

Accumulation of As, Cd, Pb, and Zn in sediment, chironomids and fish from a high-mountain lake: First insights from the Carnic Alps

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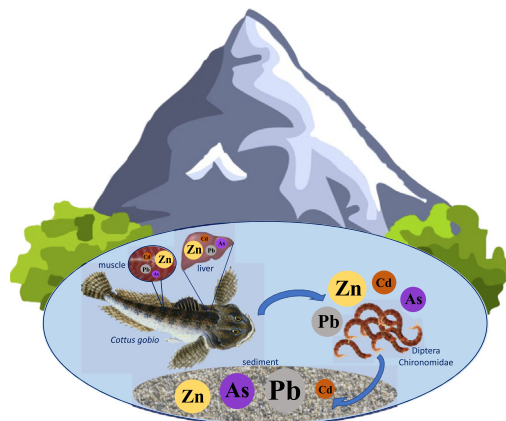
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HIGHLIGHTS

- Levels of As, Cd, Pb, and Zn were detected in a high-mountain lake dietary pathway.
- Trace element concentration decreased with increasing trophic level.
- The highest BAF values were recorded for Cd and Zn in Diptera Chironomidae.
- Very low TTF values were recorded in fish muscle and liver.
- Diptera Chironomidae seems to be a good matrix for trace element monitoring.

GRAPHICAL ABSTRACT



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ABSTRACT

Though mountain lakes are generally much less influenced by human activities than other habitats, anthropogenic threats can still alter their natural condition. A major source of global environmental pollution in mountain ecosystems is trace element contamination. For this study we investigated for the first time the accumulation of As, Cd, Pb, and Zn in sediment, Diptera Chironomidae (prey), and bullhead *Cottus gobio* (predator) in a typical high-mountain lake (Dimon Lake) in the Carnic Alps. Significant differences in trace element levels were observed between sediment, Diptera Chironomidae, and *C. gobio* liver and muscle samples (Kruskal-Wallis test; $p < .03$ for all elements). As and Pb levels were highest in sediment, Cd and Zn levels were highest in Diptera Chironomidae, and the lowest values for all elements were measured in *C. gobio* muscle and liver. Bioaccumulation factor values were much higher in Diptera Chironomidae than fish muscle and liver, with the highest values recorded for Cd (5.16) and Zn (4.37). Trophic transfer factor values were very low for all elements in fish muscle and liver, suggesting a biodilution effect along the food chain. Further studies are needed to expand on these first findings that provide useful insights to inform environmental monitoring and policy in remote high-mountain lakes.

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1. Introduction

High-mountain lakes are among the most remote aquatic environments in Europe (Catalan et al., 2006). As such, they provide a natural laboratory for ecological assessment, since their food webs are relatively simple in structural network compared to lowland lakes (Sánchez-Hernández et al., 2015). Although remote, their small size and high turnover of surface waters render mountain catchments extremely receptive and vulnerable to anthropogenic impact (Pastorino et al., 2019a). Since the 1980s, they have been affected by global anthropogenic impacts and have become a receptor for medium-range atmospheric transported (MRAT) organic and inorganic contaminants (Ferrario et al., 2017; Pastorino et al., 2020a). In the European Alps, altitudinal transport can occur over relatively short distances from sources of pollution in the industrialized areas of Germany, Switzerland, Austria, and northern Italy (Poma et al., 2017).

Aquatic ecosystems host well-adapted species and are a reservoir for organic and inorganic contaminants (Fleeger et al., 2003). Trace elements contamination is a major problem due to the persistence and accumulation of metals in the biotic and abiotic components of aquatic ecosystems (Purves, 2012; Esposito et al., 2018). Trace elements enter the aquatic environment from a variety of sources. Although most occur naturally through biogeochemical cycles, rapid industrialization has accelerated their dispersion in the environment through human activities, especially the combustion of fossil fuels (Förstner and Wittman, 1981; Borrell et al., 2016). Furthermore, trace elements attached to fine aerosols can be transported hundreds of kilometers away from the original source, washed out into the aquatic ecosystems during precipitation events, and contaminate the water column and the sediment (Pan and Wang, 2015). Trace element levels have increased in remote areas as a result of atmospheric deposition, solubilization, and mobilization of sediments which form the major sink for environmental contaminants (Karadede-Akin and Ünlü, 2007) that aquatic organisms can take up (Priju and Narayana, 2007). High-mountain lakes are therefore excellent indicators of air pollution because they are not usually subject to other forms of disturbance (e.g., land-use) (Tornimbeni and Rogora, 2012).

Moreover, due to climatic and geographical factors, high-mountain lakes may be more vulnerable to contamination than lowland lakes (Mosello et al., 2002; Catalán et al., 2009; Rogora et al., 2013). Research in European mountain areas has focused on organic pollutants in abiotic compartments (Vilanova et al., 2001), however, limited information is available concerning trace element bioaccumulation in aquatic organisms (Köck et al., 1996; Yang et al., 2007; Pastorino et al., 2019a, 2020a). Far more is known about other ecosystems, especially the accumulation of trace elements by fish (Wagner and Boman, 2003; Elia et al., 2010; Squadrone et al., 2013, 2016) and macrobenthic invertebrates (Goodyear and McNeill, 1999; Santoro et al., 2009; Pastorino et al., 2019b) in freshwater ecosystems. In addition, trace elements trapped in sediments can enter the food web through organisms taken as part of the diet, e.g., fish that prey on benthic organisms (Alhashemi et al., 2012; Zulkifli et al., 2016; Palacios-Torres et al., 2020).

Fish are at the top of the trophic chain and can accumulate large amounts of certain trace elements (Squadrone et al., 2013; Avigliano et al., 2019). The accumulation patterns of contaminants in fish and other aquatic organisms are driven by uptake and elimination rates (Güven et al., 1999). Trace elements and their compounds are taken up differentially by organs because of the affinity between them and are found at different concentrations in various organs of the body (Bervoets et al., 2001). Fish assimilate trace elements via several routes: ingestion of particulate material suspended in water, ingestion of food, ion-exchange of dissolved metals across lipophilic membranes (e.g., the gills, and adsorption via tissue and membrane surfaces). Their distribution in tissues depends on dietary or aqueous exposure or a sum of the two (Jeziarska and Witeska, 2006; Hauser-Davis et al., 2012; Pouil et al., 2018). However, in high-mountain lakes the

concentrations of heavy metals in water are often not detectable. For example, Tornimbeni and Rogora (2012) showed how Pb and Cd concentration values in water from 32 Alpine lakes were often below the detection limits of the method (0.08 and 0.01 $\mu\text{g L}^{-1}$, respectively). Thus, waterborne metals uptake can be considered negligible in these environments, especially in alkaline water where metals accumulate in sediments (Pobi et al., 2019).

Though fish muscle is not a target tissue for accumulation during acute exposure, it is a good indicator of chronic exposure (Has-Schön et al., 2006; Taweel et al., 2012). When contaminants exceed all biological defense barriers, trace elements begin to accumulate in muscle tissue (Kalay et al., 1999). But because muscle tissue is not always a good indicator of trace element accumulation in the entire body, other organs such as the liver need to be analyzed as well (Has-Schön et al., 2006).

No studies to date have investigated trace element accumulation from sediment to fish in high-mountain lakes in Alps. The aim of the present study was: a) to measure the levels of As, Cd, Pb, and Zn in sediment, the whole body of macrobenthic invertebrates (prey), and fish (predator) muscle and liver tissues; b) to determine the difference in trace element levels between these matrices; c) to evaluate the bioaccumulation factor (BAF) and the trophic transfer factor (TTF) values for prey and predator in a typical high-mountain lake (Dimon Lake) located in northeast Alps. The four trace elements (As, Cd, Pb, and Zn) were chosen based on available data for high-mountain lakes (Camarero et al., 2009; Pastorino et al., 2020a) and on their relevance for trophic levels in freshwater environments (Chernova and Lysenko, 2019).

2. Material and methods

2.1. Study site

Dimon Lake (46° 34' 4.17" N 13° 03' 43.12" E; Fig. 1) is a high-mountain lake located above the tree line in the Carnic Alps (municipality of Ligosullo, Udine Province, Friuli Venezia-Giulia, northeast Italy) at 1857 m a.s.l. Dimon Lake is a glacial-origin lake and is classified as a Site of Community Interest and Special Areas of Conservation (SCI/SAC-IT3320002 Monti Dimon e Paularo). The lake lies on sandstone and volcanic rock and has a maximum depth of 4.27 m. Originally fishless, fish were released into the lake for recreational fishing (Pastorino et al., 2020b). The fish assemblage is composed only of bullhead (*Cottus gobio*) (Pastorino et al., 2020b). The macrobenthic community consists mainly of Diptera Chironomidae, Oligochaeta, and Hirudinea (Pastorino et al., 2019a). Physicochemical and nutrient values (summer 2017) were (mean \pm standard deviation): temperature (16.11 ± 0.42 °C); conductivity (109.25 ± 2.60 $\mu\text{S cm}^{-1}$); pH (8.59 ± 0.20); dissolved oxygen (8.72 ± 0.11 mg L^{-1}); NH_4^+ (0.09 ± 0.02 mg L^{-1}); NO_3^- (19.03 ± 5.81 mg L^{-1}); PO_4^{3-} (0.042 ± 0.04 mg L^{-1}) (Pastorino et al., 2019a). Surface sediment (0–2 cm) of Dimon Lake was mainly composed by silt (74%) followed by sand (17%) and clay (9%) (Perilli, 2018).

2.2. Sampling and analysis of surface lake sediment

On 31 July 2017 integrated samples of surface sediment (0–2 cm) from five sites in the littoral zone (Fig. 1) were collected with a plastic spatula, homogenized, divided in three replicates and stored at -20 °C. Following an adaptation from EPA Method 3052 (Environmental Protection Agency, 1996), oven dried sediment samples (0.3 g) were reduced to powder with an agate mill, placed in vessels, and added with a mixture of 5 mL of nitric acid and 1 mL of hydrogen peroxide. Mineralization was performed by microwave digestion for 55 min using a Multiwave PRO Anton Paar reaction system (Anton Paar, Graz, Austria). The samples were then centrifuged to remove residue, transferred to flasks, and filled to the mark with MilliQ water (25 mL final volume). Samples were diluted to 1:20 and concentration of As, Cd, Pb, and Zn was measured by inductively coupled

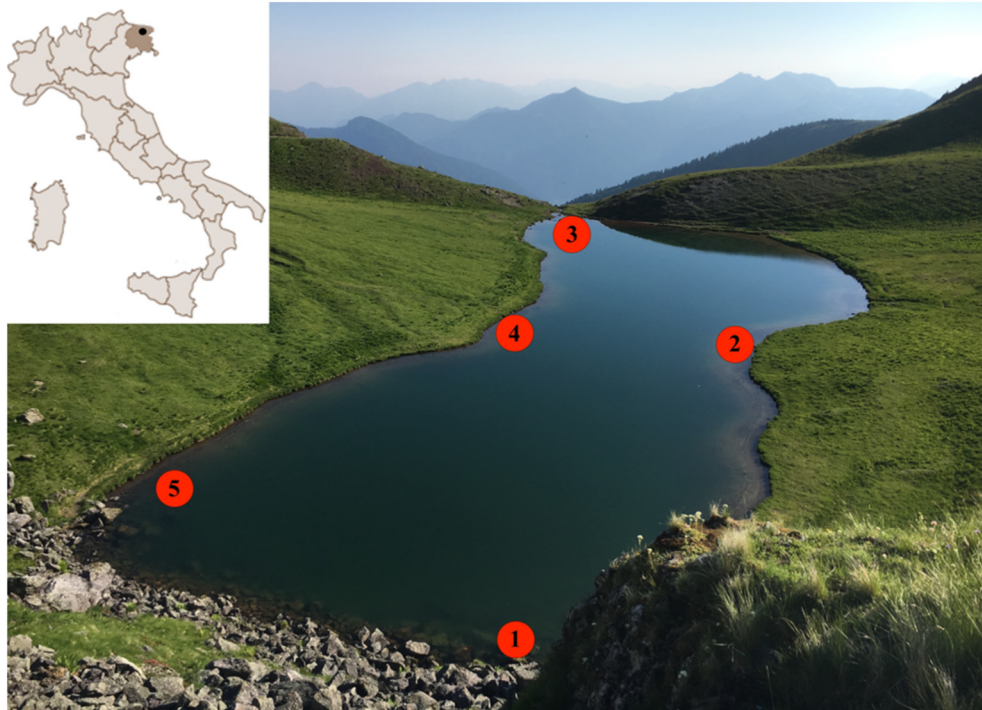


Fig. 1. Dimon Lake in Friuli Venezia-Giulia (northeast Italy) and sampling sites (1–5; red circles) for sediment and macrobenthic invertebrates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

plasma-mass spectrometry (ICP-MS NexION 350, PerkinElmer Inc., Waltham, MA, USA). Accuracy of the analytical procedures were evaluated through the analysis of the certified reference material (CRM 601) along with blank reagents in each analytical session. The measured concentrations fell within the range of certified values.

2.3. Macroinvertebrates sampling and detection of trace elements

Macrobenthic invertebrate sampling was carried out on 31 July 2017. The choice of mid-summer was dictated by the need to exploit the time in which the abundance and biomass of these organisms are greatest (Fjellheim et al., 2000, 2009). A Surber net (mesh 250 μm ; 0.1 m^2 subtended area) was used for sampling the macrobenthic invertebrates at five sites in the littoral zone (Fig. 1). Samples were sorted in the field and identified in the laboratory. Functional feeding guilds (FFG) according Merritt and Cummins (2006) were assigned for each taxon used for trace element determination. The samples were oven-dried at 70 °C for 72 h to a total dry weight of 900 mg which was divided in three subsamples of 300 mg each for trace element analysis. Levels of As, Cd, Pb, and Zn were measured by inductively coupled plasma-mass spectrometry (ICP-MS Xseries II, Thermo Scientific, Bremen, Germany) according to Squadrone et al. (2016). The uncertainty of measurements (As: 19%; Cd: 11%; Pb: 21%; Zn: 20%) were estimated according to an internal Standard Operative Procedure that follows the bottom up approach. Analytical performance was verified by processing certified reference materials (SRM 1566b), along with blank reagents in each analytical session. The analytical method was validated according to ISO/IEC 17025 (general requirements for the competence of testing and calibration laboratories).

2.4. Fish sampling and trace element detection

Fish were sampled using an electrofishing boat on the same day as macrobenthic invertebrates. The fish were sacrificed by deep anesthesia with tricaine methanesulfonate MS-222 (100 mg L^{-1}). Individuals were identified to the species level and measured for their total weight

(g) and total length (cm). The fish were put in cold boxes at 4 °C and transferred to laboratory where they were sectioned. The stomach contents were preserved in 70% alcohol to determine the frequency of prey occurrence (Fi) (Tiberti et al., 2016). Fish muscle ($n = 15$) and liver ($n = 15$) were sampled from each individual and stored at -20 °C. The fish samples were defrosted than homogenized and mineralized for trace element analysis. Trace element analysis, the uncertainty of measurements and analytical performance were performed as described for macrobenthic invertebrates. Permission for fish sampling was granted by the Ente Tutela Patrimonio Ittico del Friuli Venezia-Giulia (authorization no. 11/DIR/17/01/2017).

2.5. Statistical analysis

Kolmogorov-Smirnov test was used to determine whether our dataset was well-modeled by a normal distribution. Since the assumption of normality was not satisfied, the non-parametric Kruskal-Wallis test was used to check for significant differences in trace element levels in sediment, Diptera Chironomidae, and bullhead (muscle and liver). The Conover-Iman test was used as a post hoc test. Spearman's rank correlation coefficient was calculated to determine the relationship between trace element concentration in sediment, Diptera Chironomidae, and bullhead tissue. Principal component analysis (PCA) was performed to check for trends in trace element (As, Cd, Pb and Zn) accumulation in sediment, Diptera Chironomidae, and fish. Bioaccumulation factors (BAF) (Klavinš et al., 1998; Ruus et al., 2005) were calculated to assess trace element bioaccumulation in Diptera Chironomidae and bullhead muscle and liver tissue as follow:

$$\text{BAF} = \text{TE}_{\text{tissue}} / \text{TE}_{\text{sed}}$$

where, $\text{TE}_{\text{tissue}}$ is the trace element concentration in the tissue, and TE_{sed} is the trace element concentration in sediment.

The trophic transfer factor (TTF) was calculated for muscle and liver tissues. TTF is the ratio between trace element concentration in fish tissue (muscle and liver) and its concentration in the organism's food item

(DeForest et al., 2007). Statistical analysis was performed using RStudio software; a p-level of 0.05 was set to interpret significance of results.

3. Results

3.1. Surface sediment

Fig. 2 shows the trace element levels (mean \pm standard deviation) in the sediment samples from Dimon Lake. The mean concentration was in the order: Zn (138.8 ± 0.6) > Pb (109.6 ± 1.2) > As (39.5 ± 0.7) > Cd (0.61 ± 0.02) mg kg⁻¹.

3.2. Macrobenthic invertebrates

The macrobenthic invertebrate community was composed chiefly of Hexapoda belonging to Diptera Chironomidae (75.2%) with four subfamilies (Prodiamesinae, Chironominae, Orthocladiinae, and Tanypodinae). The Prodiamesinae subfamily was represented by the single species *Prodiamesa olivacea* (collector-gatherer-CG), while the subfamily Chironominae was almost entirely represented by the genus *Paratanytarsus* (CG). The Orthocladiinae subfamily was represented only by the genus *Cricotopus (Isocladius)* (CG), while the Tanypodinae subfamily by the genera *Macropelopia* (predator-P) and *Zavrelimyia* (P). Other taxa belonged to Oligochaeta (9.8%), and Hirudinea (14.4%). Only Diptera Chironomidae was investigated for As, Cd, Pb and Zn, since they are the sole prey found in the stomachs of bullhead. A pool of Chironomidae (whole body) was prepared for trace element analysis. The mean trace element concentration was in the order: Zn (606 ± 1.8) > Pb (49 ± 0.5) > As (10 ± 1.3) > Cd (3.2 ± 0.1) mg kg⁻¹ (Fig. 2).

3.3. Fish

Only individuals of bullhead (*Cottus gobio*) were captured from Dimon Lake. Fifteen were retained for trace element analysis. The average total length was 13.52 ± 1.29 cm and the average total weight was 28.63 ± 4.42 g. Analysis of stomach contents showed that the fish fed exclusively on Diptera Chironomidae larvae (Fi = 98.15%). The mean trace element concentration was in the order: Zn (25.3 ± 9.5) > As (0.3 ± 0.2) > Pb (0.06 ± 0.03) > Cd (0.023 ± 0.003) mg kg⁻¹ in muscle (Fig. 2), and Zn (85.6 ± 26.9) > Pb (0.3 ± 0.4) > As (1.6 ± 0.4) > Cd (0.03 ± 0.001) mg kg⁻¹ in liver (Fig. 2).

3.4. Comparisons, BAF and TTF factors

The Kruskal-Wallis test showed significant differences in trace element levels between sediment, Diptera Chironomidae, and fish tissues ($p < .002$ for all elements). The Conover Iman post-hoc test revealed a significant difference ($p < .01$) between sediment and fish tissues, between Diptera Chironomidae and fish tissues for all elements, and between fish muscle and fish liver for As, Pb and Zn ($p < .0001$) (Fig. 2). The first two principal components (Dim1; Dim2) in the PCA (Fig. 3) accounted for meaningful amounts of the total variance (99.6%): Dim1 showed 67.8% of the total variance and was positively correlated with all variables (As, Cd, Pb, and Zn). In detail, sediment samples located on the right side in order of increasing values of As and Pb. Diptera Chironomidae samples also located on the right side in order of increasing value of Cd and Zn. PCA clearly showed a separation between fish tissue samples (on the left) and Diptera Chironomidae and sediment samples (on the right) based on the difference in trace element accumulation, as already demonstrated by Kruskal-Wallis test.

Spearman's rank correlation coefficient showed no relationship between trace element concentration in the matrices. BAF factors (Table 1) were much higher in Diptera Chironomidae than in fish muscle and liver tissue, with the highest values recorded for Zn and Cd. The BAF values for muscle and liver tissues were lower than for Diptera Chironomidae. The TTF factor values were very low for all elements in both fish muscle and liver (Table 1).

4. Discussion

High-mountain lake are precious ecosystems located far from industrialized area and provide habitat for few, but well adapted species. They are a sink for contaminants from the industrialized lowland regions (Camarero, 2003, Camarero et al., 2009). Dimon Lake is a receptor of trace element contamination originating from anthropic activities, dispersed in the atmosphere, and deposited via abundant annual precipitation throughout the year (Pastorino et al., 2019a).

Camarero et al. (2009) measured the trace element concentration in sediment from 275 Alpine lakes (Piedmont, Ticino, Central, Tyrol, and Julian Alps). Arsenic levels ranged from 4 mg kg⁻¹ (Julian Alps) to 17 mg kg⁻¹ (Tyrolean Alps), much lower than the mean we recorded for Dimon Lake (39.52 mg kg⁻¹). Because it was beyond the scope, we didn't calculate the enrichment factors to assess the presence and intensity of anthropogenic contaminant deposition. Nevertheless, this high arsenic level can be attributed to both anthropogenic and lithological sources, since volcanic rock contains modest amounts of arsenic (Fuganti et al., 2005). The same trend was recorded for lead: the mean

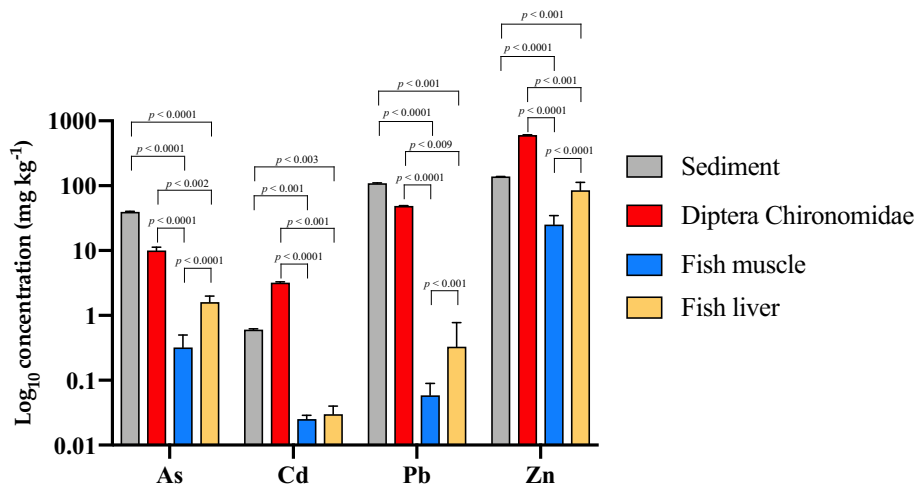


Fig. 2. Trace element concentration (Log₁₀) detected in sediment, Diptera Chironomidae, and fish muscle and liver samples. Results of post-hoc Conover Iman test are also reported to indicate significant differences between matrices.

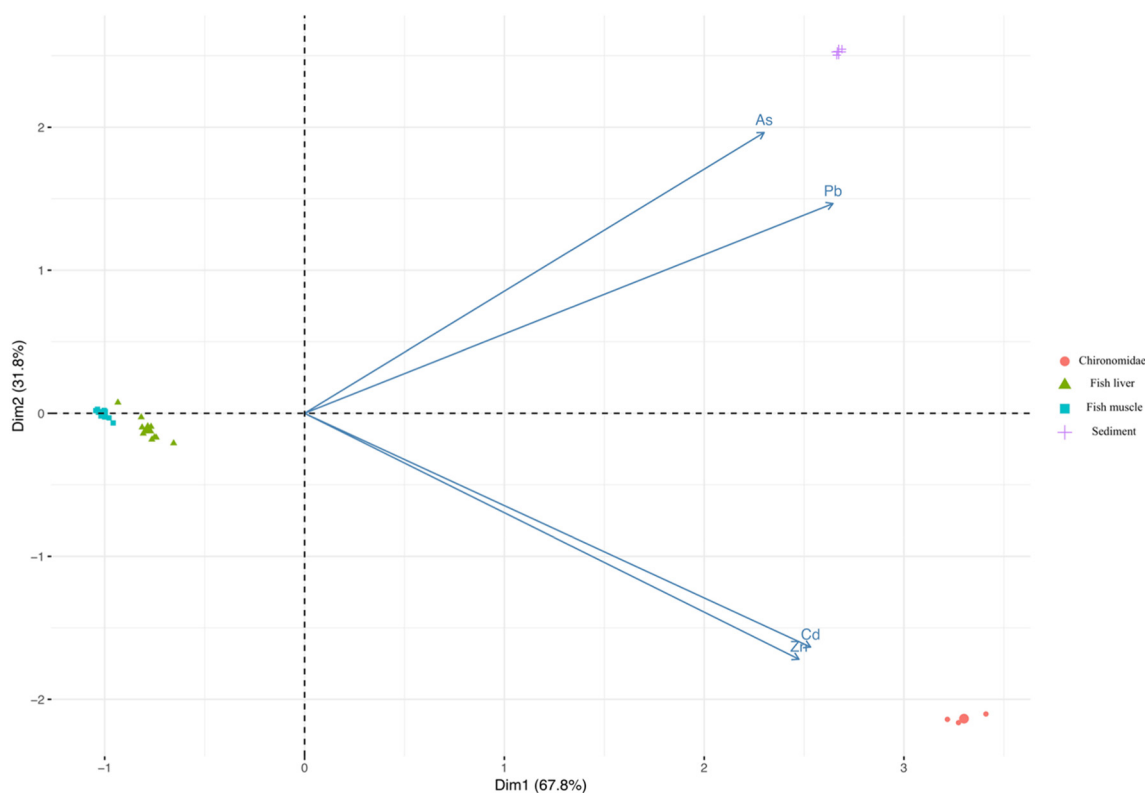


Fig. 3. Biplot of score and loadings from principal component analysis. The scores for the matrix (sediment, Diptera Chironomidae, fish liver and fish muscle) are denoted by a color and a symbol (largest symbol = average value).

(109.65 mg kg⁻¹) was higher than the levels measured for the Central (52 mg kg⁻¹), Piemonte Ticino (89 mg kg⁻¹) or Tyrolean (85 mg kg⁻¹) Alps, suggesting a flux of lead from lowland regions. Differently, mean cadmium and zinc levels were similar to those recorded for the Central and Tyrolean Alps (134 and 141 mg kg⁻¹, respectively).

Both As and Pb exceeded recommended level I and II, respectively, of sediment quality targets for the protection of sediment-dwelling organisms (Crane, 2006a, 2006b).

High concentration of trace elements recorded in sediment from Dimon Lake may be a consequence of its grain size composition (mainly silt, followed by sand and clay), since smaller grain size is associated with higher trace element concentrations (Rae, 1997; Yao et al., 2015). Furthermore, it can be also linked to the alkaline nature of Dimon Lake's water (pH > 8.5) which favors precipitation of metals and subsequent accumulation in sediments (Pobi et al., 2019).

This reflects the concentration levels recorded in Diptera Chironomidae, since it is an important taxon of sediment-bound contaminants (Hudson and Ciborowski, 1996). Chironomidae larvae spend most of their life in close contact with the sediment, making this taxon particularly relevant for biomonitoring studies (Arambourou et al., 2014). Arslan et al. (2010) also found that chironomids accumulate metals several times over their surrounding environment (e.g., sediment), which explains the higher concentration we recorded. Previous studies found that chironomids may accumulate a large amount of metals from

sediment, since they are detritus feeders that graze on sediments (Tulonen et al., 2006). The functional feeding guild (FFG) classification based on behavioral mechanisms of food acquisition is useful for understanding the differences in trace element accumulation in freshwater macrobenthic invertebrates: collector-gatherers demonstrated a greater amount of trace elements compared to the other FFG, indicating that the most effective uptake of metals occurs through sediment ingestion (Santoro et al., 2009; Pastorino et al., 2019b). Also, predator taxa seem to accumulate a large amount of trace elements compared to other FFGs (Pastorino et al., 2019b).

As and Pb concentration was decreased in order from sediment to Chironomidae, whereas the concentration of Cd and Zn was higher in Diptera Chironomidae than in sediment and fish tissues. Trace element accumulation in aquatic organisms is related not only to feeding habits but also to excretion rate among other factors (Rainbow, 2002). Indeed, Diptera Chironomidae had the highest concentrations of Cd probably because of their inefficient efflux rates or detoxification mechanisms, as reported in previous studies on aquatic invertebrates (Yu and Wang, 2002; Ng and Wang, 2004). Furthermore, a certain quantity of Zn (an essential element) is necessary for basic metabolic needs, especially as a cofactor in enzymatic reactions (Rainbow, 2002).

Aquatic organisms that accumulate trace elements from sediment may transfer them to organisms that forage on them (Soto-Jimenez, 2011). Gut content analysis discloses the feeding habits for prey and predator (Buckland et al., 2017). We found that bullhead feed exclusively on Diptera Chironomidae larvae and so assumed that the amount of ingested metals from diet originated mainly from this prey. The study by Pastorino et al. (2019b) on the feeding habits of bullhead from Dimon Lake also demonstrated that Chironomidae is the exclusively prey of bullhead all year round.

Although the relative contribution of each route varies with fish species and element considered, the predominant pathway of uptake for metals is assumed to be the diet (Wagner and Boman, 2003). Trace element amounts were lower in fish muscle and liver tissue samples than

Table 1
Bioaccumulation factor (BAF) and trophic transfer factor (TTF) values for Diptera Chironomidae (chiro) and fish muscle and liver samples.

	BAF chiro	BAF fish muscle	BAF fish liver	TTF fish muscle	TTF fish liver
As	0.26	0.008	0.04	0.03	0.16
Cd	5.16	0.04	0.05	0.008	0.009
Pb	0.45	0.0005	0.003	0.002	0.007
Zn	4.37	0.18	0.62	0.04	0.14

in either Diptera Chironomidae or sediment, indicating a dilution effect of trace elements along the food chain. Our observation that trace element levels were higher in liver than in muscle is shared by previous studies on fish (Wagner and Boman, 2003; Agah et al., 2009; Squadrone et al., 2013; Rosseland et al., 2017). The liver is particularly active in metabolizing arsenic (Authman et al., 2015), which explains the higher levels compared to muscle tissue. Furthermore, Rosseland et al. (2017) studied the trace element concentration in fish from high-altitude lakes of Nepal and found that Zn and Cd concentrations were higher in the liver compared to other organs, indicating an uptake from diet.

The most abundant trace element in both muscle and liver in fish was Zn, since it is an essential element involved in several complex metabolic pathways, such as the immune system, neurotransmission, and cell signaling (Niyogi and Wood, 2006). By comparison, Cd concentration in muscle and liver tissues was generally the lowest. Nevertheless, together with the kidney, the liver is among the several organs involved in cadmium metabolism and detoxification (Szebedinszky et al., 2001; Chowdhury et al., 2005). The lower Cd concentration measured in the liver compared to Diptera Chironomidae indicates that bullhead can avoid Cd uptake or excrete it from the body. The gastrointestinal tract of fish acts as a barrier for Cd absorption during food ingestion, and a large proportion of ingested Cd is excreted from the body via feces by mucosal sloughing (McGeer et al., 2011). Cadmium is also excreted by fish in a small proportion via bile, urine, and gills (McGeer et al., 2011). Chowdhury et al. (2005) demonstrated that only a small fraction (2–6%) of a Cd solution infused into rainbow trout (*Oncorhynchus mykiss*) stomach was absorbed across the gut wall, while the rest was eliminated from the body within 24 h. Furthermore, Khan and McGeer (2013) demonstrated that *O. mykiss* exposed to a high zinc diet showed an increase in secretion of intestinal mucus with an inhibitory effect on cadmium accumulation.

Generally, the concentration of As, Cd, Pb, and Zn in fish muscle and liver tissue samples was in line with that reported by Pastorino et al. (2020a) in the muscle of brook trout (*Salvelinus fontinalis*) from the Cottian Alps.

The highest BAF values were recorded for Cd and Zn in Diptera Chironomidae, suggesting an accumulation of these elements in the invertebrate's tissues. This may be explained by the bioavailability of trace elements that depends strongly on their forms of binding in the sediment which determine their availability for benthic organisms (Horváth et al., 2013; Roig et al., 2016). In literature it is well documented that metals forming carbonates salts or bound by ionic exchange (i.e. Cd and Zn) are most available than those bound to iron and manganese oxyhydroxides or sulfides (i.e. As) (Mosso and Davidson, 2003; Roig et al., 2016). Also, Pb is considered an immobile element linked to sulfides, justifying the lower availability and consequent bioaccumulation in chironomids.

Griboff et al. (2018) also showed that aquatic invertebrates have higher BAF for elements like Zn than fish. Generally, BAF values in fish were higher in the liver than the muscle, due to the liver's role in metal concentration and metabolism (Usero et al., 2004).

Finally, very low TTF values were recorded in muscle and liver tissue. TTF results mainly from dietary accumulation (Reinfelder et al., 1998; Mathews and Fisher, 2008).

Nevertheless, TTF indicated no magnification of trace elements in the analyzed food chain in Dimon Lake. In contrast, the trace element concentration decreased with increasing trophic level, suggesting that the trace elements are biodiluted along the food chain.

5. Conclusions

This study presents first insights into trace element accumulation and transfer along a simple food chain from a high-mountain lake. Sediment is a major sink for As and Pb, whereas Diptera Chironomidae had the highest Cd and Zn levels. Trace element levels were lowest in fish

tissues. BAF and TTF values were very low for all elements, suggesting a biodilution effect that merits future study. It is likely that fish regulate trace element intake and uptake from their environment. Environmental agencies should take in consideration results obtained from this study, since fish do not seem to be a good indicator of environmental pollution in high-mountain lakes. Instead, they should consider macrobenthic invertebrates as matrix to monitor trace elements in these ecosystems.

CRediT authorship contribution statement

Paolo Pastorino: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft, Writing - review & editing, Validation, Writing - review & editing. **Marino Prearo:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing - review & editing. **Marco Bertoli:** Investigation, Methodology, Validation, Writing - review & editing. **Maria Cesarina Abete:** Data curation, Investigation, Methodology, Validation, Writing - review & editing. **Alessandro Dondo:** Investigation, Validation, Writing - review & editing. **Gianguido Salvi:** Investigation, Validation, Writing - review & editing. **Annalisa Zaccaroni:** Investigation, Validation, Writing - review & editing. **Antonia Concetta Elia:** Conceptualization, Investigation, Writing - review & editing, Validation. **Elisabetta Pizzul:** Conceptualization, Investigation, Methodology, Supervision, Writing - review & editing, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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