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# Thiophilicity is a determinant of bioaccumulation in benthic fauna

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# 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 (BAFs) in freshwater benthic macroinvertebrates. 17 elements were quantified using ICP-MS in 16 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<sub>S</sub> 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

- 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

# 29 GRAPHICAL ABSTRACT



#### **34 INTRODUCTION**

The metallic pollution of freshwater systems has been a significant concern for many decades.<sup>1-3</sup> 35 36 Concentrations of trace metals in waterways in excess of natural background levels can be attributed to anthropogenic pressures.<sup>4,5</sup> Various metals may enter the hydrosphere through urban 37 run-off, wastewater treatment plant outfall and road run-off.<sup>6-8</sup> While direct and acute toxicity 38 39 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 40 threat that metal pollution poses to the ecosphere through the mechanism of trophic transfer.<sup>9</sup> 41 42 Benthic macroinvertebrates of various families make up the base of the aquatic food web, and are essential to the overall health of the hydrosphere.<sup>10,11</sup> Many species, including fish, regularly 43 44 predate upon these organisms and are, thus, exposed to the potential biomagnification of accumulated tissue concentrations.<sup>12-15</sup> For this reason, the accumulation of contaminants by 45 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 from their sedimentary environment.<sup>16-19</sup> It is noted that these organisms sequester the metal 48 49 contaminants in order to render them metabolically available, in the case of essential elements (eg. Cu, Fe, Zn), or toxically inert.<sup>20,21</sup> It is most commonly thought that this de-mobilization of metal 50 51 ions is through a metallothionein (MT)-regulated mechanism, wherein the thiol groups of the MT protein capture the metals,<sup>22</sup> although other thiol-mediated processes have also been proposed.<sup>23</sup> 52 While the study of MT, and its various expressions, has determined the viability of this theory, 53 54 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.<sup>24</sup> It has been widely ascertained that MT will enable the bioaccumulation of Cu, Cd, Zn and Ag,<sup>25-27</sup> and there is some evidence that it can capture Platinum-group elements,<sup>8,28-31</sup> and Pb.<sup>27,32</sup>

- Thiophilicity is defined as the affinity an element will have towards a sulfur atom.<sup>33,34</sup> Recently,
  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.<sup>35</sup> The thiophilic scale is overlaid on the periodic

61 table in Figure 1.



The Periodic Table of Thiophilicity

62

63 *Figure 1.* The Periodic Table of Thiophilicity, according to Kepp, 2016.<sup>35</sup> Higher numbers

<sup>64</sup> *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 between experimentally-derived elemental accumulation correlation 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

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.<sup>17,42-46</sup>

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.

#### 89 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<sub>3</sub> 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.

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% HNO<sub>3</sub> 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;<sup>47,48</sup> we denote this definition BAFw:

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

107 An alternative definition, that we denote BAFs, considers instead the sediment concentration:<sup>47</sup>

108 
$$BAF_S = \frac{[organism]}{[sediment]}$$
 (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 BAFs is employed exclusively in this paper to quantify accumulation.

#### 115 **Determination of thiophilicity**

The thiophilicity (S) was determined in Kepp, 2016,<sup>35</sup> using data from Haynes, 2014,<sup>49</sup> according
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)

where  $D_{\rm O}({\rm M})$  is the bond dissociation enthalpy of  ${\rm M}_{\rm O}$  and  $D_{\rm S}({\rm M})$  is the bond dissociation enthalpy of M<sub>S</sub>. 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<sup>35</sup> 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.

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).<sup>50</sup> Linear regressions were employed to investigate the hypothesis of a correlation between thiophilicity and accumulation, as follows:

133 
$$ln(BAF_{\mathcal{S}}(M)_{i}) = \beta_{1}S(M) + \beta_{0} + \epsilon_{i}$$
(5)

where  $BAF_{S}(M)_{i}$  is a single measurement datum,  $\beta_{0}$  and  $\beta_{1}$  are the regression coefficients associated with the relationship between  $BAF_{S}$  and thiophilicity, and  $\varepsilon_{i}$  is the residual.

#### 136 **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.<sup>51</sup>

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

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

148 
$$ln(BAF_{\mathcal{S}}(M)_{i}) = \sum_{k=2}^{6} \beta_{k} X_{k} + \beta_{1} S(M) + \beta_{0} + \epsilon_{i}$$
(6)

<sup>&</sup>lt;sup>1</sup> 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  $\beta_0$  and  $\beta_1$  are the regression coefficients associated with the relationship between BAFs and 150 thiophilicity,  $X_k$  are dummy variables representing studies 2 through 6,  $\beta_k$  are the corresponding 151 regression coefficients, and  $\varepsilon_i$  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 (BAF<sub>S</sub>) for 20 analytes are shown in 156 Table 2. The BAF<sub>S</sub> 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 | <i>bioaccumulation</i> | factor | from | analyzed | samples. |
|-----|----------|---------------|----|------------------------|--------|------|----------|----------|
|     |          |               |    |                        | / .    | /    | ~        | 1        |

|         |                   | BAFs         |              |
|---------|-------------------|--------------|--------------|
| 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       | <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       |

| 0.7631                |
|-----------------------|
| 11.2537               |
| 2.1328                |
| <i>n</i> . <i>d</i> . |
| n. d.                 |
| 5.5998                |
| 6.7652                |
| 4.9619                |
| 8.8327                |
| <i>n</i> . <i>d</i> . |
| <i>n</i> . <i>d</i> . |
| <i>n</i> . <i>d</i> . |
| 0.1663                |
|                       |

160

161 These calculated BAF<sub>s</sub> 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<sub>s</sub> and the 163 thiophilicity (*S*) was estimated as:

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

165



166

167 **Figure 2.** Correlation between position on the thiophilic scale (S) and sediment-associated 168 bioaccumulation factor (BAF<sub>S</sub>). 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.

The relationship was still observed when both species were stratified (*Asellus aquaticus*: p = .017,  $R^2 = 0.324$ ; *Gammarus sp*.: p = .237,  $R^2 = 0.125$ ), illustrated in Figure 3, with a relatively low pvalue for *Gammarus sp*. explained by the smaller number of detected elements in samples this species. More interestingly, there was no evidence for differing relationship in BAFs versus 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 (BAF<sub>S</sub>), 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.:  $p = .094, R^2 = 0.255$ ).

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<sub>s</sub> = 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 
$$BAF_S = e^{(3.03 \pm 0.96)S - (1.81 \pm 0.60)}$$
 (8)

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

# 199 Comparison of previously-published works



**Figure 4.** Correlation between position on the thiophilic scale (S) and sediment-associated bioaccumulation factor (BAF<sub>S</sub>), for a range of previously-published studies.<sup>36-41</sup> 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.<sup>36-41</sup> 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-thanexpected 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 BAFs for this agglomerated dataset is:

214 
$$BAF_s = e^{(3.21\pm0.24)S - (2.77\pm0.21)}$$
 (9)



Figure 5. Correlation between position on the thiophilic scale (S) and sediment-associated
bioaccumulation factor (BAFs) 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.<sup>52-54</sup>

### 226 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 producesimilar behavior to this elemental approximation.

It is known<sup>55-58</sup> 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<sub>s</sub> 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,<sup>59-62</sup> resulting in 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<sub>S</sub> of Pb was found to be well 256 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,<sup>63-</sup> 257 <sup>68</sup> leaving little indication of possible Pb-specific effects. We would like to put forward several 258 hypotheses to explain the seemingly inhibited response of the bioaccumulation mechanism to Pb: 259

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<sub>4</sub>, PbS, PbCO<sub>3</sub>, Pb<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 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<sup>2+</sup> showed a
strong thiophilic tendency to bind with the S site of methyl thiophosphate over the
corresponding O site of methyl phosphate; however, Pb<sup>2+</sup> 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

272 properties of the  $6s^2$  lone pair of  $Pb^{2+}$ , and its role in the  $Pb^{2+}$  coordination sphere.<sup>69</sup>

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. <sup>70</sup>

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.

18

281

#### 282 CONCLUSIONS

283 We have demonstrated that the thiophilic scale acts as a suitable predictor of bioaccumulation potential. The correlation between thiophilic scale and BAFs is observed across taxa from different 284 285 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)

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301

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