4.12 Data Quality, Traceability and Reproducibility

Several improvements in data handling would strengthen future work. Each reported value should be traceable to the raw instrument readings, the tissue mass, the final digest volume and dilution, and the calibration and blank corrections applied. Values below the detection or quantification limit should be flagged as such rather than reported as zero, and RSDs should be calculated from unrounded means and SDs.

Quality control should include reagent and procedural blanks, duplicate digestions, regular calibration checks and a certified reference material or spike recovery test, in line with established AAS practice [United States Environmental Protection Agency, 2007]. These checks make it possible to separate real differences between samples from contamination, losses during digestion or instrument drift.

4.13 Recommended Follow-up Monitoring Design

A follow-up study could use three to five mapped stations, including an upstream reference site, reaches influenced by urban or industrial discharges, and downstream sites. At each station, several independent pools of snails of a defined size class should be collected together with water and surface sediment. Snails should be rinsed, depurated, measured and analysed on a defined wet- or dry-weight basis, with shells and soft tissue analysed separately if partitioning is of interest.

Water samples should be analysed for pH, conductivity, hardness, dissolved oxygen, temperature and, where possible, dissolved organic carbon, because these variables control metal bioavailability. Sediment analysis should include grain size and organic matter [Khan *et al.,* 2016].

Sampling in at least two contrasting seasons, repeated at the same stations in later years, would allow bioconcentration and biota–sediment accumulation factors to be calculated, spatial and seasonal patterns to be tested, and the reliability of *P. acuta* as a biomonitor to be assessed [Li *et al.,* 2018; Orabi & Khalifa, 2020; Mullen *et al.,* 2025]. The minimum design elements are summarized in Table 8.

Table 8: Minimum design elements recommended for a confirmatory Shah Alam River snail-biomonitoring study

Component	Recommended approach	Reason
Field replication	Mapped stations with independent snail pools	Supports spatial inference and valid statistics.
Snail characterization	Species voucher, shell size, pooled count, wet/dry mass basis	Controls biological variation and improves cross-study comparison.
Depuration / washing	Standardized and documented	Reduces uncertain contribution from gut contents and adhering particles.
Environmental matrices	Matched water and sediment at same stations and dates	Enables BCF/BSAF and source-pathway interpretation.
Water chemistry	pH, hardness, conductivity, temperature, dissolved oxygen; DOC if feasible	Improves interpretation of metal bioavailability.
Analytical QA/QC	Blanks, duplicates, calibration checks, CRM or spike recovery, LOD/LOQ	Separates environmental signal from analytical bias or imprecision.
Reporting	Final digest volume, dilution factor, tissue concentration in mg/kg	Provides a biologically interpretable concentration basis.
Temporal design	Contrasting seasons and repeated years	Allows seasonal and trend assessment.
Optional biology	Condition indices or validated biomarkers	Adds effect information without replacing chemical measurements.

4.14 Analytical and Study Limitations

The main limitation of this study is that all six samples were prepared from a single pooled collection of snails taken from one area of about 100 m² during one week. The samples are therefore replicate portions of the same material rather than independent field replicates, and the results cannot be used to compare contaminated and uncontaminated sites, to describe variation along the river, or to identify seasonal or long-term trends.

Analytical quality control was not fully documented. Calibration data, procedural blanks, recovery tests, certified reference materials and detection limits were not reported, and these would be needed to confirm the accuracy of the measured concentrations [United States Environmental Protection Agency, 2007]. The Cr result for Sample 4 should also be checked against the original instrument readings.

Preservation in 70% ethanol before analysis, the absence of a depuration step and the lack of a defined wet- or dry-weight basis may have affected the measured concentrations and limit comparison with other studies. Metals can leach into the preservative, and gut contents and attached particles may add to the measured values. The final digest volume and dilution factor are also needed to convert the mg/L results into mg/kg tissue concentrations. No water, sediment or shell samples were analysed, so accumulation factors could not be calculated.

Without a reference site it is also not possible to separate human inputs from natural background levels, since metals can enter rivers through the weathering of local rocks and essential metals are always present in living tissue.

Finally, no biological effects such as mortality, growth, reproduction or biochemical markers were measured, so the results describe metal occurrence in the snails rather than their health. Statements about toxicity in this paper are based on published studies and should be read as context rather than as observed effects in the collected *P. acuta*.

5. CONCLUSION

This study measured Pb, Ni, Zn, Cr and Cu in soft-tissue digests of *Physella acuta* collected from the Shah Alam River, Peshawar, in February 2023. Pb was by far the most abundant metal (1.53–5.73 mg/L), followed by Zn (0.146–0.295 mg/L), Cr (0.089–0.110 mg/L), Ni (0.044–0.090 mg/L) and Cu (0.041–0.096 mg/L). Compared with WHO (2022) drinking-water guideline values used as a screening reference, Pb and Cr were above the guideline in all six samples and Ni was above it in half of the samples, with a mean equal to the guideline value, whereas Zn and Cu remained well within their reference values.

These results indicate that *P. acuta* in the Shah Alam River is exposed to elevated levels of Pb, Cr and Ni, and they support the potential use of this snail as a biomonitor of metal pollution. Because the values were measured in tissue digests rather than in river water, and because water, sediment and complete quality-control data were not available, they should be regarded as a preliminary assessment. A follow-up study using mapped reference and impacted stations, independent snail pools, a defined tissue mass basis, matched water and sediment samples, full AAS quality control and repeated seasonal sampling would allow tissue concentrations, accumulation factors and ecological risk to be determined, and would confirm whether *P. acuta* can serve as a reliable local sentinel of metal pollution.

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