

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
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1 Thiophilicity is a determinant of bioaccumulation 2 in benthic fauna

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

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

5 ² School of Chemistry, University College Cork, Ireland

6 ³ Environmental Research Institute, University College Cork, Ireland

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

8 **KEYWORDS**

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

10 **ABSTRACT**

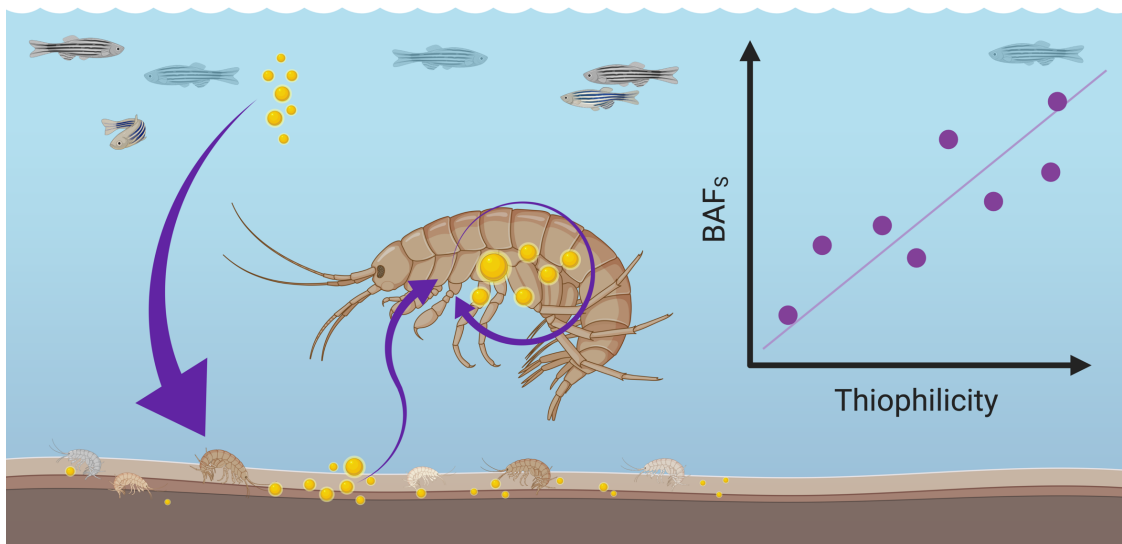
11 Aquatic contamination can settle into sediments, where it complexes with organic matter and
12 becomes bioavailable. The resulting bioaccumulation of these contaminants by benthic fauna
13 poses a serious threat due to the potential for trophic transfer. This paper offers an insight into the
14 heterogenous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

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

25 HIGHLIGHTS

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

29 GRAPHICAL ABSTRACT



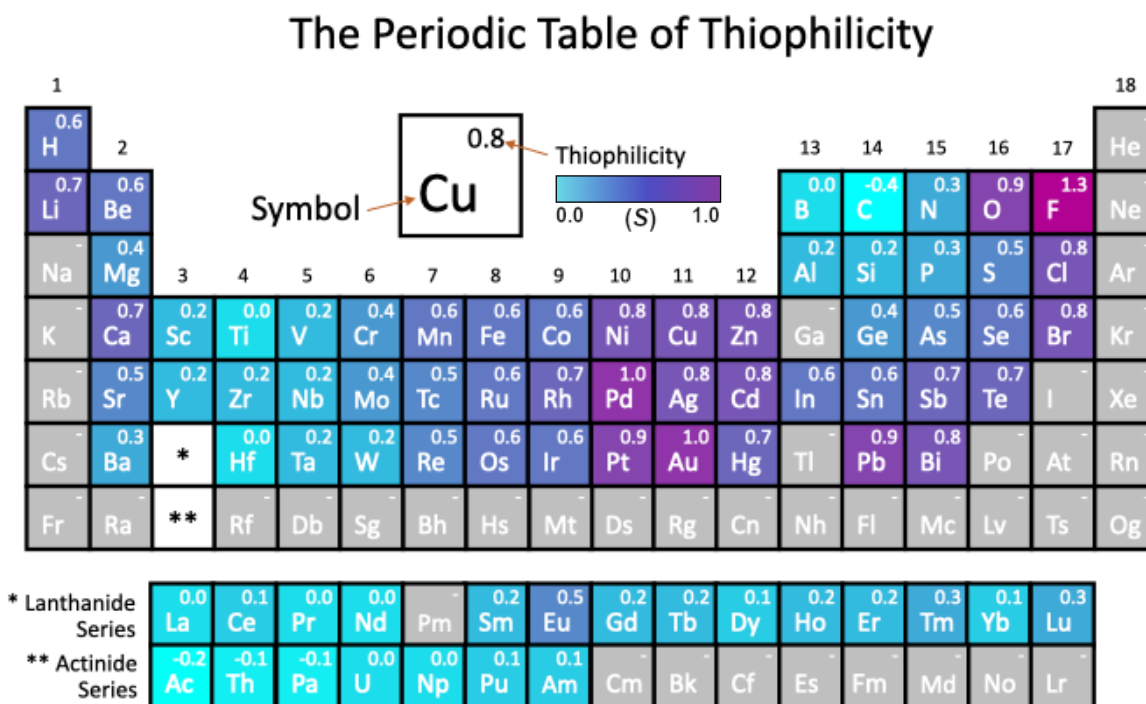
34 INTRODUCTION

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

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

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

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



62

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

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

72 **MATERIALS AND METHODS**

73 **Environmental sampling**

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

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

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

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

89 **Chemical analysis**

90 Solid samples (sediment and macroinvertebrate) were dried and approximately 25 mg (dry weight)
91 of each per site were digested with 2 ml conc. HNO₃ and 1 ml HF, then heated to reflux overnight.
92 HF content was subsequently evaporated, and the remaining solutions were diluted to 100 ml with
93 deionized water.

94 In order to determine the elemental concentrations of Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As,
95 Pd, Ag, Cd, Sb, W, Pt, Au, Hg and Pb in the samples, analysis was carried out using a PerkinElmer
96 NexION 2000B ICP-MS. External calibration was carried out using serial dilutions from 10 ppm
97 standards. Diluent consisted of a 2% HNO₃ solution, prepared using in-house distilled nitric acid
98 and 18.2 Mohm deionized water. SLRS-6 (riverine water, Canada) was used as a certified
99 reference material, and instrument drift was normalized by spiking laboratory blanks, standards
100 and samples with 1 ppb of Rh, In and Ir.

101 **Determination of bioaccumulation factor (BAF)**

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

106 $BAF_W = \frac{[organism]}{[water]}$ (1)

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

108 $BAF_S = \frac{[organism]}{[sediment]}$ (2)

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

115 **Determination of thiophilicity**

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

118 $\Delta D_{OS}(M) = D_O(M) - D_S(M)$ (3)

119 $S(M) = 1 - \frac{\Delta D_{OS}(M) - \Delta D_{OS}(Au)}{\Delta D_{OS}(Hf) - \Delta D_{OS}(Au)}$ (4)

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

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

125 **Statistical analysis**

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

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

$$133 \ln(BAF_S(M)_i) = \beta_1 S(M) + \beta_0 + \epsilon_i \quad (5)$$

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

136 **Comparative analysis**

137 Data from a number of previously-published studies, outlined in Table 1, were analyzed in a
138 similar manner to the data presented in the study to verify this phenomenon over a wider range of
139 conditions, taxa and analytical approaches. Data collection was limited to studies that considered
140 multiple analytes and multiple taxa and/or sites. The resulting dataset contains data on over 20
141 taxonomic orders and 11 elements. Where the data was not presented in numerical form,
142 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.

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

153 RESULTS AND DISCUSSION

154 Correlation between thiophilicity and BAFs

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

159 **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

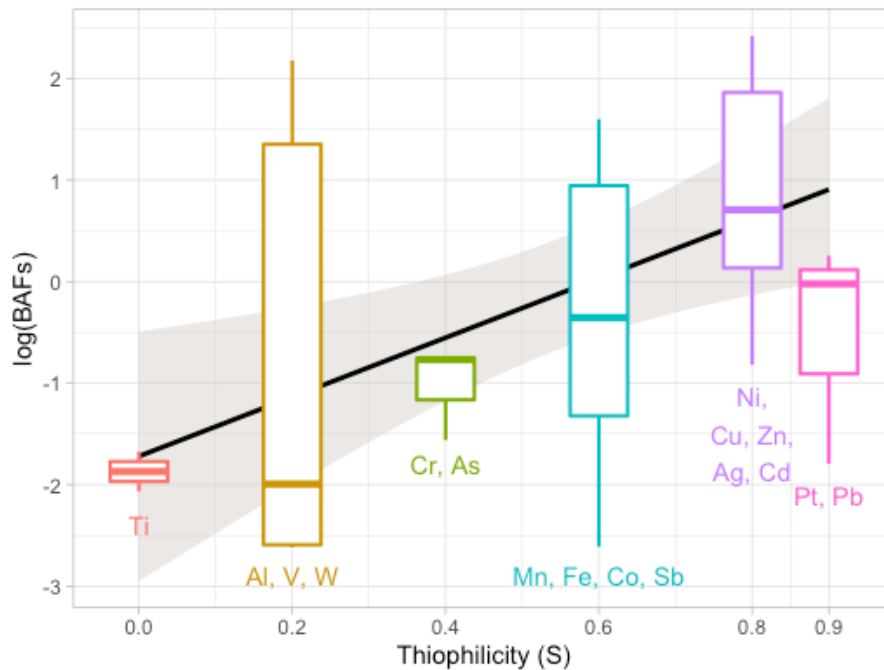
Co	0.6	0.3778	<i>n. d.</i>
Ni	0.8	0.4410	0.7631
Cu	0.8	7.6285	11.2537
Zn	0.8	1.9318	2.1328
As	0.5	0.4641	<i>n. d.</i>
Pd	1.0	<i>n. d.</i>	<i>n. d.</i>
Ag	0.8	1.0538	5.5998
Cd	0.8	1.4695	6.7652
Sb	0.7	4.6726	4.9619
W	0.2	3.8723	8.8327
Pt	0.9	1.2910	<i>n. d.</i>
Au	1.0	<i>n. d.</i>	<i>n. d.</i>
Hg	0.7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

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

165



166

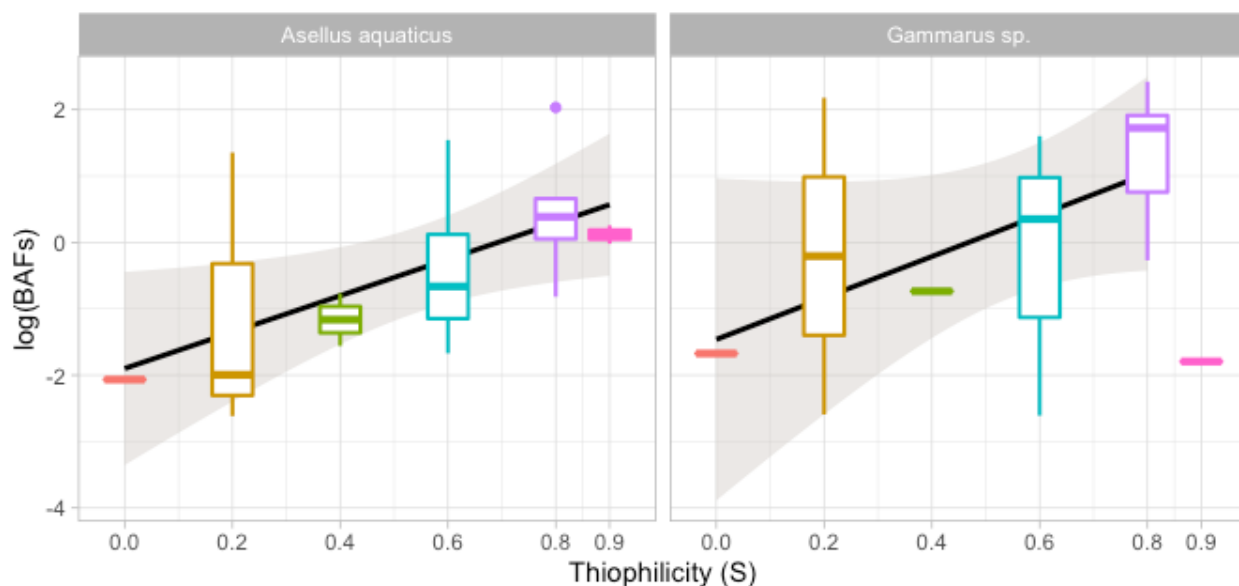
167 **Figure 2.** Correlation between position on the thiophilic scale (S) and sediment-associated
 168 bioaccumulation factor (BAF_s). With the exception of the elements with $S=0.9$, S values have been
 169 rounded down to the nearest multiple of 0.2 for illustrative clarity, and elements with the 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 (*BAFs*), 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, $R^2 = 0.255$).

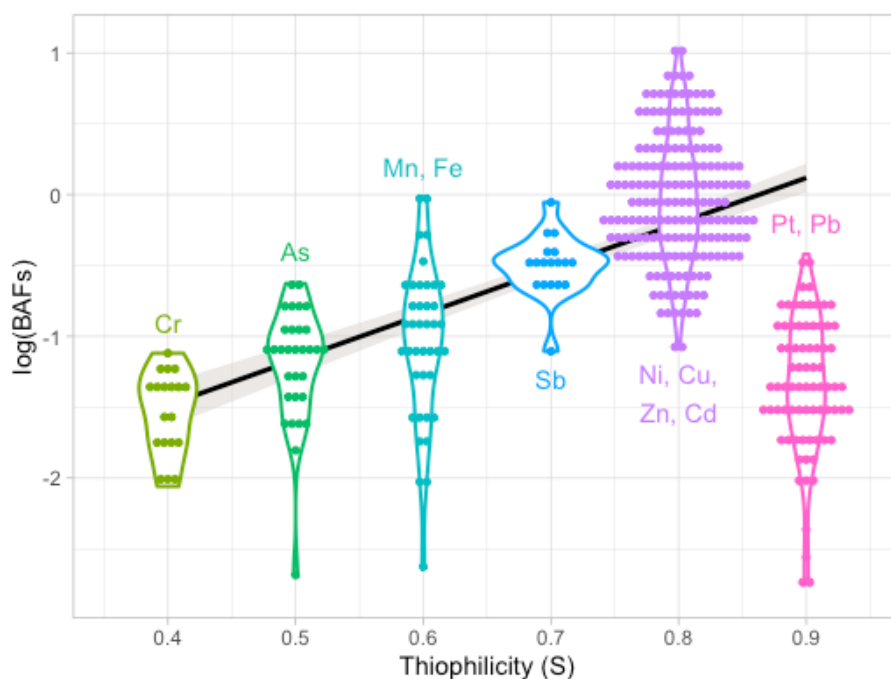
191 Examination of the adherence of each element to the trend described above showed that *Pb*,
192 although scoring highly on the thiophilicity scale ($S = 0.9$), was not readily accumulated by either

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

$$197 \quad BAF_s = e^{(3.03 \pm 0.96)S - (1.81 \pm 0.60)} \quad (8)$$

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

199 Comparison of previously-published works

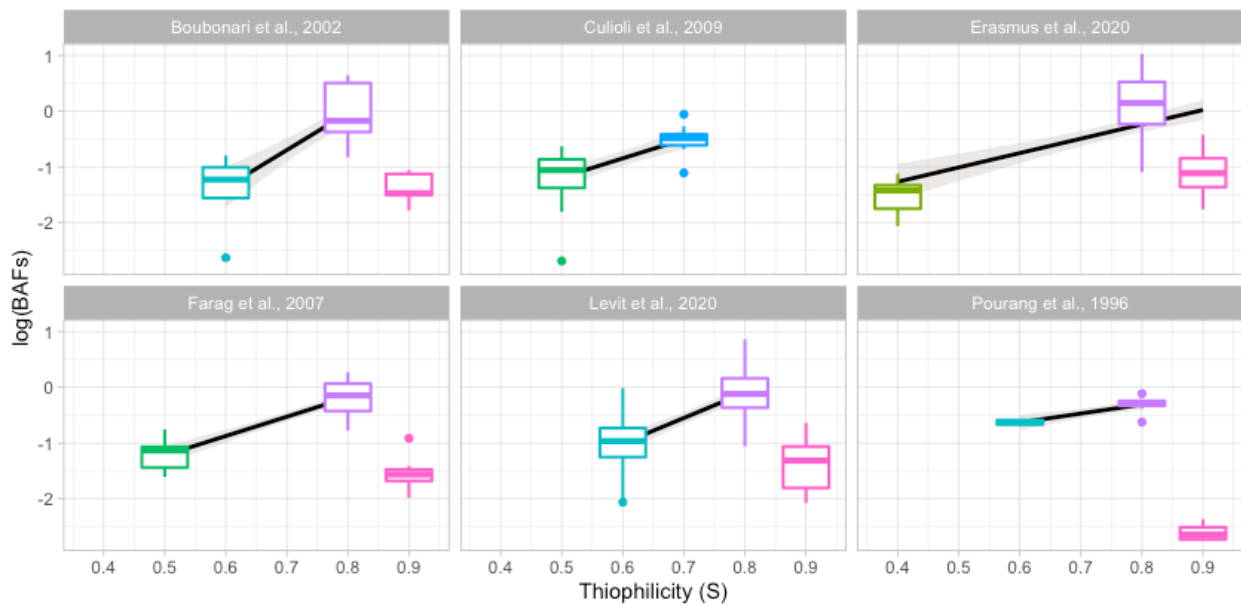


200 **Figure 4.** Correlation between position on the thiophilic scale (S) and sediment-associated
 201 bioaccumulation factor (BAF_s), for a range of previously-published studies.³⁶⁻⁴¹ Each dot
 202 represents a single data point. Pb was omitted from the analysis and determination of the trend
 203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

217 *study. Pb was omitted from the analysis and determination of the trend line (shaded area showing*
218 *95% confidence interval), but is shown here for illustration. The elements included in each study*
219 *are listed in Table 1.*

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

226 **Further discussion**

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

232 The analysis presented above assumes that the elemental thiophilicity offers a suitable
233 approximation for the probability of the thiol capturing the metallic component of a molecule.
234 Needless to say, there may be differences between the thiophilicity of a given element and a
235 molecule containing that element, but we make the simplifying assumption that the elemental
236 properties are representative. In an unspecified environment, where there is little certainty about
237 the molecular composition of contaminants present, and where the elemental accumulation is of
238 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:

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

281

282 **CONCLUSIONS**

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

291 **SUPPORTING INFORMATION**

292 The following supplementary file is available online:

293 Data listing (PDF)

294 **ACKNOWLEDGMENTS**

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

301

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