

Title	Thiophilicity is a determinant of bioaccumulation in benthic fauna
Authors	O'Callaghan, Irene;Fitzpatrick, Dara;Sullivan, Timothy
Publication date	2021-12-07
Original Citation	O'Callaghan, I., Fitzpatrick, D. and Sullivan, T. (2021) 'Thiophilicity is a determinant of bioaccumulation in benthic fauna', Environmental Pollution, 294, 118641 (7pp). doi: 10.1016/j.envpol.2021.118641
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.envpol.2021.118641
Rights	© 2021, Elsevier Ltd. All rights reserved. This manuscript version is made available under the CC BY-NC-ND 4.0 license. - https://creativecommons.org/licenses/by-nc-nd/4.0/
Download date	2024-04-25 10:42:49
Item downloaded from	https://hdl.handle.net/10468/12396

Thiophilicity is a determinant of bioaccumulation in benthic fauna

Irene O'Callaghan^{1,2}, Dara Fitzpatrick² and Timothy Sullivan^{1,3}*

¹ School of Biological, Earth & Environmental Sciences, University College Cork, Ireland

² School of Chemistry, University College Cork, Ireland

³ Environmental Research Institute, University College Cork, Ireland

* Correspondence should be addressed to: irene.ocallaghan@ucc.ie

KEYWORDS

Bioavailability; Metallothionein; Macroinvertebrate; Metal contamination; Trophic transfer.

ABSTRACT

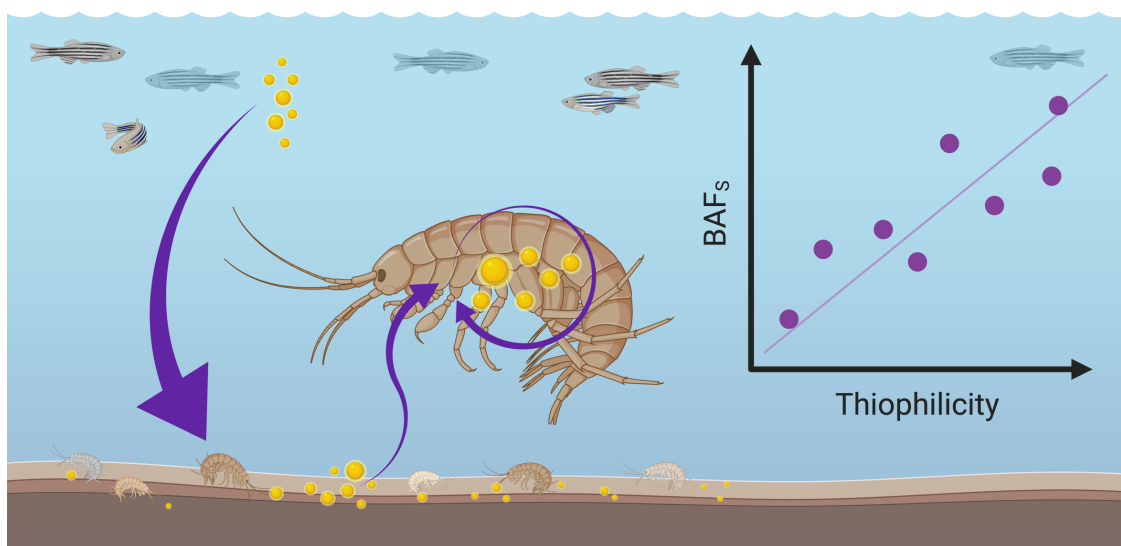
Aquatic contamination can settle into sediments, where it complexes with organic matter and becomes bioavailable. The resulting bioaccumulation of these contaminants by benthic fauna poses a serious threat due to the potential for trophic transfer. This paper offers an insight into the heterogenous accumulation behavior of different elements, and the consequences for ecological risk. In this study, we present field quantification of sediment-associated bioaccumulation factors (BAFs) in freshwater benthic macroinvertebrates. 17 elements were quantified using ICP-MS in sediment and *Asellus aquaticus* and *Gammarus sp.* samples. Previously published reports of contaminant concentrations in freshwater and marine sediments and benthic fauna were likewise analyzed to provide a complementary picture of bioaccumulation across contaminants and taxa. We demonstrate that the BAFs correlates strongly with the thiophilicity of the elemental

contaminants, as defined by (Kepp, 2016), for all strata examined. These findings support the hypothesis that thiol-mediated processes, such as that of metallothionein, play a larger role in bioaccumulation than typically afforded. In conclusion, we demonstrate the potential for the thiophilic scale to act as a predictor of accumulation potential.

HIGHLIGHTS

- Accumulation correlates strongly with thiophilicity across orders and environments
- Results support a thiol-based bioaccumulation process
- Bioavailability of contaminants can be predicted using the thiophilic scale

GRAPHICAL ABSTRACT



INTRODUCTION

The metallic pollution of freshwater systems has been a significant concern for many decades.¹⁻³ Concentrations of trace metals in waterways in excess of natural background levels can be attributed to anthropogenic pressures.^{4,5} Various metals may enter the hydrosphere through urban run-off, wastewater treatment plant outfall and road run-off.⁶⁻⁸ While direct and acute toxicity resulting from exposure to metal contamination is of the greatest concern, bioaccumulation of metallic pollutants at sub-lethal levels in benthic fauna is equally relevant when considering the threat that metal pollution poses to the ecosphere through the mechanism of trophic transfer.⁹ Benthic macroinvertebrates of various families make up the base of the aquatic food web, and are essential to the overall health of the hydrosphere.^{10,11} Many species, including fish, regularly predate upon these organisms and are, thus, exposed to the potential biomagnification of accumulated tissue concentrations.¹²⁻¹⁵ For this reason, the accumulation of contaminants by benthic fauna is a key ecotoxicological pathway in freshwater ecosystems.

Benthic macroinvertebrates have been shown to uptake and accumulate a range of trace metals from their sedimentary environment.¹⁶⁻¹⁹ It is noted that these organisms sequester the metal contaminants in order to render them metabolically available, in the case of essential elements (eg. Cu, Fe, Zn), or toxically inert.^{20,21} It is most commonly thought that this de-mobilization of metal ions is through a metallothionein (MT)-regulated mechanism, wherein the thiol groups of the MT protein capture the metals,²² although other thiol-mediated processes have also been proposed.²³ While the study of MT, and its various expressions, has determined the viability of this theory, there remain questions about the selectivity of the protein in the case of freshwater benthic fauna

and the relative importance of this particular detoxification mechanism.²⁴ It has been widely ascertained that MT will enable the bioaccumulation of Cu, Cd, Zn and Ag,²⁵⁻²⁷ and there is some evidence that it can capture Platinum-group elements,^{8,28-31} and Pb.^{27,32}

Thiophilicity is defined as the affinity an element will have towards a sulfur atom.^{33,34} Recently, a scale has been proposed for determining the thiophilicity of each element, based on the relative affinities of the element to sulfur and oxygen.³⁵ The thiophilic scale is overlaid on the periodic table in Figure 1.

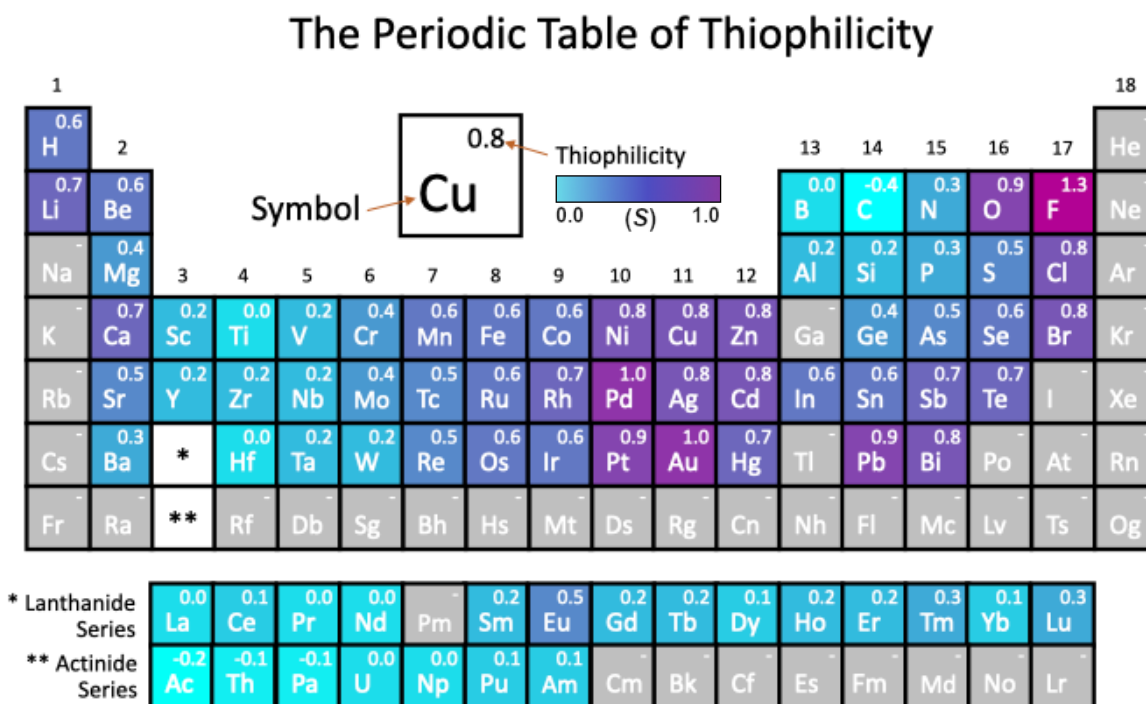


Figure 1. The Periodic Table of Thiophilicity, according to Kepp, 2016.³⁵ Higher numbers indicate a higher affinity to S versus O.

To date, there has been no application of the thiophilic scale to the field of environmental chemistry. In the sections that follow, we present an argument to support the use of the thiophilic scale to predict the bioaccumulation rate of differing pollutants. We determine the strength of the correlation between experimentally-derived elemental accumulation across multiple macroinvertebrate taxa, and provide a meta-analysis of previously-published studies in this context. Ultimately, we demonstrate that the thiophilic scale strongly determines the bioaccumulation of metal contaminants in sediment-dwelling organisms.

MATERIALS AND METHODS

Environmental sampling

Samples were acquired in the field to ensure that the conclusions of this study are environmentally relevant. In this manner, we side-step disagreements regarding the comparability of lab-based exposures and natural environmental processes.^{17,42-46}

Sampling was carried out at two sites on the Tramore River, on the periphery of Cork City, Ireland. All water, sediment and macroinvertebrate samples of *Asellus aquaticus* and *Gammarus sp.* were obtained during the month of July 2021.

Macroinvertebrate samples were obtained through kick-net sampling, using an aquatic net (1 mm mesh size, #175601), purchased from NHBS Ltd. (Devon, UK). Sterile plastic sample bottles (300 ml, NHBS Ltd., #223954) were used to take water and sediment (from the top 1-2 cm) samples, and all samples were transferred to sterile 50 ml Eppendorf tubes (DNA LoBind, #15581312), from Fisher Scientific Ireland Ltd. (Dublin, Ireland), for subsequent ICP analysis. A 50 ml water sample was taken at each site, and sediment was taken from the top 3 cm of the riverbed at each

site. Macroinvertebrate specimens were identified and separated in a white plastic tray. A sufficient number of adult specimens were obtained of each macroinvertebrate species in order to ensure that there was enough biomass for analysis.

Chemical analysis

Solid samples (sediment and macroinvertebrate) were dried and approximately 25 mg (dry weight) of each per site were digested with 2 ml conc. HNO_3 and 1 ml HF, then heated to reflux overnight. HF content was subsequently evaporated, and the remaining solutions were diluted to 100 ml with deionized water.

In order to determine the elemental concentrations of Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Pd, Ag, Cd, Sb, W, Pt, Au, Hg and Pb in the samples, analysis was carried out using a PerkinElmer NexION 2000B ICP-MS. External calibration was carried out using serial dilutions from 10 ppm standards. Diluent consisted of a 2% HNO_3 solution, prepared using in-house distilled nitric acid and 18.2 Mohm deionized water. SLRS-6 (riverine water, Canada) was used as a certified reference material, and instrument drift was normalized by spiking laboratory blanks, standards and samples with 1 ppb of Rh, In and Ir.

Determination of bioaccumulation factor (BAF)

The Bioaccumulation Factor (BAF) is a measure of the rate of uptake of a pollutant by an organism, normalized to the concentration in an external, environmental compartment. The BAF is typically defined as the ratio of the accumulated contaminant concentration to the contaminant concentration in the water;^{47,48} we denote this definition BAF_w :

$$BAF_W = \frac{[organism]}{[water]} \quad (1)$$

An alternative definition, that we denote BAF_S , considers instead the sediment concentration:⁴⁷

$$BAF_S = \frac{[organism]}{[sediment]} \quad (2)$$

Sediment concentrations in this study were consistently several orders of magnitude larger than water concentrations. Casual mediation analysis of measured water, sediment and accumulated macroinvertebrate concentrations, described below, supported the hypothesis of uptake directly from sediment to macroinvertebrate ($p < .001$), but did not support the hypothesis of uptake directly from water from macroinvertebrate ($p = .106$). For this reason, the BAF_S is employed exclusively in this paper to quantify accumulation.

Determination of thiophilicity

The thiophilicity (S) was determined in Kepp, 2016,³⁵ using data from Haynes, 2014,⁴⁹ according to the following equations:

$$\Delta D_{OS}(M) = D_O(M) - D_S(M) \quad (3)$$

$$S(M) = 1 - \frac{\Delta D_{OS}(M) - \Delta D_{OS}(Au)}{\Delta D_{OS}(Hf) - \Delta D_{OS}(Au)} \quad (4)$$

where $D_O(M)$ is the bond dissociation enthalpy of M_O and $D_S(M)$ is the bond dissociation enthalpy of M_S . Au and Hf were chosen as reference elements to obtain a scale that is mostly contained within the range of 0 to 1, although some exceedances of these limits occur.

Where there is a lack of experimental data to support the application of the equation for a given analyte, the estimated value for the thiophilicity determined in Kepp, 2016³⁵ was employed here.

Statistical analysis

Data pre-processing was carried out using Excel (Microsoft Corp.), and statistical analysis was carried out using RStudio software. A number of statistical packages were employed, namely mediation, ggplot2 and tidyverse.

Estimation of the relative contributions of direct uptake from water and uptake from sediment was carried out using the mediate function (10,000 simulations with robustSE set to TRUE).⁵⁰ Linear regressions were employed to investigate the hypothesis of a correlation between thiophilicity and accumulation, as follows:

$$\ln(BAF_s(M)_i) = \beta_1 S(M) + \beta_0 + \epsilon_i \quad (5)$$

where $BAF_s(M)_i$ is a single measurement datum, β_0 and β_1 are the regression coefficients associated with the relationship between BAF_s and thiophilicity, and ϵ_i is the residual.

Comparative analysis

Data from a number of previously-published studies, outlined in Table 1, were analyzed in a similar manner to the data presented in the study to verify this phenomenon over a wider range of conditions, taxa and analytical approaches. Data collection was limited to studies that considered multiple analytes and multiple taxa and/or sites. The resulting dataset contains data on over 20 taxonomic orders and 11 elements. Where the data was not presented in numerical form, webplotdigitizer was employed to extract the values from figures.⁵¹

143 **Table 1.** Studies considered as part of the meta-analysis.¹

Reference	Environment	Taxa considered	Analytes considered
Boubonari <i>et al.</i> , 2009 ³⁶	Marine	<i>Alismatales, Amphipoda, Cardiida, Decapoda, Littorinimorpha, Phyllodocida, Ulvales</i>	Fe, Cu, Zn, Cd, Pb
Culioli <i>et al.</i> , 2009 ³⁷	Freshwater	<i>Coleoptera, Ephemeroptera, Hygrophila, Plecoptera, Trichoptera, Tricladida</i>	As, Sb
Erasmus <i>et al.</i> , 2020 ³⁸	Freshwater	<i>Decapoda, Diptera, Ephemeroptera, Haplotaxida, Hygrophila, Odonata, Trichoptera</i>	Cr, Ni, Cu, Zn, Cd, Pt, Pb
Farag <i>et al.</i> , 2007 ³⁹	Freshwater	“Benthic macroinvertebrates”	Cu, Zn, As, Cd, Pb
Levit <i>et al.</i> , 2020 ⁴⁰	Marine	<i>Amphipoda, Bivalvia, Gastropoda, Hirudinea, Oligochaeta</i>	Mn, Fe, Cu, Zn, Cd, Pb
Pourang, 1996 ⁴¹	Freshwater	<i>Diptera, Haplotaxida, Mytilida, Venerida</i>	Mn, Cu, Zn, Pb

144 As bioaccumulation factor can be site- and species-specific, these contributions have been
 145 accounted for through normalization. In studies considering species from different orders, BAF_S
 146 values were first normalized by taxonomic order. Normalization of site-specific contributions was
 147 then performed using the following multivariate linear regression:

$$148 \ln(BAF_S(M)_i) = \sum_{k=2}^6 \beta_k X_k + \beta_1 S(M) + \beta_0 + \epsilon_i \quad (6)$$

¹ Where possible, taxa classification is given as the order; however, where taxonomic irregularities or unspecific reporting prevented order-level identification of an order, the closest taxonomic level is given.

where β_0 and β_1 are the regression coefficients associated with the relationship between BAFs and thiophilicity, X_k are dummy variables representing studies 2 through 6, β_k are the corresponding regression coefficients, and ε_i is the residual. This allowed for the contribution of thiophilicity to be decoupled from other contributing factors.

RESULTS AND DISCUSSION

Correlation between thiophilicity and BAFs

Calculated sediment-associated Bioaccumulation Factors (BAFs) for 20 analytes are shown in Table 2. The BAFs could not be calculated for Pd, Au or Hg due to some concentrations being below the lower limit of detection, and also could not be calculated from *Gammarus sp.* samples for a further 4 elements for the same reason.

Table 2. Determination of bioaccumulation factor from analyzed samples.

Analyte	Thiophilicity (S)	BAFs	
		<i>A. aquaticus</i>	<i>Gammarus sp.</i>
Al	0.2	0.0731	0.0749
Ti	0.0	0.1266	0.1876
V	0.2	0.1361	<i>n. d.</i>
Cr	0.4	0.2101	0.4789
Mn	0.6	0.7023	1.4206
Fe	0.6	0.1879	0.0735

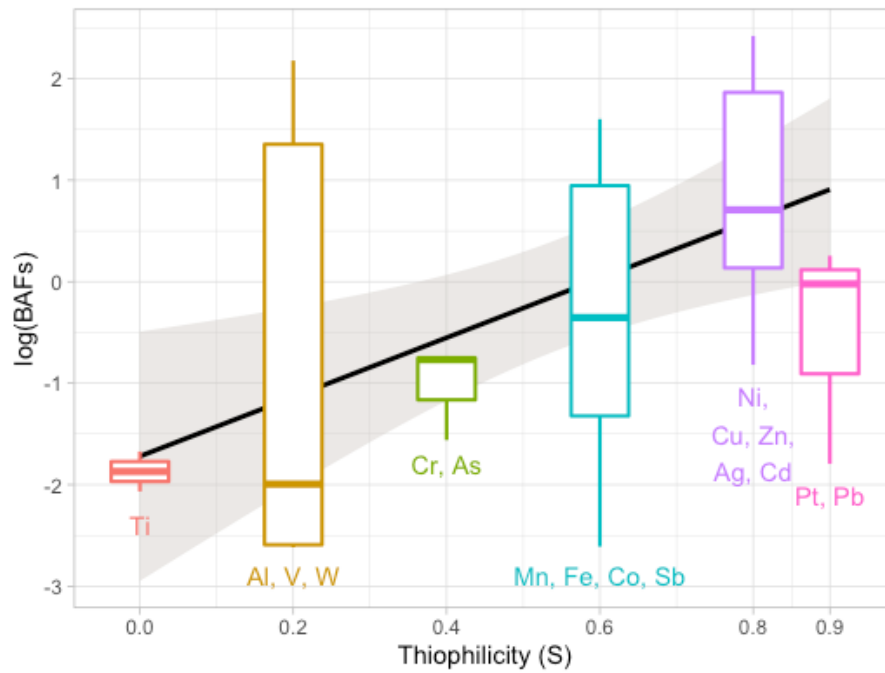
Co	0.6	0.3778	<i>n. d.</i>
Ni	0.8	0.4410	0.7631
Cu	0.8	7.6285	11.2537
Zn	0.8	1.9318	2.1328
As	0.5	0.4641	<i>n. d.</i>
Pd	1.0	<i>n. d.</i>	<i>n. d.</i>
Ag	0.8	1.0538	5.5998
Cd	0.8	1.4695	6.7652
Sb	0.7	4.6726	4.9619
W	0.2	3.8723	8.8327
Pt	0.9	1.2910	<i>n. d.</i>
Au	1.0	<i>n. d.</i>	<i>n. d.</i>
Hg	0.7	<i>n. d.</i>	<i>n. d.</i>
Pb	0.9	0.9788	0.1663

160

161 These calculated BAF_S values showed a strong correlation ($p = .012$, $R^2 = 0.203$) with the position
162 of the element on the thiophilic scale, as seen in Figure 2. The relationship between BAF_S and the
163 thiophilicity (S) was estimated as:

164
$$BAF_S = e^{(2.52 \pm 0.94)S - (1.64 \pm 0.61)} \quad (7)$$

165



166

167 **Figure 2.** Correlation between position on the thiophilic scale (*S*) and sediment-associated
 168 bioaccumulation factor (BAFs). With the exception of the elements with *S*=0.9, *S* values have been
 169 rounded down to the nearest multiple of 0.2 for illustrative clarity, and elements with the same
 170 thiophilicity have been amalgamated. Pb has been excluded from the calculation of the trend line
 171 (shaded area showing 95% confidence interval; $p = .004$, $R^2 = 0.276$).

172 This strong correlation between BAFs and *S* points to the predominance of thiol-mediated
 173 processes in the sequestration and accumulation of metal contaminants.

174 The relationship was still observed when both species were stratified (*Asellus aquaticus*: $p = .017$,
 175 $R^2 = 0.324$; *Gammarus sp.*: $p = .237$, $R^2 = 0.125$), illustrated in Figure 3, with a relatively low *p*-
 176 value for *Gammarus sp.* explained by the smaller number of detected elements in samples this
 177 species. More interestingly, there was no evidence for differing relationship in BAFs versus
 178 thiophilicity between species, shown by a high *p*-value and low effect size ($p = .387$, $R^2 = 0.022$),

despite each species belonging to a different macroinvertebrate order. It is known that MT is present in a wide range of flora and fauna, in which it generally performs a metal-scavenging function. Although species-to-species differences could be expected, the uniformity of the relationship between *A. aquaticus* and *Gammarus sp.* suggests that the thiol-mediated uptake mechanisms operate similarly across freshwater crustaceans. Additionally, these results suggest that differences in life history and behavior have a less important role than the thiol-mediated process in determining the rate of uptake and accumulation.

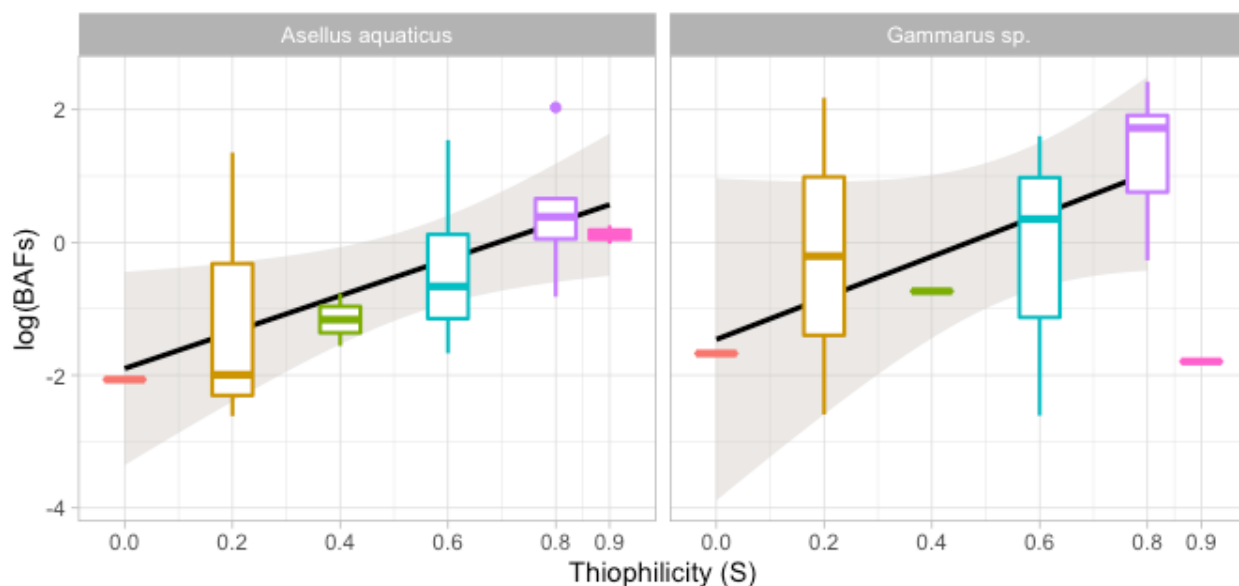


Figure 3. Correlation between position on the thiophilic scale (*S*) and sediment-associated bioaccumulation factor (*BAFs*), after stratification by species. *Pb* has been excluded from the calculation of the trend line (shaded area showing 95% confidence interval; *Asellus aquaticus*: $p = .020$, $R^2 = 0.332$; *Gammarus sp.*: $p = .094$, $R^2 = 0.255$).

Examination of the adherence of each element to the trend described above showed that *Pb*, although scoring highly on the thiophilicity scale ($S = 0.9$), was not readily accumulated by either

Asellus aquaticus or *Gammarus sp.* ($BAF_s = 0.979$ and 0.166 , respectively, compared to an expected value of 1.875). Exclusion of Pb from the above analysis showed a stronger contribution of thiophilicity to the accumulation of sediment contamination ($p = .004$, $R^2 = 0.276$). This lead-free relationship is given by:

$$BAF_s = e^{(3.03 \pm 0.96)S - (1.81 \pm 0.60)} \quad (8)$$

and is represented by the trend lines in Figures 2 and 3.

Comparison of previously-published works

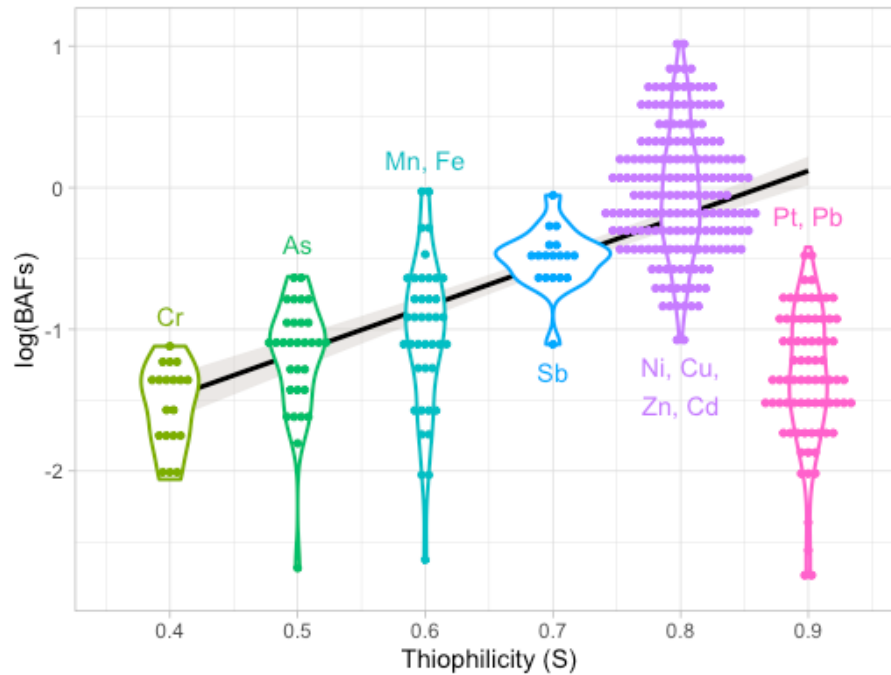
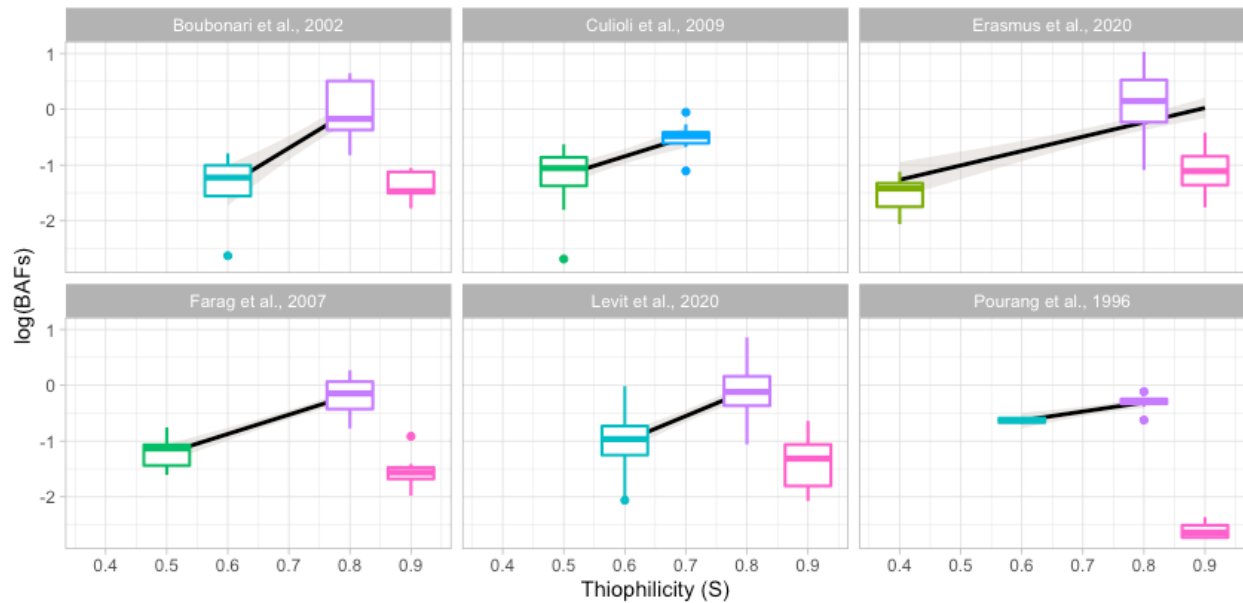


Figure 4. Correlation between position on the thiophilic scale (S) and sediment-associated bioaccumulation factor (BAF_s), for a range of previously-published studies.³⁶⁻⁴¹ Each dot represents a single data point. Pb was omitted from the analysis and determination of the trend line (shaded area showing 95% confidence interval; $p < .001$, $R^2 = 0.409$), but is shown here for illustration.

205 A meta-analysis was carried out using previously-published data.³⁶⁻⁴¹ The studies considered
 206 spanned a wide range of sediment-dwelling taxa with differing life histories, and from both marine
 207 and freshwater environments. This aggregated data, shown in Figure 4, also showed a lower-than-
 208 expected accumulation of Pb with respect to its position on the thiophilic scale, so this element has
 209 been omitted from the following analysis, although it is included in the accompanying figures for
 210 completeness.

211 Analysis of this data shows a similarly robust relationship between accumulation and proposed
 212 thiophilicity ($p < .001$, $R^2 = 0.409$). The estimated relationship between thiophilicity and BAFs for
 213 this agglomerated dataset is:

$$214 \quad BAF_S = e^{(3.21 \pm 0.24)S - (2.77 \pm 0.21)} \quad (9)$$



215 **Figure 5.** Correlation between position on the thiophilic scale (S) and sediment-associated
 216 bioaccumulation factor (BAF_s) for a range of previously-published studies, after stratification by

study. Pb was omitted from the analysis and determination of the trend line (shaded area showing 95% confidence interval), but is shown here for illustration. The elements included in each study are listed in Table 1.

This relationship was found to remain significant for each individual study after stratification (Figure 5). The strength of the effect despite the inclusion of such a diverse range of taxa provides further evidence in favor of the dominance of thiol-mediated accumulation across benthic fauna. It also suggests that differences in uptake (due to, for example, differences in morphology, behavior and life history) between species is less of a contributor to the total accumulated concentration than is frequently suggested.⁵²⁻⁵⁴

Further discussion

The strong correlation between bioaccumulation and the thiophilicity of the element, along with the lack of a significant correlation between sediment concentrations and thiophilicity, points to the key role of a metal-sulfur bond in the uptake and bioaccumulation process within the macroinvertebrates. This provides strong evidence for the importance of a thiol-based accumulation process, supporting extant hypotheses of MT-ruled detoxification mechanisms.

The analysis presented above assumes that the elemental thiophilicity offers a suitable approximation for the probability of the thiol capturing the metallic component of a molecule. Needless to say, there may be differences between the thiophilicity of a given element and a molecule containing that element, but we make the simplifying assumption that the elemental properties are representative. In an unspecified environment, where there is little certainty about the molecular composition of contaminants present, and where the elemental accumulation is of most concern, this assumption would appear to be justified. In the complex environment of the

239 freshwater benthos, it would be expected that molecule-specific effects average out to produce
240 similar behavior to this elemental approximation.

241 It is known⁵⁵⁻⁵⁸ that the structure and characteristics of MT can vary between species, but all rely
242 on the thiol group for the capture of the metal analyte. The consistency of the correlation between
243 thiophilicity and accumulation supports the hypothesis that MT-regulation is a common and
244 equally important detoxification mechanism across species.

245 While it is notable from the aforementioned results that a similar relationship between thiophilicity
246 and BAF_s is seen across taxa from different orders, it is expected that the nature of this relationship
247 would vary by species. It is likely that some species or higher taxonomic groupings may exhibit
248 specific effects, such as differing accumulation saturation points, wherein accumulated
249 concentrations reach a horizontal asymptote, or independent differences in accumulation rate.
250 Furthermore, the expression of metallothionein can also differ between species,⁵⁹⁻⁶² resulting in
251 further element-specific effects.

252 The results presented in this article support the hypothesis that the accumulation of elemental
253 contaminants in benthic fauna correlates significantly with the analyte's position on the thiophilic
254 scale. However, Pb was not found to follow this trend; despite occupying the highest position on
255 the thiophilic scale of any element considered in this paper, the BAF_s of Pb was found to be well
256 below the observed trend. Unfortunately, while a number of metallothionein isoforms have been
257 characterized, the metal-detoxification role of non-mammalian MT remains poorly understood,⁶³⁻
258 ⁶⁸ leaving little indication of possible Pb-specific effects. We would like to put forward several
259 hypotheses to explain the seemingly inhibited response of the bioaccumulation mechanism to Pb:

- The elemental thiophilicity of Pb may not necessarily relate to the thiophilicities of the dominant compounds in which Pb is found in the environment. Pb is expected to persist in the environment as a free ion or in a complex such as PbO, PbSO₄, PbS, PbCO₃, Pb₃(PO₄)₂ or as a halide (e.g. PbBrCl). It may also complex to other anions or colloids in the aqueous environment. It is possible that the form in which the element is found in the environment prevents the formation of a thiol bond.
- Species-specific metallothionein may exhibit stereochemistry not conducive to a bond with a large Pb atom. A recent study illustrated an analogous example, where Pb²⁺ showed a strong thiophilic tendency to bind with the S site of methyl thiophosphate over the corresponding O site of methyl phosphate; however, Pb²⁺ also showed an oxophilic tendency to bind with the O site of uridylyl-(5'→3')-[5']-uridylate over the corresponding S site of *P*-thiouridylyl-(5'→3')-[5']-uridylate. This observation was attributed to the properties of the 6s² lone pair of Pb²⁺, and its role in the Pb²⁺ coordination sphere.⁶⁹
- It is possible that a separate Pb-specific detoxification mechanism exists that causes Pb to be excreted at a rate higher than other elements. A previous study examining the ecdysis of the crab species *Uca pugnax* found Cu and Zn concentrations in the exoskeleton replaced with Pb concentrations from the soft tissue immediately prior to molting of the exoskeleton, thus depurating Pb from the body.⁷⁰
- The thiophilic scale employs the bond dissociation enthalpy of MS as a proxy for affinity of the thiol to bind with an element. In the case of Pb, this relationship may not necessarily hold.

281

282

283
284
285
286
287
288
289
290

291
292
293

294
295
296
297
298
299
300

301

CONCLUSIONS

We have demonstrated that the thiophilic scale acts as a suitable predictor of bioaccumulation potential. The correlation between thiophilic scale and BAF_s is observed across taxa from different orders, in multiple studies addressing both freshwater and marine environments. In the context of environmental modelling and impact prediction, this observation can inform predictions of bioavailability and the corresponding contribution to toxicological risk of hazardous substances, especially in the case of lesser-studied analytes and in situations where the feasibility of direct measurement is limited. It also strongly suggests the predominant role of thiol-mediated accumulation mechanisms, such as have been reported with metallothionein.

SUPPORTING INFORMATION

The following supplementary file is available online:
Data listing (PDF)

ACKNOWLEDGMENTS

This study was funded by the Irish Research Council and Environmental Protection Agency (Ireland), under Grant no. GOIPG/2018/3351. The authors would like to thank Trang To (Oxford University Centre for the Environment) for carrying out sample analysis, as well as Sally Sheard and Lisbeth Bjerva (Oxford Innovation) for their assistance. The authors also wish to express their gratitude to the anonymous reviewers for their time and constructive comments. The table of contents graphic was created using BioRender.com.

- (1) Förstner, U.; Prosi, F. *Biological Aspects of Freshwater Pollution*; Elsevier, 1979; pp 129–161.
- (2) Nriagu, J. O. Global metal pollution: poisoning the biosphere? *Environment: Science and Policy for Sustainable Development* **1990**, *32*, 7–33.
- (3) Sahu, C.; Basti, S. Trace metal pollution in the environment: a review. *International Journal of Environmental Science and Technology* **2021**, *18*, 211–224.
- (4) Saleem, M.; Iqbal, J.; Shah, M. H. Geochemical speciation, anthropogenic contamination, risk assessment and source identification of selected metals in freshwater sediments—a case study from Mangla Lake, Pakistan. *Environmental Nanotechnology, Monitoring & Management* **2015**, *4*, 27–36.
- (5) Thevenon, F.; Graham, N. D.; Chiaradia, M.; Arpagaus, P.; Wildi, W.; Poté, J. Local to regional scale industrial heavy metal pollution recorded in sediments of large freshwater lakes in central Europe (lakes Geneva and Lucerne) over the last centuries. *Science of the Total Environment* **2011**, *412*, 239–247.
- (6) Buzier, R.; Tusseau-Vuillemin, M.-H.; Keirsbulck, M.; Mouchel, J.-M. Inputs of total and labile trace metals from wastewater treatment plants effluents to the Seine River. *Physics and Chemistry of the Earth, Parts A/B/C* **2011**, *36*, 500–505.
- (7) Maceda-Veiga, A.; Monroy, M.; de Sostoa, A. Metal bioaccumulation in the Mediterranean barbel (*Barbus meridionalis*) in a Mediterranean River receiving effluents from urban and industrial wastewater treatment plants. *Ecotoxicology and Environmental Safety* **2012**, *76*, 93–101.
- (8) Moldovan, M.; Rauch, S.; Gómez, M.; Palacios, M. A.; Morrison, G. M. Bioaccumulation of palladium, platinum and rhodium from urban particulates and sediments by the freshwater isopod *Asellus aquaticus*. *Water Research* **2001**, *35*, 4175–4183.
- (9) Suedel, B.; Boraczek, J.; Peddicord, R. Trophic transfer and biomagnification potential of contaminants in aquatic ecosystems. *Reviews of Environmental Contamination and Toxicology* **1994**, *136*, 21–89.
- (10) Swartwout, M. C.; Keating, F.; Frimpong, E. A. A survey of macroinvertebrates colonizing bluehead chub nests in a Virginia stream. *Journal of Freshwater Ecology* **2016**, *31*, 147–152.
- (11) Thorp, J. H.; Bergey, E. Field experiments on responses of a freshwater, benthic macroinvertebrate community to vertebrate predators. *Ecology* **1981**, *62*, 365–375.
- (12) Croteau, M.-N.; Luoma, S. N.; Stewart, A. R. Trophic transfer of metals along freshwater food webs: evidence of cadmium biomagnification in nature. *Limnology and Oceanography* **2005**, *50*, 1511–1519.

- 339 (13) Griboff, J.; Horacek, M.; Wunderlin, D. A.; Monferran, M. V. Bioaccumulation and
340 trophic transfer of metals, As and Se through a freshwater food web affected by
341 anthropic pollution in Córdoba, Argentina. *Ecotoxicology and Environmental Safety*
342 **2018**, *148*, 275–284.
- 343 (14) Lau, S.; Mohamed, M.; Yen, A. T. C.; Su'Ut, S. Accumulation of heavy metals in
344 freshwater molluscs. *Science of the Total Environment* **1998**, *214*, 113–121.
- 345 (15) Watras, C.; Back, R.; Halvorsen, S.; Hudson, R.; Morrison, K.; Wente, S. Bioaccumulation
346 of mercury in pelagic freshwater food webs. *Science of the Total Environment* **1998**,
347 *219*, 83–208.
- 348 (16) Canivet, V.; Chambon, P.; Gibert, J. Toxicity and bioaccumulation of arsenic and
349 chromium in epigeal and hypogean freshwater macroinvertebrates. *Archives of*
350 *Environmental Contamination and Toxicology* **2001**, *40*, 345–354.
- 351 (17) Hare, L. Aquatic insects and trace metals: bioavailability, bioaccumulation, and toxicity.
352 *Critical Reviews in Toxicology* **1992**, *22*, 327–369.
- 353 (18) Oberholster, P. J.; Myburgh, J. G.; Ashton, P. J.; Coetzee, J. J.; Botha, A.-M.
354 Bioaccumulation of aluminium and iron in the food chain of Lake Loskop, South
355 Africa. *Ecotoxicology and Environmental Safety* **2012**, *75*, 134–141.
- 356 (19) Santoro, A.; Blo, G.; Mastrolitti, S.; Fagioli, F. Bioaccumulation of heavy metals by
357 aquatic macroinvertebrates along the Basento River in the south of Italy. *Water, Air, and*
358 *Soil Pollution* **2009**, *201*, 19–31.
- 359 (20) Isani, G.; Carpenè, E. Metallothioneins, unconventional proteins from unconventional
360 animals: a long journey from nematodes to mammals. *Biomolecules* **2014**, *4*, 435–457.
- 361 (21) Truchet, D. M.; Buzzi, N. S.; Simonetti, P.; Marcovecchio, J. E. Uptake and detoxification
362 of trace metals in estuarine crabs: insights into the role of metallothioneins. *Environmental*
363 *Science and Pollution Research* **2020**, *27*, 31905–31917.
- 364 (22) Ngu, T. T.; Stillman, M. J. Metal-binding mechanisms in metallothioneins. *Dalton*
365 *Transactions* **2009**, 5425–5433.
- 366 (23) Cobbett, C.; Goldsbrough, P. Phytochelatins and metallothioneins: roles in heavy metal
367 detoxification and homeostasis. *Annual Review of Plant Biology* **2002**, *53*, 159–182.
- 368 (24) Palmiter, R. D. The elusive function of metallothioneins. *Proceedings of the National*
369 *Academy of Sciences* **1998**, *95*, 8428–8430.
- 370 (25) Samuel, M. S.; Datta, S.; Khandge, R. S.; Selvarajan, E. A state of the art review on
371 characterization of heavy metal binding metallothioneins proteins and their widespread
372 applications. *Science of the Total Environment* **2021**, 145829.
- 373 (26) Wang, C.; Shu, F.; Hong, Y.; Wang, J.; Peng, K.; Sheng, J.; Wu, D.; Hu, B.; Shi, J.; Jian,
374 S. Analysis of the structure and activity of the promoter regions of the metallothionein

- 375 genes of the freshwater pearl mussel *Hyriopsis schlegelii*. *Bioscience, Biotechnology, and*
376 *Biochemistry* **2018**, 82, 780–791.
- 377 (27) Yang, H. Z.; Gu, W. J.; Chen, W.; Hwang, J. S.; Wang, L. Metal binding characterization
378 of heterologously expressed metallothionein of the freshwater crab *Sinopotamon*
379 *henanense*. *Chemosphere* **2019**, 235, 926–934.
- 380 (28) Bongers, J.; Richardson, D. E.; Bell, J. U. Platinum (II) binding to metallothioneins.
381 *Journal of Inorganic Biochemistry* **1988**, 34, 55–62.
- 382 (29) O’Callaghan, I.; Harrison, S.; Fitzpatrick, D.; Sullivan, T. The freshwater isopod *Asellus*
383 *aquaticus* as a model biomonitor of environmental pollution: A review. *Chemosphere*
384 **2019**, 235, 498–509.
- 385 (30) Rauch, S.; Morrison, G. M. Platinum uptake by the freshwater isopod *Asellus aquaticus* in
386 urban rivers. *Science of the Total Environment* **1999**, 235, 261–268.
- 387 (31) Wong, D. L.; Stillman, M. J. Metallothionein: an aggressive scavenger—the metabolism
388 of rhodium (II) tetraacetate (Rh₂ (CH₃CO₂)₄). *ACS Omega* **2018**, 3, 16314–16327.
- 389 (32) Bordon, I. C.; Emerenciano, A. K.; Melo, J. R. C.; da Silva, J. R. M. C.; Favaro, D. I. T.;
390 Gusso-Choueri, P. K.; de Campos, B. G.; de Souza Abessa, D. M. Implications on the Pb
391 bioaccumulation and metallothionein levels due to dietary and waterborne exposures: the
392 *Callinectes danae* case. *Ecotoxicology and Environmental Safety* **2018**, 162, 415–422.
- 393 (33) Moreno-Alcántar, G.; Romo-Islas, G.; Flores-Alamo, M.; Torrens, H. Auophilicity vs.
394 thiophilicity: directing the crystalline supramolecular arrangement in luminescent gold
395 compounds. *New Journal of Chemistry* **2018**, 42, 7845–7852.
- 396 (34) Porath, J.; Maisano, F.; Belew, M. Thiophilic adsorption—a new method for protein
397 fractionation. *FEBS Letters* **1985**, 185, 306–310.
- 398 (35) Kepp, K. P. A quantitative scale of oxophilicity and thiophilicity. *Inorganic Chemistry*
399 **2016**, 55, 9461–9470.
- 400 (36) Boubonari, T.; Kevrekidis, T.; Malea, P. Metal (Fe, Zn, Cu, Pb and Cd) concentration
401 patterns in components of a macrophyte-based coastal lagoon ecosystem. *Hydrobiologia*
402 **2009**, 635, 27–36.
- 403 (37) Culioli, J.-L.; Fouquoire, A.; Calendini, S.; Mori, C.; Orsini, A. Trophic transfer of
404 arsenic and antimony in a freshwater ecosystem: a field study. *Aquatic Toxicology* **2009**,
405 94, 286–293.
- 406 (38) Erasmus, J.; Malherbe, W.; Zimmermann, S.; Lorenz, A.; Nachev, M.; Wepener, V.;
407 Sures, B.; Smit, N. Metal accumulation in riverine macroinvertebrates from a platinum
408 mining region. *Science of the Total Environment* **2020**, 703, 134738.
- 409 (39) Farag, A. M.; Nimick, D. A.; Kimball, B. A.; Church, S. E.; Harper, D. D.;
410 Brumbaugh, W. G. Concentrations of metals in water, sediment, biofilm, benthic
411 macroinvertebrates, and fish in the Boulder River watershed, Montana, and the role of

- colloids in metal uptake. *Archives of Environmental Contamination and Toxicology* **2007**, 52, 397–409.
- (40) Levit, R.; Shigaeva, T.; Kudryavtseva, V. Heavy Metals in Macrozoobenthos and Sediments of the Coastal Zone of the Eastern Gulf of Finland. *Russian Journal of General Chemistry* **2020**, 90, 2700–2707.
- (41) Pourang, N. Heavy metal concentrations in surficial sediments and benthic macroinvertebrates from Anzali wetland, Iran. *Hydrobiologia* **1996**, 331, 53–61.
- (42) Belzunce-Segarra, M. J.; Simpson, S. L.; Amato, E. D.; Spadaro, D. A.; Hamilton, I. L.; Jarolimek, C. V.; Jolley, D. F. The mismatch between bioaccumulation in field and laboratory environments: Interpreting the differences for metals in benthic bivalves. *Environmental Pollution* **2015**, 204, 48–57.
- (43) Burkhard, L. P. Factors influencing the design of bioaccumulation factor and biotasediment accumulation factor field studies. *Environmental Toxicology and Chemistry: An International Journal* **2003**, 22, 351–360.
- (44) Burkhard, L. P.; Cowan-Ellsberry, C.; Embry, M. R.; Hoke, R. A.; Kidd, K. A. Bioaccumulation data from laboratory and field studies: are they comparable? *Integrated Environmental Assessment and Management* **2012**, 8, 13–16.
- (45) Selck, H.; Drouillard, K.; Eisenreich, K.; Koelmans, A. A.; Palmqvist, A.; Ruus, A.; Salvito, D.; Schultz, I.; Stewart, R.; Weisbrod, A., et al. Explaining differences between bioaccumulation measurements in laboratory and field data through use of a probabilistic modeling approach. *Integrated Environmental Assessment and Management* **2012**, 8, 42–63.
- (46) Weisbrod, A. V.; Woodburn, K. B.; Koelmans, A. A.; Parkerton, T. F.; McElroy, A. E.; Borg'a, K. Evaluation of bioaccumulation using in vivo laboratory and field studies. *Integrated Environmental Assessment and Management: An International Journal* **2009**, 5, 598–623.
- (47) DeForest, D. K.; Brix, K. V.; Adams, W. J. Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. *Aquatic Toxicology* **2007**, 84, 236–246.
- (48) Mackay, D.; Arnot, J. A.; Gobas, F. A.; Powell, D. E. Mathematical relationships between metrics of chemical bioaccumulation in fish. *Environmental Toxicology and Chemistry* **2013**, 32, 1459–1466.
- (49) Haynes, W. M. *CRC Handbook of Chemistry and Physics*; CRC press, 2014.
- (50) Tingley, D.; Yamamoto, T.; Hirose, K.; Keele, L.; Imai, K. Mediation: R package for causal mediation analysis. *Journal of Statistical Software* **2014**, 59, 1–38.
- (51) Rohatgi, A. WebPlotDigitizer, Version 4.2. URL <https://automeris.io/WebPlotDigitizer> 2020.

- 449 (52) Cid, N.; Ibáñez, C.; Palanques, A.; Prat, N. Patterns of metal bioaccumulation in two filter-
 450 feeding macroinvertebrates: exposure distribution, inter-species differences and
 451 variability across developmental stages. *Science of the Total Environment* **2010**, *408*,
 452 2795–2806.
- 453 (53) Fletcher, D. E.; Lindell, A. H.; Stillings, G. K.; Mills, G. L.; Blas, S. A.; McArthur, J. V.
 454 Spatial and taxonomic variation in trace element bioaccumulation in two herbivores
 455 from a coal combustion waste contaminated stream. *Ecotoxicology and Environmental*
 456 *Safety* **2014**, *101*, 196–204.
- 457 (54) Goodyear, K.; McNeill, S. Bioaccumulation of heavy metals by aquatic
 458 macroinvertebrates of different feeding guilds: a review. *Science of the Total*
 459 *Environment* **1999**, *229*, 1–19.
- 460 (55) Amiard, J.-C.; Amiard-Triquet, C.; Barka, S.; Pellerin, J.; Rainbow, P. Metallothioneins in
 461 aquatic invertebrates: their role in metal detoxification and their use as biomarkers.
 462 *Aquatic Toxicology* **2006**, *76*, 160–202.
- 463 (56) Engel, D. W.; Brouwer, M. Trace metal-binding proteins in marine molluscs and
 464 crustaceans. *Marine Environmental Research* **1984**, *13*, 177–194.
- 465 (57) Mao, H.; Wang, D.-H.; Yang, W.-X. The involvement of metallothionein in the
 466 development of aquatic invertebrate. *Aquatic Toxicology* **2012**, *110*, 208–213.
- 467 (58) Pedersen, S. N.; Pedersen, K. L.; Højrup, P.; Depledge, M. H.; Knudsen, J. Primary
 468 structures of decapod crustacean metallothioneins with special emphasis on freshwater and
 469 semi-terrestrial species. *Biochemical Journal* **1996**, *319*, 999–1003.
- 470 (59) Beil, A.; Jurt, S.; Walser, R.; Schönhut, T.; Güntert, P.; Palacios, Ò.; Atrian, S.;
 471 Capdevila, M.; Dallinger, R.; Zerbe, O. The solution structure and dynamics of Cd-
 472 metallothionein from *Helix pomatia* reveal optimization for binding Cd over Zn.
 473 *Biochemistry* **2019**, *58*, 4570–4581.
- 474 (60) Homa, J.; Klimek, M.; Kruk, J.; Cocquerelle, C.; Vandenbulcke, F.; Plytycz, B. Metal-
 475 specific effects on metallothionein gene induction and riboflavin content in coelomocytes
 476 of *Allolobophora chlorotica*. *Ecotoxicology and Environmental Safety* **2010**, *73*, 1937–
 477 1943.
- 478 (61) Le Croizier, G.; Lacroix, C.; Artigaud, S.; Le Floch, S.; Raffray, J.; Penicaud, V.;
 479 Coquillé, V.; Autier, J.; Rouget, M.-L.; Le Bayon, N., et al. Significance of
 480 metallothioneins in differential cadmium accumulation kinetics between two marine fish
 481 species. *Environmental Pollution* **2018**, *236*, 462–476.
- 482 (62) Pérez-Rafael, S.; Mezger, A.; Lieb, B.; Dallinger, R.; Capdevila, M.; Palacios, Ò.;
 483 Atrian, S. The metal binding abilities of *Megathura crenulata* metallothionein (McMT) in
 484 the frame of Gastropoda MTs. *Journal of Inorganic Biochemistry* **2012**, *108*, 84–90.
- 485 (63) He, Y.; Wang, L.; Ma, W.; Lu, X.; Li, Y.; Liu, J. Secretory expression, immunoaffinity
 486 purification and metal-binding ability of recombinant metallothionein (ShMT) from

- 487 freshwater crab *Sinopotamon henanense*. *Ecotoxicology and Environmental Safety* **2019**,
488 *169*, 457–463.
- 489 (64) Park, H.; Ahn, I.-Y.; Choi, H. J.; Pyo, S. H.; Lee, H. E. Cloning, expression and
490 characterization of metallothionein from the Antarctic clam *Laternula elliptica*. *Protein*
491 *Expression and Purification* **2007**, *52*, 82–88.
- 492 (65) Rocha, T. L.; Bilbao, E.; Cardoso, C.; Soto, M.; Bebianno, M. J. Changes in
493 metallothionein transcription levels in the mussel *Mytilus galloprovincialis* exposed to
494 CdTe quantum dots. *Ecotoxicology* **2018**, *27*, 402–410.
- 495 (66) Soazig, L.; Marc, L. Potential use of the levels of the mRNA of a specific metallothionein
496 isoform (MT-20) in mussel (*Mytilus edulis*) as a biomarker of cadmium contamination.
497 *Marine Pollution Bulletin* **2003**, *46*, 1450–1455.
- 498 (67) Vergani, L.; Grattarola, M.; Borghi, C.; Dondero, F.; Viarengo, A. Fish and molluscan
499 metallothioneins: a structural and functional comparison. *The FEBS Journal* **2005**,
500 *272*, 6014–6023.
- 501 (68) Vergani, L.; Grattarola, M.; Grasselli, E.; Dondero, F.; Viarengo, A. Molecular
502 characterization and function analysis of MT-10 and MT-20 metallothionein isoforms from
503 *Mytilus galloprovincialis*. *Archives of Biochemistry and Biophysics* **2007**, *465*, 247–253.
- 504 (69) Sigel, A.; Operschall, B. P.; Sigel, R. K.; Sigel, H. Metal ion complexes of nucleoside
505 phosphorothioates reflecting the ambivalent properties of lead (II). *New Journal of*
506 *Chemistry* **2018**, *42*, 7551–7559.
- 507 (70) Bergey, L. L.; Weis, J. S. Molting as a mechanism of depuration of metals in the fiddler
508 crab, *Uca pugnax*. *Marine Environmental Research* **2007**, *64*, 556–562.