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Thiophilicity is a determinant of bioaccumulation in benthic fauna

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KEYWORDS

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

ABSTRACT

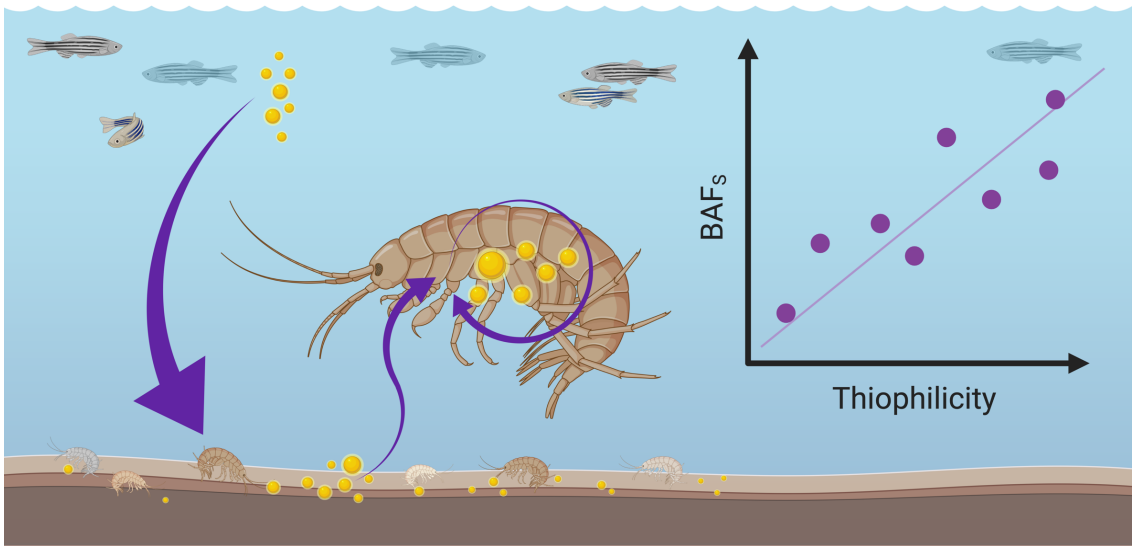
Aquatic contamination can settle into sediments, where it complexes with organic matter and becomes bioavailable. The resulting bioaccumulation of these contaminants by benthic fauna poses a serious threat due to the potential for trophic transfer. This paper offers an insight into the heterogenous accumulation behavior of different elements, and the consequences for ecological risk. In this study, we present field quantification of sediment-associated bioaccumulation factors (BAF_S) 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 BAF_S 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.

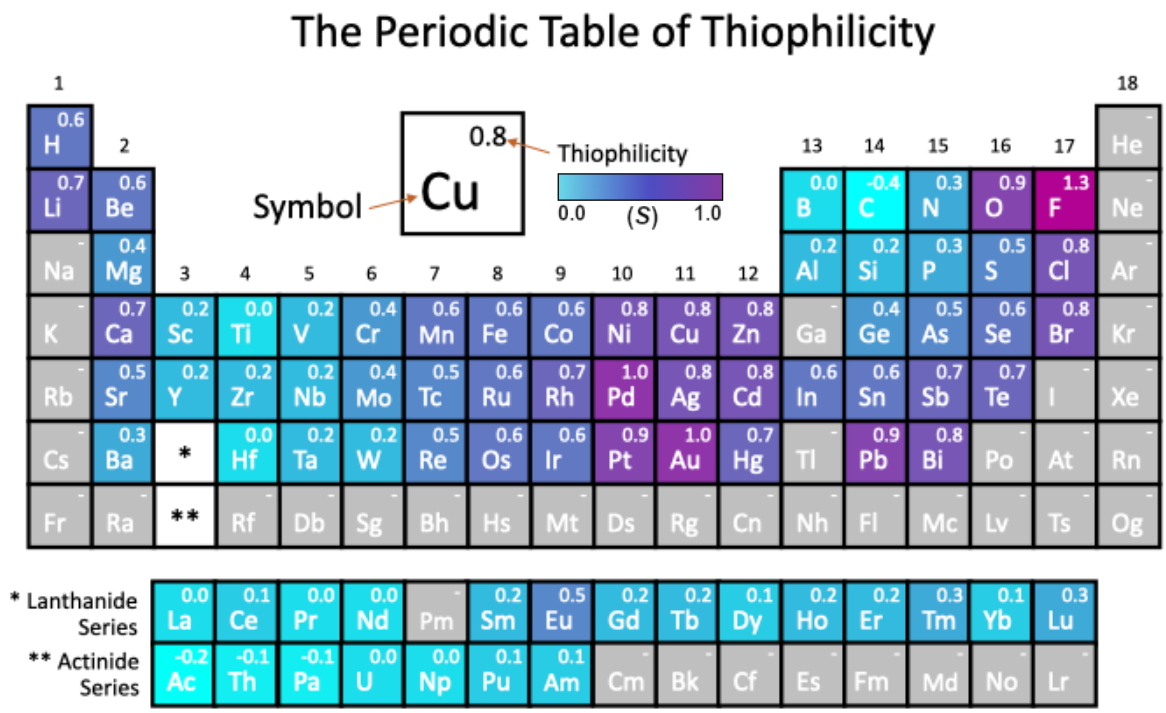


Figure 1. *The Periodic Table of Thiophilicity, according to Kepp, 2016.[35] Higher numbers indicate a higher affinity to S versus O.*

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, 2016[35] 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:

$$ln\left(BAF_S(M)_i\right) = \beta_1 S(M) + \beta_0 + \epsilon_i \quad (5)$$

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 *et al.*, 2009[36]	Marine	*Alismatales*, *Amphipoda*, *Cardiida*, *Decapoda*, *Littorinimorpha*, *Phyllodocida*, *Ulvales*	Fe, Cu, Zn, Cd, Pb
Culioli *et al.*, 2009[37]	Freshwater	*Coleoptera*, *Ephemeroptera*, *Hygrophila*, *Plecoptera*, *Trichoptera*, *Tricladida*	As, Sb
Erasmus *et al.*, 2020[38]	Freshwater	*Decapoda*, *Diptera*, *Ephemeroptera*, *Haplotaxida*, *Hygrophila*, *Odonata*, *Trichoptera*	Cr, Ni, Cu, Zn, Cd, Pt, Pb
Farag *et al.*, 2007[39]	Freshwater	"Benthic macroinvertebrates"	Cu, Zn, As, Cd, Pb
Levit *et al.*, 2020[40]	Marine	*Amphipoda*, *Bivalvia*, *Gastropoda*, *Hirudinea*, *Oligochaeta*	Mn, Fe, Cu, Zn, Cd, Pb
Pourang, 1996[41]	Freshwater	*Diptera*, *Haplotaxida*, *Mytilida*, *Venerida*	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 $$ln\left(BAF_S(M)_i\right) = \sum_{k=2}^{6} \beta_k X_k + \beta_1 S(M) + \beta_0 + \epsilon_i \quad (6)$$

[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 BAF_S 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 BAF_S

Calculated sediment-associated Bioaccumulation Factors (BAF_S) for 20 analytes are shown in Table 2. The BAF_S 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.

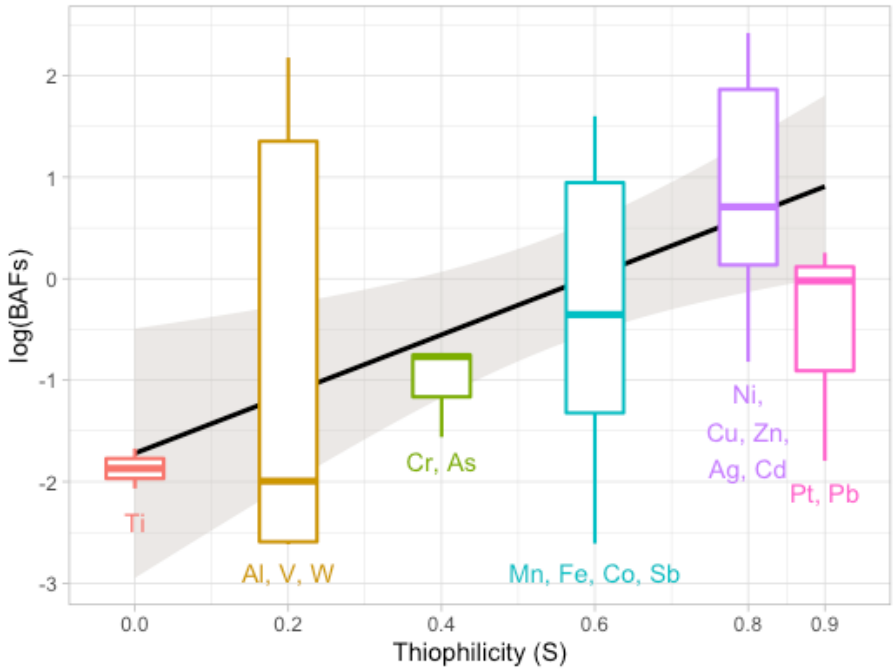
Table 2. *Determination of bioaccumulation factor from analyzed samples.*

		BAF_S	
Analyte	Thiophilicity (*S*)	*A. aquaticus*	*Gammarus sp.*
Al	0.2	0.0731	0.0749
Ti	0.0	0.1266	0.1876
V	0.2	0.1361	*n. d.*
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	*n. d.*
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	*n. d.*
Pd	1.0	*n. d.*	*n. d.*
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	*n. d.*
Au	1.0	*n. d.*	*n. d.*
Hg	0.7	*n. d.*	*n. d.*
Pb	0.9	0.9788	0.1663

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

$$BAF_S = e^{(2.52\pm0.94)S-(1.64\pm0.61)} \quad (7)$$



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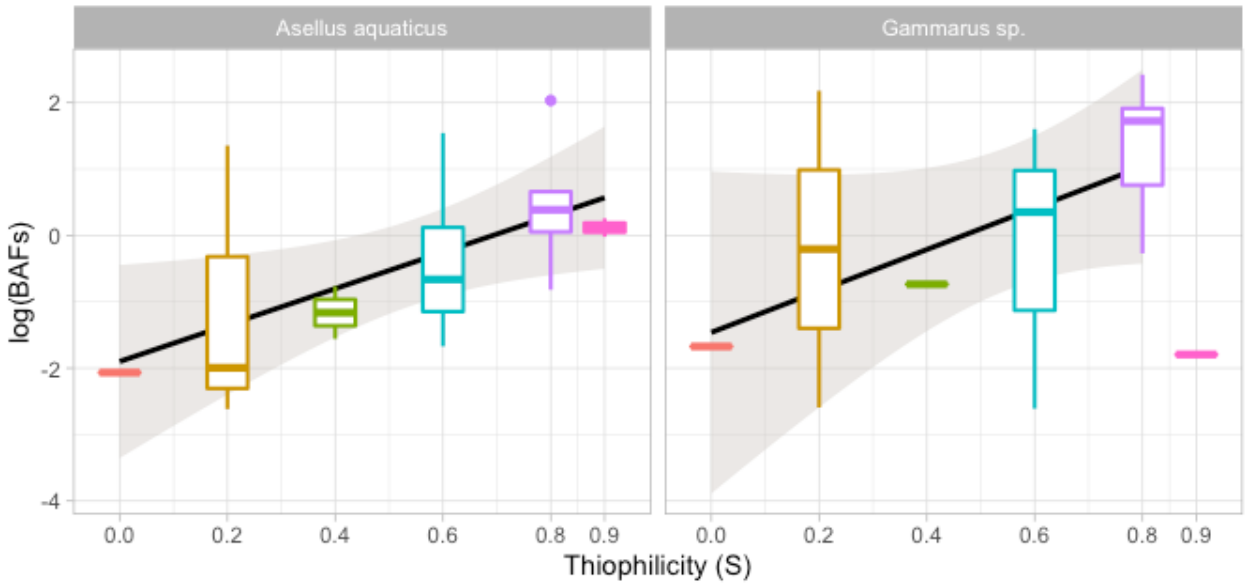
167 ***Figure 2.*** *Correlation between position on the thiophilic scale (S) and sediment-associated*
168 *bioaccumulation factor (BAF_S). With the exception of the elements with S=0.9, S values have been*
169 *rounded down to the nearest multiple of 0.2 for illustrative clarity, and elements with the 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 BAF_S 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 BAF_S versus
178 thiophilicity between species, shown by a high *p*-value and low effect size ($p = .387$, $R^2 = 0.022$),

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

186



187 ***Figure 3.*** *Correlation between position on the thiophilic scale (S) and sediment-associated*
188 *bioaccumulation factor (BAF_S), after stratification by species. Pb has been excluded from the*
189 *calculation of the trend line (shaded area showing 95% confidence interval; Asellus aquaticus*: p
190 $= .020$, $R^2 = 0.332$; *Gammarus sp.*: $p = .094$, $R^2 = 0.255$).

191 Examination of the adherence of each element to the trend described above showed that Pb,
192 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:

$$BAF_S = e^{(3.03\pm0.96)S-(1.81\pm0.60)} \quad (8)$$

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

Comparison of previously-published works

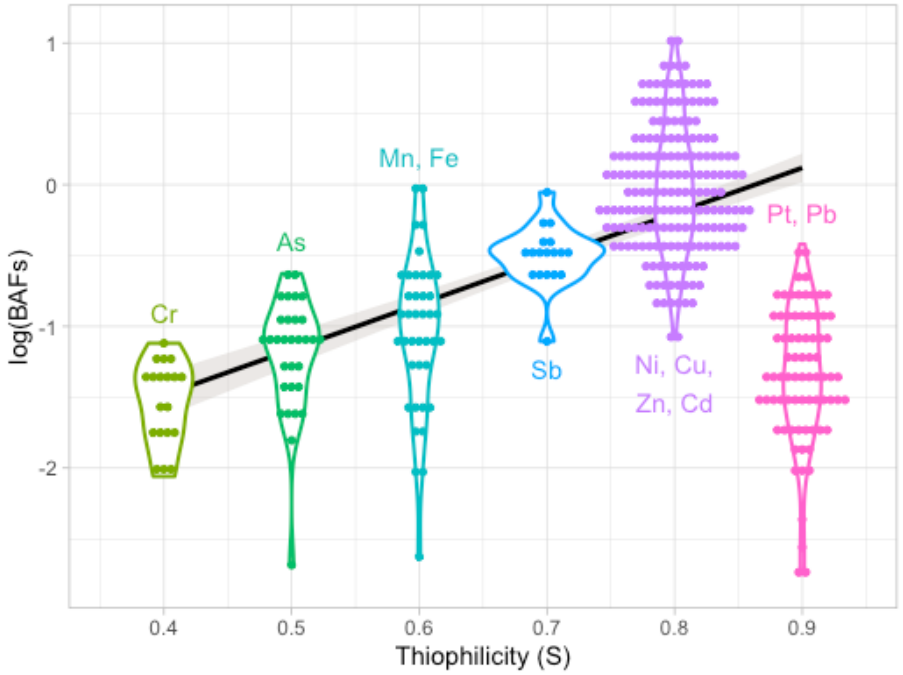


Figure 4. *Correlation between position on the thiophilic scale (S) and sediment-associated bioaccumulation factor (BAF_S), for a range of previously-published studies.*[36-41] *Each dot represents a single data point. Pb was omitted from the analysis and determination of the trend line (shaded area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

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

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

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

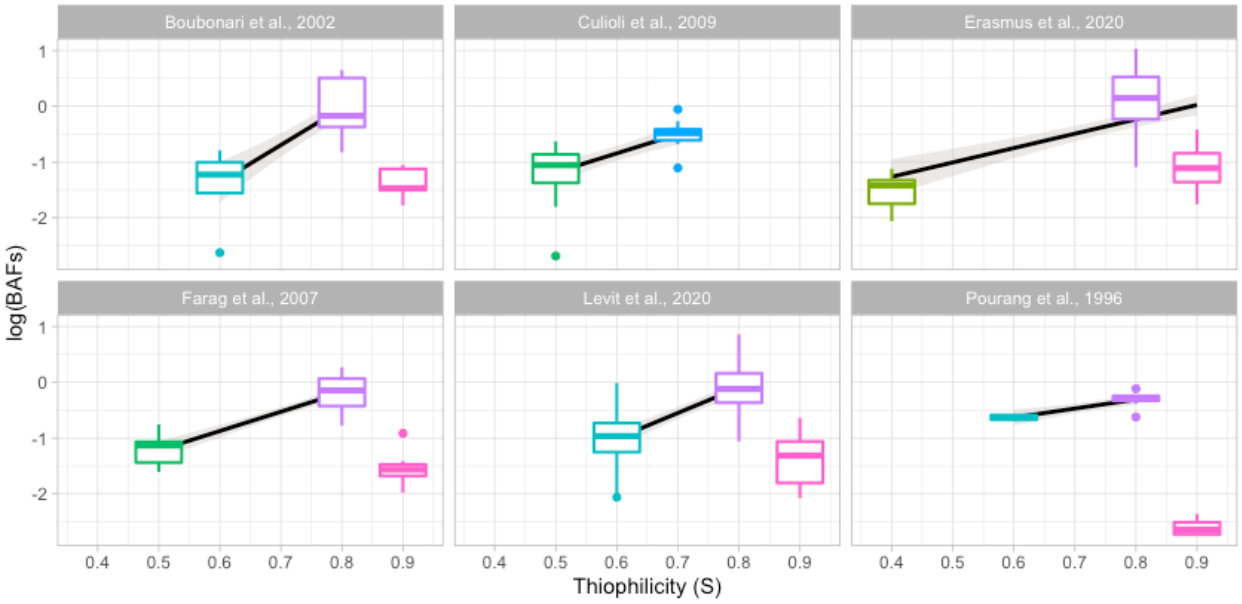


Figure 5. *Correlation between position on the thiophilic scale (S) and sediment-associated bioaccumulation factor (BAF_S) for a range of previously-published studies, after stratification by*

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

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

It is known[55-58] that the structure and characteristics of MT can vary between species, but all rely on the thiol group for the capture of the metal analyte. The consistency of the correlation between thiophilicity and accumulation supports the hypothesis that MT-regulation is a common and equally important detoxification mechanism across species.

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

The results presented in this article support the hypothesis that the accumulation of elemental contaminants in benthic fauna correlates significantly with the analyte's position on the thiophilic scale. However, Pb was not found to follow this trend; despite occupying the highest position on the thiophilic scale of any element considered in this paper, the BAF_S of Pb was found to be well below the observed trend. Unfortunately, while a number of metallothionein isoforms have been characterized, the metal-detoxification role of non-mammalian MT remains poorly understood,[63-68] leaving little indication of possible Pb-specific effects. We would like to put forward several 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, $PbSO_4$, PbS, $PbCO_3$, $Pb_3(PO_4)_2$ 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 Pb^{2+} showed a strong thiophilic tendency to bind with the S site of methyl thiophosphate over the corresponding O site of methyl phosphate; however, Pb^{2+} 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 $6s^2$ lone pair of Pb^{2+}, and its role in the Pb^{2+} 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.

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)

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