

Profiling metal contamination from ultramafic sediments to biota along the Albanian shoreline of Lake Ohrid (Albania/Macedonia)

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METHODS

Surface water characterization

Surface water samples were collected and preserved in polyethylene containers (acid-washed for trace element analysis). Samples were either directly acidified by adding 1% (v/v) concentrated nitric acid (VWR, Suprapur) for later analyses of total metal contents, or first filtered through syringe Millex filters (PVDF; 0.45 µm pore size) and then acidified as above. Immediately before use, filters were rinsed with 10 mL of a 10% v/v HCl (VWR, Suprapur) followed by three times 10 mL of ultrapure water. Filtration blanks were obtained by filtering 10 mL aliquot of ultrapure waters through pre-washed filters. In the case of water samples, the first 2–3 mL of filtrate were discarded. Another fraction of raw water samples was filtered through pre-combusted GF/F filters (Whatman), and was not acidified to quantify dissolved organic carbon.

Assessment of metal availability in sediments

Mobile fraction

This mobile fraction was obtained by adding 25 mL of 0.01 M CaCl₂ solution to 2.5 g of sediments (Houba et al., 1990). The resulting suspensions were then shaken in a mechanical, end-over-end shaker for 2h at room temperature. The extracts were separated from the solid residues by centrifugation at 4000 rpm for 10 min. Supernatants were removed and filtered (Millipore 0.45 µm filters). Metals were analyzed in filtrates.

Phytoavailable fraction

This phytoavailable fraction was obtained from sediment subsamples of 2.5 g leached with 25 mL of a 0.05 M EDTA solution adjusted to pH 7 (Ure et al., 1996). The mixtures were then shaken in a mechanical, end-over-end shaker during 2h at room temperature. The extracts were separated from the solid residues by centrifugation at 4000 rpm for 10 min. Supernatants were removed and filtered (Millipore 0.45 µm filters). Metals were analyzed in filtrates.

Mobilizable fraction

This mobilizable fraction was obtained from sediment subsamples of 1 g leached with 40 mL of 0.11 M acetic acid (Quevauviller et al., 1997). The mixtures were then shaken in a mechanical, end-over-end shaker during 16h at room temperature. The extracts were separated from the solid residues by centrifugation at 4000 rpm for 10 min. Supernatants were removed and filtered (Millipore 0.45 µm filters). Metals were analyzed in filtrates.

Amorphous oxides

Iron, Mn and Al oxides were obtained from sediment subsamples of 1 g treated with 100 mL of 0.2 M NH_4 -oxalate and 0.2 M oxalic acid mixture at pH 3 in the dark (Schwertmann, 1964). The mixtures were then shaken in a mechanical and end-over-end shaker during 4 h at room temperature in the dark. The extracts were separated from the solid residues by centrifugation at 4000 rpm for 10 min. Supernatants were removed and filtered (Millipore 0.45 μm filters). Metals were analyzed in filtrates.

Chronic bioassays

OstracodTox test

Test were carried out in 6-well microplates. Ten neonates were introduced in each well containing 2 mL of standard fresh water (SFW) (NaHCO_3 : 96 mg L^{-1} ; $\text{CaSO}_4 \times 2 \text{H}_2\text{O}$: 120 mg L^{-1} ; $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$: 123 mg L^{-1} and KCl : 4 mg L^{-1}), 1.5 mL of algal food suspension (*Scenedesmus* spp.), prepared in SFW and 1 mL of wet sediment. Plates were incubated at $25 \pm 1^\circ\text{C}$ in darkness for 6 days. At the end of the exposure period, ostracods were recovered and the percentage of mortality as well as body lengths were determined under a stereomicroscope. Controls were performed under the same procedure with standard sand.

Toxicity test with *Chironomus riparius* larvae

Chironomids had been cultured prior to the experiments according to standard methods. First instar larvae of *Chironomus riparius* were obtained from the breeding at laboratory Chrono-Environnement (Besançon, France). Briefly, egg masses were removed from the culture four days before the experiment and left to hatch at room temperature for two days in 20 mL pillboxes filled with standard water. Synchronized newborn organisms were then transferred to 3 L containers filled with 2 L standard water and a thin layer of sand, and were fed with organic oat flour during two days. Then, first instar larvae were individually sorted, pooled by 20 individuals and introduced into exposure beakers.

Larvae were exposed in static conditions in 0.8 L beakers, filled with 0.1 L sediments and 0.5 L of water (pH = 7.8 and conductivity 250 $\mu\text{S cm}^{-1}$). Beakers were set at 20°C with a 16:8 h light:dark photoperiod and left to stabilized for five days before the introduction of larvae. Water was oxygenated through a continuous air bubbling in each beaker and regularly checked for pH, conductivity and dissolved oxygen. For each sediment, six replicate beakers containing 20 larvae were used and fed *ad libitum* (1 mg organic oat flour larva $^{-1}$ day $^{-1}$). After 10 days of exposure, the larvae were sampled in three beakers by gently sieving the sediment to recover organisms. Then, living larvae were dried on an absorbent paper sheet, individually measured

for their length, then pooled per replicate in cryogenic tubes, weighed and finally sacrificed at -80°C. The three remaining beakers were monitored daily for the emergence of adults. For the Pog sediment, this monitoring was done only on one replicate since high and unexplained mortality was observed from 15-16 days of exposure in two beakers out of three. Adults were counted, their gender determined and then sacrificed as described for larvae.

Organic carbon

In all filtered water samples, dissolved organic carbon (DOC) was analyzed using a Shimadzu total organic carbon analyzer (TOC 5000, Tokyo, Japan) and the non-purgeable organic carbon determination method was employed. In sediments, the amount of organic matter was determined by loss on ignition at 550°C (LOI) (APHA, 2005).

TABLES AND FIGURES

Table S1. Abiotic and biotic compartments sampled at each site and the number of samples analyzed for metal quantification and stable isotopes.

Samples	Metal analysis					Stable isotopes
	Pog	Mem	Poj	Pis	Lin	
<u>Abiotic compartments</u>						
Water*	1	1	1	1	1	-
Sediment*	1	1	1	1	1	-
<u>Primary producers</u>						
Phytoplankton* ^a	1	1	1	1	1	5
Periphytic biofilm ^a	-	3	3	3	3	9
<i>Myriophyllum spicatum</i>	3	3	4	4	3	8
<u>Invertebrates</u>						
Zooplankton* ^a	1	1	1	1	1	5
<i>Radix relict</i>	-	3	10	-	-	3
<i>Dreissena carinata</i>	12	10	12	12	14	4
<i>Unio tumidus</i>	12	12	-	-	-	6
<i>Gammarus</i> sp.	-	9	8	7	6	3
<u>Fish^b</u>						
<i>Alburnus albidus</i>			8			4
<i>Barbus meridionalis petenyi</i>			6			4
<i>Leuciscus cephalus albus</i>			5			4
<i>Rutilus ohridanus</i>			7			4
<i>Salmo letnica</i>			3			3

* Composite samples, ^a Analysis on filters, ^b Fish species cannot be specific to one site (mobility)

Table S2. Parameters of the logistic model fitted to the time course of the cumulative percentage of *Chironomus riparius* emergence for the different treatments.

Sediments	Maximal emergence percentage reached A (± se) (%)	Emergence rate k (± se) (d ⁻¹)		Time at the inflection point I (± se) (d)		
Reference (sand)	85.53 ± 2.81	a	0.80 ± 0.08	a	18.88 ± 0.16	a
Pog	85.95 ± 1.65	a	1.67 ± 0.21	b	17.57 ± 0.09	b
Mem	89.82 ± 3.83	a	1.27 ± 0.20	b	17.29 ± 0.70	bc
Poj	86.30 ± 4.66	a	1.50 ± 0.12	b	16.60 ± 0.09	c
Pis	83.77 ± 4.82	a	1.58 ± 0.26	b	16.88 ± 0.15	bc

Values that share the same letters are not significantly different (p<0.05).
se: standard error.

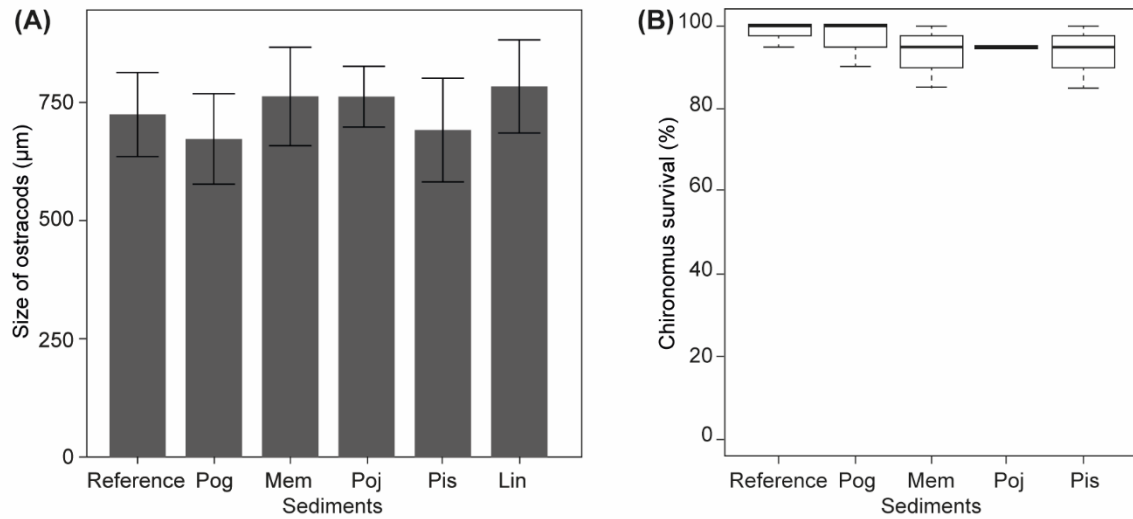


Figure S1. Ecotoxicity assessment of lake sediments on ostracods (A) and chironomids (B). A reference non-toxic sediment (sand) was also tested. (A) Mean size of ostracods *H. incongruens* (+ 1 sd), (B) Survival of *C. riparius*. Box plots show the median values, quartiles ("hinges"), extremes ("whiskers") and outliers (dots). Different letters indicate significant differences.

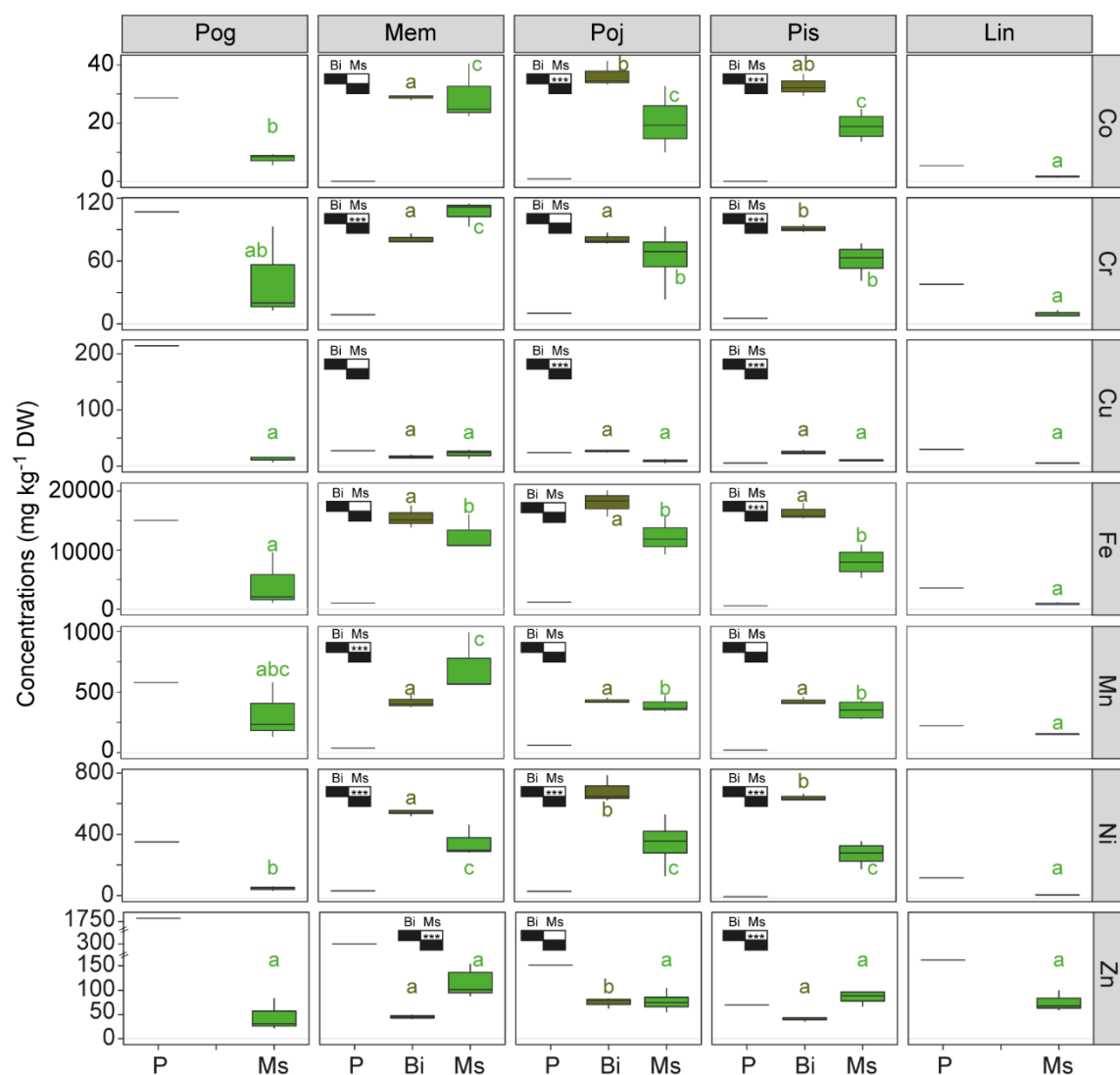


Figure S2. Metal concentrations (mg kg⁻¹ dry weight) bioaccumulated by primary producers in the five study sites. Box plots show the median values, quartiles (“hinges”), extremes (“whiskers”) and outliers (dots). Only one composite sample was analyzed for phytoplankton (P). Other primary producers are periphytic biofilm (Bi) and aquatic plants *Myriophyllum spicatum* (Ms). Significant differences in metal concentrations between sites for a given primary producer are illustrated by different lowercase letters. Significant intra-site differences are marked as *** for $p < 0.001$ in the correlation matrices inside the corresponding panel.

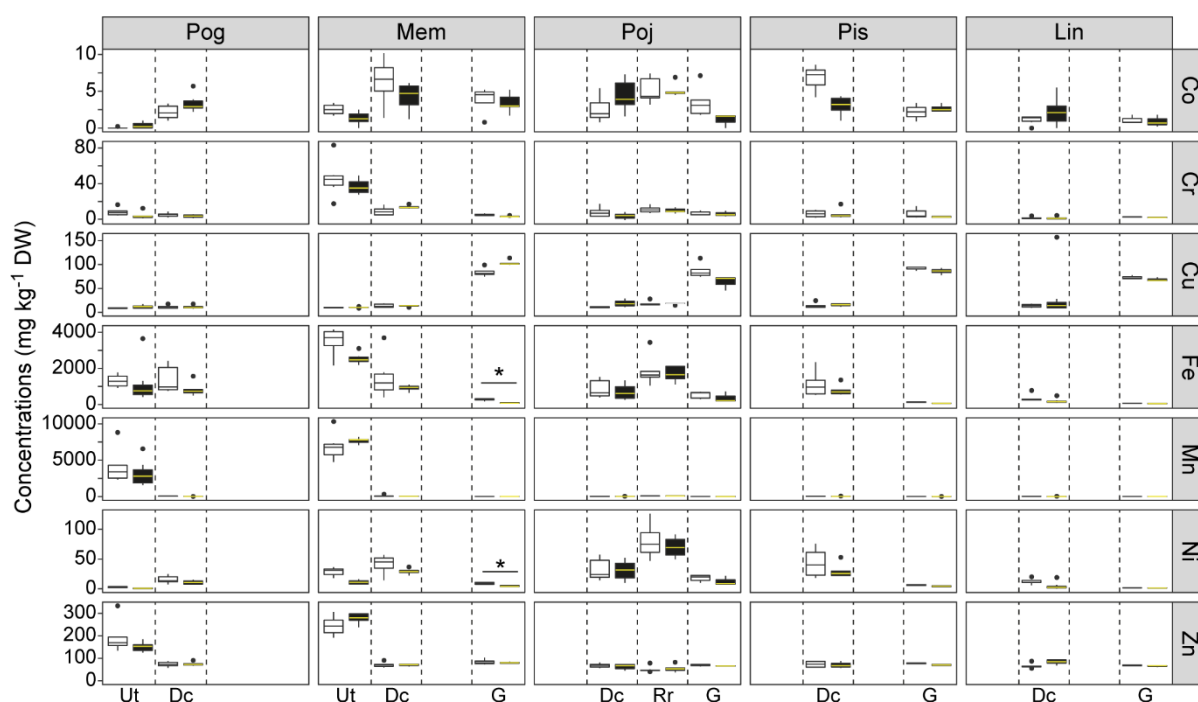


Figure S3. Concentrations of metals (mg kg⁻¹ dry weight) in invertebrates that were either depurated (black box) or not depurated (white box). * for p<0.05. Invertebrates are two bivalves, *D. carinata* (Dc) and *U. tumidus* (Ut), a gastropod *R. relictus* (Rr) and gammarids *Gammarus* sp. (G). Box plots show the median values, quartiles (“hinges”), extremes (“whiskers”) and outliers (dots).

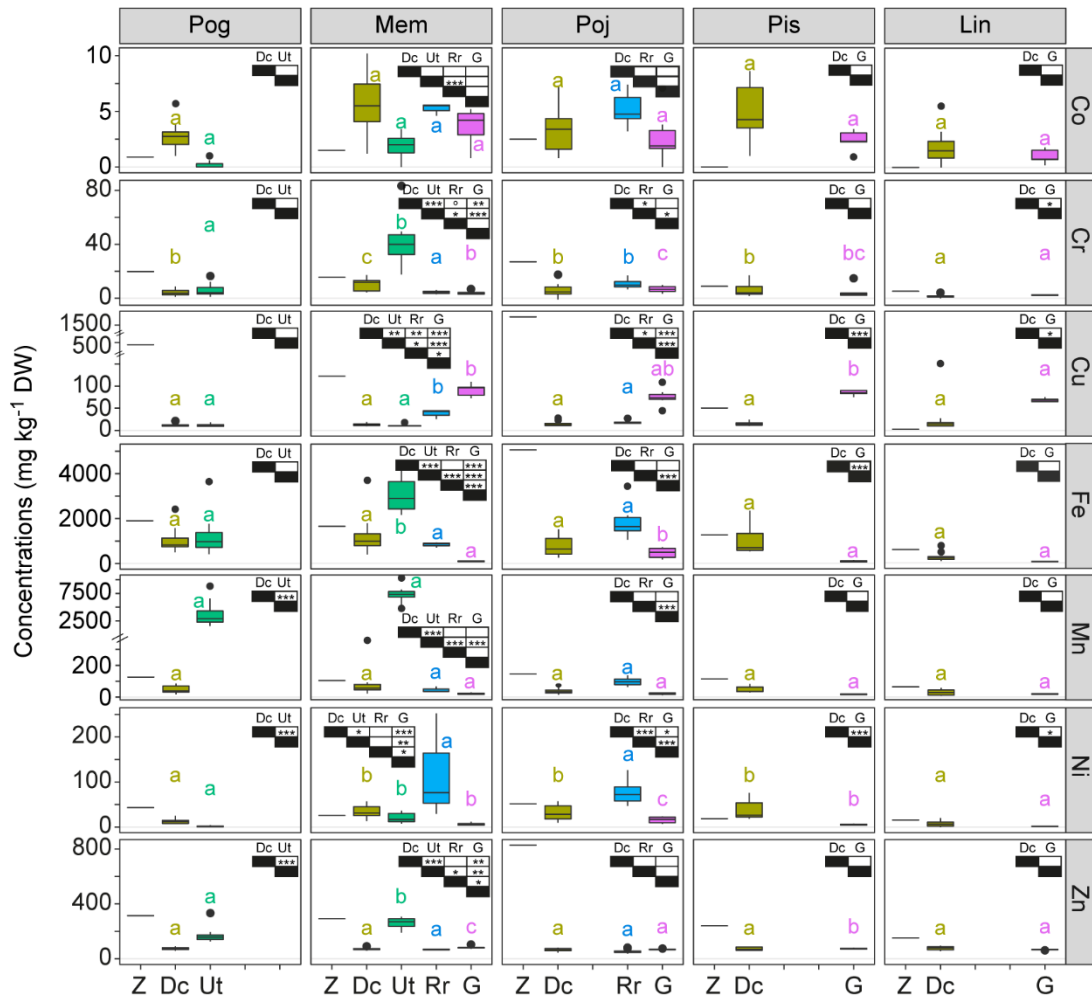


Figure S4. Metal concentrations (mg kg⁻¹ dry weight) bioaccumulated by invertebrates in the five study sites. Only for zooplankton (Z), a composite sample was analyzed. Other invertebrates are two bivalves, *D. carinata* (Dc) and *U. tumidus* (Ut), a gastropod *R. relicta* (Rr) and gammarids *Gammarus* sp. (G). Significant differences in metal concentrations between sites for a given invertebrate species are illustrated by different lowercase letters. Significant Intra-site differences are marked as *** for p<0.001, ** for p<0.01, and * for p<0.05 in the correlation matrices inside the corresponding panel. Box plots show the median values, quartiles ("hinges"), extremes ("whiskers") and outliers (dots).

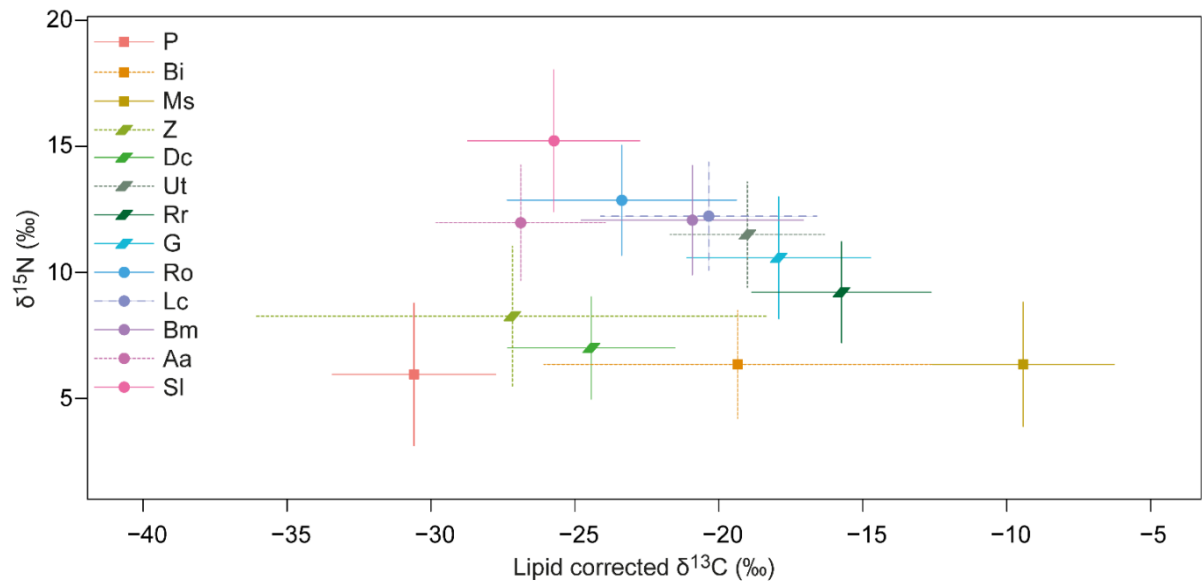


Figure S5. Average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures of primary producer, invertebrate and fish samples. Horizontal and vertical error bars represent standard errors. Nitrogen and carbon isotope enrichment factors of 3.4‰ (± 1) and 0.4‰ (± 1.3), respectively (Post, 2002) were added. Legend: Phytoplankton (P), periphytic biofilm (Bi), *M. spicatum* (Ms), zooplankton (Z), *D. carinata* (Dc), *U. tumidus* (Ut), *R. relictus* (Rr), *Gammarus* sp. (G), *R. ohridanus* (Ro), *L. cephalus albus* (Lc), *B. meridionalis petenyi* (Bm), *A. albidus* (Aa) and *S. letnica* (Sl).

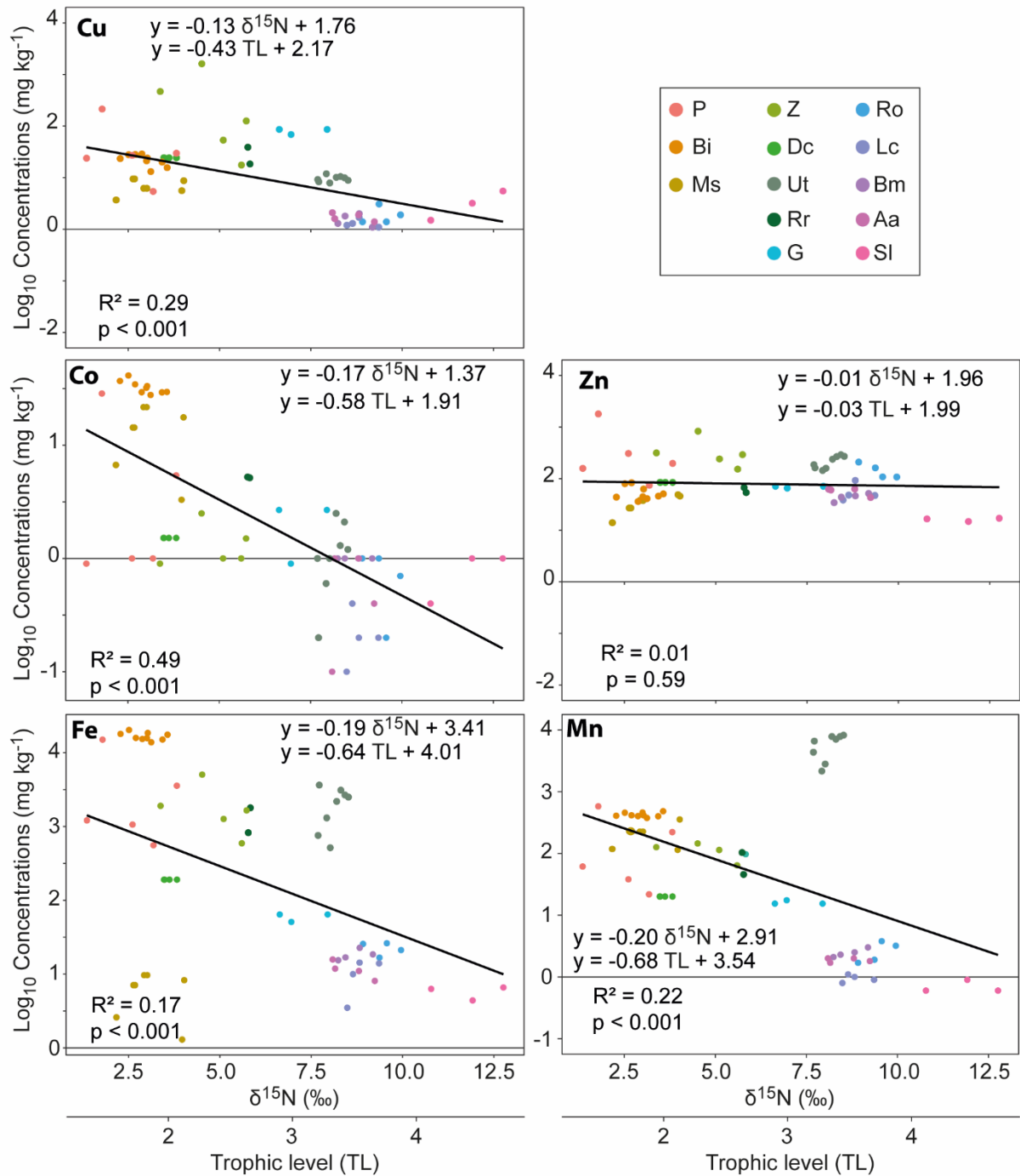


Figure S6. Logarithm of Cu, Co, Zn, Fe and Mn concentrations (mg kg⁻¹ D.W.) as a function of $\delta^{15}\text{N}$ values and trophic level (TL). Legend: Phytoplankton (P), periphytic biofilm (Bi), *M. spicatum* (Ms), zooplankton (Z), *D. carinata* (Dc), *U. tumidus* (Ut), *R. relicta* (Rr), *Gammarus* sp. (G), *R. ohridanus* (Ro), *L. cephalus albus* (Lc), *B. meridionalis petenyi* (Bm), *A. albidus* (Aa) and *S. letnica* (Sl).