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1 **Spatial variation of metal bioaccumulation in the hydrothermal vent mussel**
2 *Bathymodiolus azoricus*

3
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15

16 **Abstract**

17
18 The variability of the bioaccumulation of metals (Ag, Cd, Cu, Fe, Mn, Zn) was extensively
19 studied in the mussel *Bathymodiolus azoricus* from five hydrothermal vent sites inside three
20 main vent fields of increasing depth along the Mid Atlantic Ridge: Menez-Gwen, Lucky-
21 Strike and Rainbow. Metal bioaccumulation varied greatly between vent fields and even
22 between sites inside a vent field with *B. azoricus* showing a great capacity to accumulate
23 metals. The bioaccumulation of these metals also varied significantly among tissues. The
24 main target was the gills where metals were mainly associated with soluble compounds
25 whereas in the digestive gland they were mainly associated with insoluble compounds.
26 Storage of metals under insoluble forms in *B. azoricus* seems to be a major pathway for the
27 detoxification of both essential and non-essential metals. Mussels from the studied fields can
28 be discriminated following their metallic load but the segregation relies partially on the
29 composition of the metal-enriched fluids.
30

31 **Keywords:** Bioaccumulation; Metals; Azores; Hydrothermal Vents; Mytilids; *Bathymodiolus*

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37

38 **1. Introduction**

39

40 Marine molluscs have a large capacity to accumulate metals and therefore are commonly
41 suggested as biomonitors of metal contamination of marine ecosystems (Phillips, 1977, 1985;
42 Goldberg et al., 1978; Rainbow, 1995; Lauenstein and Daskalalis, 1998; Langston and
43 Bebianno, 1998; Neff, 2002). Some metals (Cu, Fe, Mn and Zn) are essential for living
44 organisms as they are components of enzymes, respiratory proteins and structural elements of
45 organisms. Although they become toxic when present in excess, it has been shown that the
46 coastal mussel, *Mytilus edulis*, regulates zinc and partially copper bioaccumulation over a
47 wide range of concentrations in the environment sea water (Langston et al., 1998).
48 Conversely, other metals like Ag and Cd, are non essential metals, their accumulation by
49 molluscs is almost universally determined by the degree of exposure (Langston et al., 1998).
50 The cellular detoxification of trace metals is then achieved by several mechanisms including
51 their binding to metallothioneins (e.g. Ag, Cd, Cu, Hg and Zn) and/or their incorporation to
52 metabolically inert granules (Dallinger and Rainbow, 1993).

53 The hydrothermal environments are characterized by high concentrations of several metals
54 like Cd, Cu, Fe, Hg, Mn, Zn originating from the discharge of hydrogen sulfide-rich vent
55 waters (Corliss et al., 1979; Von Damm, 1990). These peculiar ecosystems represent natural
56 laboratories for studies on the potential deleterious effects of the cited metals (Cosson and
57 Vivier, 1995; Cosson and Vivier, 1997; VENTOX, 2003). Bivalve molluscs living in these
58 environments are known to accumulate tremendous amounts of metals with surprisingly no
59 evident deleterious effects. Bioaccumulation was reported for the clams *Calyptogena*
60 *magnifica* (Roesijadi and Crecelius, 1984; Roesijadi et al., 1985; Cosson-Mannevy et al.,
61 1988) and *Vesicomya gigas* (Ruelas-Inzunza et al., 2003), and an unidentified Japanese clam
62 (Kim et al., 1991) from the East Pacific Rise. Similar results were obtained when studying the
63 mussels *Bathymodiolus thermophilus* from the East Pacific Rise (Smith and Flegel, 1989;

64 Rouse, 1999) and *B. azoricus* from the Mid-Atlantic Ridge (Geret et al., 1998; Rouse et al.,
65 1998).

66 The Mytilidae of the genus *Bathymodiolus* are almost ubiquitous among the organisms
67 associated with hydrothermal vents (Tunnicliffe, 1991) and are appropriate models for
68 assessing the responses to the metallic enrichment of such ecosystems. The Atlantic species *B.*
69 *azoricus* is commonly found at the Azores Triple Junction hydrothermal vents on the Mid-
70 Atlantic Ridge (ATJ-MAR) where it is frequently the dominant species, extended in high
71 densities at the base or even on the entire walls of the chimneys (Van Dover et al., 1996;
72 Colaço et al., 1998; Desbruyères et al., 2001). Preliminary results from individuals sampled at
73 two sites indicated that the levels of some elements (i.e. Ag, Ba, Cd, Cu, Fe, Mn, Sr and Zn)
74 within the gills and the digestive gland were high compared to the levels measured in tissues
75 of the mussel *Mytilus edulis* (Rouse et al., 1998). Moreover, the authors suggested that the
76 observed differences between the levels of metals in the mussels from the two vent sites could
77 be related to differences within the chemical composition of respective hydrothermal fluids.

78 This paper details the bioaccumulation of essential (Cu, Fe, Mn and Zn) and non-essential
79 metals (Ag and Cd) in three tissues (i.e. gills, mantle and digestive gland) of *B. azoricus*
80 sampled at five sites located among three different vent fields from the ATJ-MAR. The aims
81 of this study were, (i) to describe the variations of metal levels between tissues, (ii) to address
82 their intracellular distribution between soluble and insoluble fractions in relation with
83 bioaccumulation and detoxification processes, and (iii) to establish an eventual correlation
84 between their abundance within and among vent fields and their bioaccumulation by *B.*
85 *azoricus*.

86

87 **2. Materials and Methods**

88

89 **2.1. Sampling and sample preparation**

90

91 Mussels were collected during dives using the Ifremer Remotely Operated Vehicle
92 "Victor6000" during the "ATOS 2001" cruise. Three vent fields were explored located on
93 three segments of the south-eastern limb of the ATJ (Ondréas et al. 1997): Menez Gwen (MG,
94 37°51'N, 31°31'W, 850m), Lucky Strike (LS, 37°17'N, 32°16'W, 1650m) and Rainbow
95 (36°13'N, 33°54'W, 2350m) (Fig. 1).

96 The vent fields differ by their depth (850 to 2350m), their tectonic settings, the composition
97 of their host rocks (mantle-derived serpentinized peridotite vs. basalt), the nature of associated
98 volcanism, their fluid composition and the dominance of different key faunal assemblages
99 (Fouquet et al. 1998, Desbruyères et al. 2001). Samples were collected using the grab of the
100 submersible. Two sites were visited at Menez Gwen (ATOS 8 - A8 and ATOS 10 - A10, North
101 East of the top of the volcano), two at Lucky Strike (Eiffel Tower - ET and Bairro Alto – BA,
102 respectively South East and North West of the lava lake) and one in Rainbow (Rb).

103 At each site, about ten mussels of comparable size (A8: $4.76 \pm 0.15\text{cm}$, A10: $4.99 \pm 0.37\text{cm}$,
104 BA: $7.67 \pm 0.66\text{cm}$, ET: $6.50 \pm 0.38\text{cm}$, Rb: $6.76 \pm 0.57\text{cm}$) were sampled from a very small
105 area delimited by a 50x50cm frame deployed by the submersible. Mussels were dissected on
106 board. Tissues (i.e. gills, mantle and digestive gland) were isolated, stored in liquid nitrogen
107 and lyophilised when back to the laboratory. Each sample was weighed, homogenized in
108 TRIS buffer (100mM, 10mM β mercaptho-ethanol, pH 8.6) and centrifuged (30 000g, 30min,
109 4°C). For each sample, the pellet containing the insoluble compounds (named insoluble
110 fraction hereafter) and an aliquot of the supernatant, containing the soluble compounds
111 (named soluble fraction hereafter) were digested with nitric acid at 60°C for metal analysis.

112

113 2.2. Analytical procedure

114

115 Metal quantification (i.e. Ag, Cd, Cu, Fe, Mn and Zn) was determined by Electrothermal

116 Atomic Absorption Spectrometry (E-AAS: Hitachi Z-5000 and Solaar Unicam 989QZ), by
117 Flame Atomic Absorption Spectrometry (F-AAS: Hitachi Z-5000) or by Inductive Coupled
118 Plasma Atomic Emission Spectrometry (ICP-AES JY-238 sequential) depending on the ratio,
119 concentration vs volume, of the liquid aliquots. The accuracy of the analytical procedure was
120 checked using certified reference material (TORT-2; DORM-2; DOLT-2) from the National
121 Research Council, Canada. Our results were in good agreement with the certified values
122 except for Ag. The concentration of Ag in the nitric digested supernatants (soluble fractions)
123 of homogenized reference material was too close to our detection limit. The levels of metals
124 are expressed as $\mu\text{g g}^{-1}$ dry weight of soft tissue.

125

126 2.3. Statistical analysis

127

128 In a first step, univariate analyses were performed to assess the variations in the levels of
129 metals among sites for each tissue. One-way ANOVAs with site as a fixed factor were used on
130 log-transformed data $[\ln(x+1)]$. Normality was judged by normal probability plots and
131 homogeneity of variances was verified with the Bartlett's test ($\alpha= 0.05$). Post-hoc comparison
132 tests for means were made using the Tukey's honestly significant difference method for
133 unequal sample size. When a log transformation failed to meet ANOVA assumptions, raw data
134 were analysed by a Kruskal-Wallis nonparametric test. Nonparametric multiple comparisons
135 were performed using Nemenyi and Dunn's test (Zar, 1999). Analyses were carried out on
136 total levels of each metal.

137 In a second step, principal components analysis (PCA) was used to investigate the spatial
138 patterns of the relative levels of soluble and insoluble fractions of metals over the individuals
139 (Legendre and Legendre, 1998). One analysis was performed for each tissue. Metal levels
140 were $\ln(x+1)$ transformed prior to analysis.

141

142 3. Results

143

144 3.1. Whole metal levels

145

146 Detailed variations of metal bioaccumulation in the tissues between vent fields and sites are
147 given in Table 1.

148

149 3.1.1. Intra-site comparison

150 At Menez Gwen, the gills and the digestive gland showed a similar pattern regarding the
151 mean levels of analysed metals (Table 1). Ag, Cd and Mn levels were higher at ATOS 8 (A8)
152 than at ATOS 10 (A10), while the contrary was observed for Cu. No significant difference
153 was noticed for Fe and Zn. Concerning the mantle, a pattern similar to that of the gills and
154 digestive gland was observed for Cu and Fe, while similar levels of Ag, Cd and Mn were
155 measured in mussels from A8 or A10. Consequently, with the exception of Cu, tissues of
156 mussels from A8 presented metal levels (e.g., Ag, Cd, Fe, Mn, Zn) higher than or equal to
157 those observed for mussels from A10.

158 At Lucky Strike, the levels of Ag in the gills and the mantle were higher at Bairro Alto (BA)
159 than at Eiffel Tower (ET). Higher levels of Cd and Zn were observed respectively for the gills
160 and the mantle of mussels collected at BA. Beside these observations, no significant
161 difference was evidenced between the levels of metals measured in gills and mantle from BA
162 or ET mussels.

163

164 3.1.2. Inter-sites comparison

165 In general, mean levels of Ag, Cd, Cu and Zn in mussel tissues were higher at both sites of
166 MG than at Rainbow or did not differ significantly (Table 1). Conversely, Fe and Mn were
167 more abundant in mussels from Rb than from MG sites.

168 Similarly mussels collected at Rb generally contained less Ag, Cd, Cu and Zn than those
169 collected at LS. The digestive gland was the only tissue where higher levels of Fe and Mn
170 were observed for mussels collected at Rb.

171 Gills and mantle of mussels from both BA and A8 presented similar high levels of Ag, while
172 this metal was less abundant in mussels collected at both A10 and ET. Cd was more abundant
173 in the tissues of the mussels collected at LS than in those collected at MG. Cu was particularly
174 abundant in mussels collected at A10 whereas Zn was more abundant in mussels collected at
175 LS, especially at BA. The same observation goes for Fe and Mn with equal mean levels
176 observed for BA and ET in the gills and the mantle.

177 Mussels from Rb bioaccumulated more Fe and Mn than those from MG or LS. Conversely,
178 they showed lower mean levels of Ag, Cd, Cu and Zn. Higher levels of Cd, Zn, Fe and Mn
179 were recorded for mussels collected at LS than for those collected at MG. At both fields (LS
180 and MG) high levels of Ag were observed in mussels from BA and A8, while the highest
181 level of Cu was recorded in mussels from A10 site.

182

183 3.2. Metal compartmentalization

184

185 The amount (expressed as percentages – mean values) of metals associated with insoluble
186 compounds in the tissues of mussels collected at the studied ATJ sites are given in Table 2.

187 This amount ranges between 24 and 97%. However, with a few exceptions (bold in the table),
188 whatever the site, the tissue and the metal, there is a predominant association of the metals
189 with insoluble compounds with mean amounts > 50%.

190 From all the metals traced at the mussel gills, Zn was the only one that was not predominantly
191 associated with insoluble compounds, being equally distributed between the soluble and
192 insoluble fractions in mussels from MG and Rb.

193 In the mantle Ag is preferably immobilized as soluble form with the exception of mussels
194 from Eiffel Tower site (Lucky Strike field), the ratio of Ag stored under insoluble form of
195 which reached 50%. Cd was essentially associated with insoluble compounds in ATJ mussels.
196 The ratio of Cu associated with insoluble compounds was low in mussels from Rb, while it
197 was high for MG and LS mussels. Fe and Mn insoluble percentages were high for ATJ mussel
198 mantles with the exception of Mn at MG. The percentage of Zn associated with insoluble
199 compounds in mussels from Rb was lower than that of MG and LS mussels.
200 The association of Ag with insoluble compounds varied greatly in the digestive gland of
201 mussels from ATJ sites resulting in the following ranking LS>MG>Rb. Cd was essentially
202 associated with insoluble compounds in mussels from MG and LS, while the association was
203 moderate at Rb. The same observations fit with the association of Cu and Zn. Similarly, the
204 ratios of Fe and Mn immobilized as insoluble form in the digestive gland were elevated.

205

206 3.3. PCA analysis

207

208 A principal components analysis (PCA) was used to investigate the spatial patterns of the
209 relative levels of soluble and insoluble fractions of metals over the individuals. For each
210 tissue, principal component analyses clearly separated individuals between the sampling vent
211 fields and even between the vent sites within a field according to their relative concentrations
212 in the soluble and insoluble fractions of the different metals (Fig. 2-4).

213 The first 2 principal components accounted for 64.2% of the variability in the metal
214 concentrations in the gill, with 38.9% on axis 1 and 25.3% on axis 2 (Fig. 2A). Several groups
215 of mussels were discriminated. Going from the right of axis 1 we found LS mussels (BA then
216 ET) followed by MG mussels (A8 and A10) and Rb mussels towards the left. This axis
217 discriminates according to the relative levels of Zn and Cd, insoluble Ag and Fe, and soluble

218 Mn (Fig. 2B). On the second axis, MG discriminated itself from Rb and LS vent fields with
219 respect to relative levels of Fe, insoluble Mn and soluble Cu.

220 In the mantle, the PCA showed that the first two axes accounted for 64.6% of variability
221 between the mussels, with 36.2% explained by axis 1 and 28.4% by axis 2 (Fig. 3A). The first
222 axis takes into account the relative levels of Cd and Fe, soluble Zn and insoluble Mn, while
223 the second one discriminates positively the Cu levels and negatively the levels of Mn. LS (BA
224 then ET) and MG (A8 and A10) separated out to the right and left of axis 1, respectively (Fig.
225 3B). Rainbow showed some separation from MG along the second axis.

226 Axes 1 and 2 of the PCA accounted for 72.8% of the variability in metal levels in the
227 digestive gland between individuals, with 50.3% on axis 1 and 22.5% on axis 2 (Fig. 4A).

228 Metals with the greatest influence on variability between groups of mussels along axis 1 are
229 Zn, soluble Mn and Ag, and insoluble Cu (Fig. 4B). Along axis 2 the discrimination follows
230 the levels of insoluble Fe and Mn. On the first plane PCA ordination, Rb separated out from
231 LS and MG along axis 1, while this later field separated from LS and Rb along axis 2.

232 The main features that can be extracted from the 3 PCA are: (i) there is a clear spatial
233 segregation in the metal bioaccumulation by *B. azoricus*; (ii) this site segregation is observed
234 for the 3 organs studied; (iii) the behaviour of each metal or metal fraction is different for
235 each organ studied.

236

237 **4. Discussion**

238

239 Hydrothermal fluids contain high concentrations of metals, the proportions of which vary
240 greatly from one field to another, even at the scale of the ATJ zone (Charlou et al., 2000,
241 2002; Douville et al., 2002). These variations are related both to the chemical composition of
242 the basement rocks, the dynamic specificity of each hydrothermal field (Bonatti, 1984; Von
243 Damn 1990) and even the depth bringing about phase separation processes (Charlou et al.,

244 2000, 2002; Douville et al., 2002). Vent mussels do not live directly in contact with the
245 potentially harmful hot fluid but in the highly chemically reactive mixing zone. Here, the
246 mineral-rich solution, characterized by elevated methane, hydrogen and reduced sulfides,
247 mixes dynamically with the bottom oxygen-rich seawater. Metals present in the fluid form
248 precipitates with sulfide in plumes, chimney or in conduits surfaces (Trefry and Trocine,
249 1985; Von Damm et al., 1995b). Iron forms preferentially fine grained sulfide particles that
250 are exported in the buoyant and neutrally buoyant plume. Cu and Zn sulfides form large sized
251 grains and more crystalline particles that settle rapidly in the near field region (Feely et al.,
252 1994).

253 The bioaccumulation of metals in the mussels depends on the abundance and the
254 bioavailability of the latter in their proximal environment. Moreover, metal bioaccumulation
255 and storage forms are governed by the physiological functioning of the mussels. Taking into
256 account these abiotic and biotic factors, the goal of our work was to ascertain if the amounts
257 of metals bioaccumulated by the vent mussel *B. azoricus* in its tissues reflect the relative
258 abundance of the metals present in the emitted fluids at 5 sites (A8, A10, ET, BA, Rb) located
259 within 3 hydrothermal fields (Menez Gwen, Lucky Strike and Rainbow).

260

261 Our results highlighted that vent mussels bioaccumulate higher metal levels (Ag, Cd, Cu, Fe,
262 Mn, Zn) in their tissues (gills, mantle, digestive gland) than the coastal mussels, *Mytilus*
263 *edulis* (Fung et al., 2004) and *M. galloprovincialis* (Bebianno and Machado, 1997; Besada et
264 al., 2002; Kalpaxis et al., 2004; Szefer et al., 2004) in similar tissues. These results are in
265 accordance with scattered studies that reported whole levels of various metals in *B. azoricus*
266 from the same fields (Rousse et al., 1998; Geret et al., 1998; Kadar et al., 2005; Colaço et al.,
267 2006). As a general rule, there are specific pathways of metal bioaccumulation in tissues
268 depending upon their direct contact with the surrounding environment, the bioavailability
269 (chemical forms) of metals, the efficiency of uptake, storage, transfer to inner organs,

270 excretion mechanisms and physiological status. Despite its very peculiar environment and
271 physiology *B. azoricus* presents a metal organotropism similar to the main trends observed in
272 coastal mussels (Langston and Bebianno, 1998). The gills are directly exposed to dissolved
273 and particulate metals and show the highest levels of metals while the mantle, similarly
274 exposed, shows the lowest levels. This discrepancy could be related to a lesser involvement of
275 the mantle in exchanges, its participation to shell production as well as storage and its lack of
276 symbionts. The digestive gland of *B. azoricus* is supposed to be involved at a lesser degree in
277 the nutrition owing to the importance of the symbiosis as a source of energy, but confirms its
278 participation in metal metabolism as a pathway and storage organ. However, the fact that *B.*
279 *azoricus* can thrive with such metallic loads is noteworthy knowing that non essential metal
280 presence or excessive amounts of essential metals result generally in the perturbation of the
281 enzymatic functions (via constitutive ion substitutions on metalloenzymes) and the
282 antioxidant defence system (Bebianno et al., 2005; Company et al., 2006).

283

284 A comparison of the levels of studied metals in *B. azoricus* whole organs was established with
285 data regarding vent bivalves from the East Pacific Rise (EPR): *Bathymodiolus thermophilus*
286 and two vesicomids *Calyptogena magnifica* and *Vesicomya gigas* to assess the metal load of
287 *B. azoricus* (Fig. 5).

288 Main features emerge from this comparison between *B. azoricus* and related species from the
289 Pacific vents. The high load of Fe and Zn in the gills of mussels collected at Rb and LS,
290 respectively has to be attributed to the high concentration of these metals in corresponding
291 end-members (Charlou et al., 2000, 2002; Douville et al., 2002). However it should be noted
292 that, even though Fe and Zn are as abundant in the fluids from the EPR (Michard et al., 1984;
293 Von Damm, 1995; Cosson 1996; Le Bris et al., 2003) as in Rb and LS fluids, the availability
294 of both metals in the surrounding environment of *B. thermophilus* seems to be lower than in
295 *B. azoricus* environment (Table 3). Conversely, *C. magnifica* and *V. gigas*, present high levels

296 of Fe and Zn. Several hypothesis can be proposed to explain this discrepancy. First of all, *C.*
297 *magnifica* and *B. thermophilus*, even if they share the same location in some EPR sites, are
298 not positioned identically with regard to the emission of the fluid-seawater mixture. *C.*
299 *magnifica* lives in cracks in the basalt crust with its foot extending towards the input of fluid
300 enriched with reduced compounds, while *B. thermophilus* is an epibiotic organism living on
301 bare basalt having a more versatile relationship to the reduced compound-providing fluid. In
302 the Guaymas Basin, *V. gigas* is encountered in a sedimentary environment enriched with
303 particulate mineral and organic matter which could be ingested and partially responsible for
304 the important metal bioaccumulation (Ruelas-Inzunza et al., 2003). The high amounts of Zn in
305 *C. magnifica* tissues could also be attributed to the occurrence of a Zn-protein involved in
306 sulphide transport from their foot to the endosymbiont host in their gills (Childress et al.,
307 1993). The existence of such a protein has been suggested by Ruelas-Inzunza et al. (2003) as
308 a reason for the high amounts of Zn observed in *V. gigas* tissues. Moreover, the degree of
309 dependence of the bivalve genus regarding their endosymbionts could interfere with the
310 uptake of metals from their environment under soluble or particulate forms, in relation with
311 their feeding habit. Copper in the end-member and immediate neighbourhood of mussels at
312 MG is low compared to recorded values for the EPR or LS and Rb sites (Charlou et al., 2000,
313 2002; Cosson 1996; Douville et al., 2002; Michard et al., 1984; Von Damm, 1995), but
314 however Cu is abundant in both gills and mantle of MG mussels reflecting a higher
315 bioavailability.

316 The association of Ag with insoluble compounds in the gills of *B. azoricus* is less important
317 than for *B. thermophilus* but similar to that observed in *M. edulis*. Mussels from LS are
318 presenting intermediate values (Table 2). Conversely, the storage of Cd under insoluble forms
319 is more efficient for *B. azoricus*. It is of interest to note that compared to *M. edulis* both
320 *Bathymodiolus* species present higher percentages of Cd associated with insoluble
321 compounds. The proportion of Cu in an insoluble form in *B. azoricus* is generally lower than

322 the very high value reported for *B. thermophilus* and higher than the intermediate value
323 reported for *M. edulis*. The percentage of insoluble Zn in the gills of mussels from LS is the
324 highest reported, but mussels from MG or Rb show percentages similar to that reported for *B.*
325 *thermophilus* and *M. edulis*.

326 In the mantle of mussels from ATJ, the proportion of Ag immobilized in an insoluble form is
327 low compared to the corresponding ratio observed for *B. thermophilus*. Mussels from Rb
328 present a percentage of Ag bound to insoluble compounds equivalent to that observed in *M.*
329 *edulis* mantle, while the values reported for MG and LS mussels are higher. Unlike *B.*
330 *thermophilus* Cd is essentially associated with insoluble compounds in *B. azoricus* mantle.
331 Owing to the very low and erratic levels of Cd in the mantle of *M. edulis* used as controls in
332 cited metal exposure experiments, no comparison can be established with *B. azoricus* Cd
333 partitioning. The proportion of Cu associated with insoluble compounds in *B. azoricus* mantle
334 is generally below that reported for *B. thermophilus*. ATJ mussels present percentages of Cu
335 associated with insoluble compounds in their mantle equivalent to those reported for *M.*
336 *edulis*. Generally, the association of Zn with insoluble compounds is more effective in *B.*
337 *azoricus* than in *B. thermophilus* or *M. edulis*.

338 The percentage of Ag associated with insoluble compounds in the digestive gland of Rb
339 mussels is equivalent to that recorded for *M. edulis*, while that observed at MG and LS are
340 higher and much higher, respectively. Mussels from ATJ present a high association of Cd, Cu
341 and Zn with insoluble compounds in the digestive gland compared to *M. edulis*.

342 In *B. azoricus* the partitioning of metals between soluble and insoluble fractions varies among
343 sites and tissues as whole metal levels do. But, as mentioned in earlier work, metals are
344 preferentially associated with insoluble compounds (Rousse et al., 1998; Geret et al., 1998).
345 However it is noticeable that the distribution of metals in *B. azoricus* tissues often does not
346 differ from that of corresponding tissues of non-exposed *M. edulis*, underlying that the
347 compartmentalization of metals in bivalves is a complex phenomenon not directly related to

348 their abundance in the surrounding seawater and/or the whole tissue. The comparison of the
349 proportion of metals stored as insoluble forms in the tissues of both *Bathymodiolus* species
350 (with higher percentages observed for Ag and Cu in *B. thermophilus*, while higher
351 percentages of Cd and Zn are observed in *B. azoricus*) supports also the former statement.
352 Nevertheless, the efficient storage of metals as chemical forms known to be less toxic is a
353 mechanism allowing *Bathymodiolus* to cope with a highly metal-loaded environment.
354 The PCA analysis (Fig. 2-4) allows discrimination of the bioaccumulation of metals by *B.*
355 *azoricus* in relation to the sampling fields (RB, LS and MG) and even to the sampling sites
356 (MG-A8 vs A10, LS-ET vs BA). This discrimination can be related to the large differences
357 observed in the chemical composition of the hydrothermal fluids emitted in the 3 fields (Table
358 3). The fluids venting in Rainbow are characterized by unique Fe and Mn composition,
359 largely higher than generally encountered in the MAR hydrothermal vents. This metallic
360 enrichment of the fluid is also observed for Ag, Cu, Zn and Pb (Douville et al., 2002). In the
361 same way, the discrimination observed at the site scale in Lucky Strike between Eiffel Tower
362 and Bairro Alto can be explained by the differences in end-member concentrations between
363 the vents located around the LS lava lake area (Charlou et al., 2000). Bairro Alto is situated in
364 the Northern part of the lake where the emitted fluids have a salinity similar to seawater.
365 Eiffel Tower is in the Southern part of the lake where the fluid exhibits a salinity lower than
366 seawater. The same conclusion could be applied to Menez Gwen: the two different sampling
367 sites (A8 and A10) are situated in the NW of the volcano and could be under the influence of
368 two chemically different fluids not studied by Charlou et al., 2000.

369 However, the chemical composition of the pure fluid is not sufficient to explain the
370 discrimination observed in the PCA. For example, concerning gills and mantle, the LS
371 mussels are separated from Rb mussels owing to their high levels of Cd and Zn that are not
372 consistent with data regarding the fluid concentration at LS. In the same way, the variables Fe
373 and Mn are often opposed to Zn and Cu, whereas the metal concentrations in the fluid follow

374 the same trend with higher concentrations at Rainbow and smaller ones in Menez Gwen.
375 Consequently, the metal burden of *B. azoricus* tissues is under the dependency of factors
376 belonging to different scales. At the site scale, the composition of the emitted fluid governs
377 the input of metals in the ecosystem and their potential bioaccumulation by mussels. At the
378 scale of mussel's immediate environment, uptake and storage of metals is more dependent of
379 their physico-chemical forms (dissolved vs particulate), the potential accumulation of
380 particulate matter, their association with inert or organic material, and the physiological needs
381 and behaviour of the mussels. However, even if not directly related to their concentration in
382 the pure fluid, the analysis of the metallic load of *B. azoricus* allows their separation among
383 the three studied ATJ fields.

384

385 Taking into account that the bioaccumulation of metals in bivalves results mainly from their
386 trophic uptake (e.g. Geret 2000, Wang 2002) the high amounts of metals observed in the
387 mixotrophic *B. azoricus* are surprising. Several explanations can be proposed: (i) *B. azoricus*
388 are exposed continuously to a metal enriched environment (Desbruyères et al., 2001); (ii)
389 metals in *B. azoricus* surrounding water are in extremely available forms; (iii) *B. azoricus*
390 uptake rates from the environment are extremely high in relation with the need of energy
391 sources; (iv) the trophic uptake of metal by *B. azoricus* is particularly important despite the
392 relevant role of the symbiosis in its nutrition. Data regarding the bioavailability of metals in
393 mussels proximate environment are needed. Further work has to be developed regarding
394 metal uptake pathways, direct or trophic, in relation with the feeding habits of *B. azoricus* and
395 the occurrence of metal containing particles in its digestive tract and the putative participation
396 of symbionts in trace element detoxication in the gills.

397

398 **5. Conclusions**

399

400 Our study confirms the existence of an equilibrium between the levels of metals in the end-
401 members, the proximate environment of *B. azoricus* and mussel tissues. The recorded
402 variations are due to local modification of metal bioavailability generated by abiotic or biotic
403 factors, and the physiological status of the organisms influenced by the physico-chemical
404 characteristics of the three studied fields.

405

406

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413

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415

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Table 1 : Mean levels and standard deviation of metals in tissues of the vent mussel *Bathymodiolus azoricus* collected at three fields (five sites) on the Mid Atlantic Ridge-Azores Triple Junction. For each line, similar letters indicate no statistical difference among tissues ($p > 0.05$). Levels are expressed as $\mu\text{g}\cdot\text{g}^{-1}$ dry weight of soft tissue. A8 and A10 (Menez Gwen field), BA and ET (Bairro Alto and Eiffel Tower - Lucky Strike field).

	MENEZ GWEN		LUCKY STRIKE		RAINBOW	
	A8	A10	BA	ET		
GILLS						
Ag	4.5 ± 1.2 a	1.9 ± 1.3 b	5.2 ± 1.3 a	2.1 ± 0.7 b	1.8 ± 1.1	b
Cd	7.4 ± 2.5 c	3.2 ± 1.0 d	47.2 ± 9.8 a	17.6 ± 2.2 b	1.8 ± 0.7	e
Cu	57 ± 12 b	130 ± 82 a	80 ± 21 ab	109 ± 69 a	52 ± 27	b
Fe	183 ± 46 c	206 ± 45 bc	361 ± 185 ab	488 ± 570 ab	2066 ± 956	a
Mn	6.3 ± 0.8 a	4.8 ± 0.7 b	9.5 ± 6.5 a	7.2 ± 1.7 a	9.1 ± 2.6	a
Zn	168 ± 33 c	197 ± 57 bc	1977 ± 605 a	768 ± 578 ab	106 ± 46	c
MANTLE						
Ag	0.7 ± 0.3 a	0.5 ± 0.3 ab	0.8 ± 0.3 a	0.4 ± 0.1 bc	0.1 ± 0.1	c
Cd	0.5 ± 0.3 bc	0.5 ± 0.4 bc	2.4 ± 1.2 a	1.2 ± 0.6 ab	0.04 ± 0.03	c
Cu	9 ± 4 bc	81 ± 76 a	15 ± 6 abc	28 ± 27 ab	1 ± 1	c
Fe	28 ± 35 c	40 ± 47 bc	229 ± 113 a	141 ± 74 ab	186 ± 94	ab
Mn	3.0 ± 0.7 bc	2.4 ± 1.8 c	6.4 ± 2.4 a	5.5 ± 1.1 ab	8.4 ± 2.5	a
Zn	46 ± 13 cd	100 ± 63 ab	145 ± 67 a	69 ± 27 bc	28 ± 8	d
DIGESTIVE GLAND						
Ag	0.9 ± 0.3 a	0.4 ± 0.1 b		0.5 ± 0.2 b	0.5 ± 0.2	b
Cd	4.0 ± 1.3 a	2.3 ± 0.5 b		4.5 ± 2.1 a	1.5 ± 1.0	b
Cu	30 ± 15 b	172 ± 81 a		48 ± 27 b	11 ± 10	c
Fe	198 ± 91 c	200 ± 60 c		919 ± 699 b	1854 ± 1048	a
Mn	3.8 ± 2.1 c	2.1 ± 0.6 d		5.4 ± 1.6 b	8.1 ± 1.9	a
Zn	82 ± 42 b	111 ± 49 b		276 ± 81 a	45 ± 12	c

Table 2 : Mean levels and standard deviation of amounts (expressed as percentages) of metals associated with insoluble compounds in the tissues of vent mussels collected at the studied Azores Triple Junction sites. A8 and A10 (Menez Gwen field), BA and ET (Bairro Alto and Eiffel Tower - Lucky Strike field). *B. t.*: *Bathymodiolus thermophilus* collected during the HOPE99 cruise at the East Pacific Rise (EPR) 9°N vent site (Cosson, unpublished data), *M. edulis*: *Mytilus edulis*, from Bay of Bourgneuf and Normandy shellfish farms used as control in laboratory experiments, except for Ag data obtained from Ag-exposed mussels (Geret, 2000). **: levels were too low to allow the determination of Ag levels in soluble and/or insoluble fractions.

	<i>Bathymodiolus azoricus</i>					<i>B. t.</i>	<i>M. edulis</i>
	MENEZ GWEN		LUCKY STRIKE		RAINBOW	EPR	Geret
	A8	A10	BA	ET		9°N	2000
GILLS							
Ag	27 ± 6	24 ± 14	43 ± 10	43 ± 15	31 ± 25	89	33 / 13 / **
Cd	57 ± 5	47 ± 7	80 ± 7	73 ± 7	47 ± 18	43	17 / ** / 24
Cu	64 ± 5	76 ± 9	80 ± 5	86 ± 4	65 ± 10	91	54 / 38 / 44
Fe	82 ± 4	82 ± 4	74 ± 8	79 ± 8	75 ± 5		
Mn	61 ± 3	68 ± 5	86 ± 5	71 ± 5	68 ± 7		
Zn	45 ± 8	45 ± 13	94 ± 2	84 ± 7	37 ± 15	38	48 / 55 / 47
MANTLE							
Ag	31 ± 16	38 ± 26	36 ± 7	50 ± 14	22 ± 15	91	24 / 26 / **
Cd	61 ± 18	85 ± 14	57 ± 11	42 ± 22	67 ± 25	45	** / ** / **
Cu	52 ± 17	89 ± 6	66 ± 12	69 ± 14	34 ± 24	89	60 / 55 / 29
Fe	58 ± 24	79 ± 17	68 ± 17	68 ± 14	80 ± 7		
Mn	49 ± 7	45 ± 17	62 ± 7	50 ± 3	57 ± 9		
Zn	66 ± 9	82 ± 7	81 ± 8	68 ± 11	48 ± 10	59	42 / 35 / 51
DIGESTIVE GLAND							
Ag	54 ± 19	41 ± 19		80 ± 14	24 ± 22		18 / 23 / 24
Cd	79 ± 8	80 ± 11		94 ± 5	48 ± 23		22 / 18 / 15
Cu	88 ± 7	98 ± 1		95 ± 5	68 ± 18		32 / 29 / 09
Fe	96 ± 3	98 ± 1		95 ± 6	77 ± 11		
Mn	87 ± 8	89 ± 5		92 ± 5	61 ± 18		
Zn	92 ± 6	96 ± 2		97 ± 2	61 ± 20		32 / 33 / 44

Table 3 : End-member concentrations in the Mid Atlantic Ridge-Azores Triple Junction (Menez Gwen, Lucky Strike and Rainbow), East Pacific Rise (EPR) and Guaymas Basin vent fluids. Adapted from Charlou et al., 2002; Cosson, 1996; Douville et al., 2002; LeBris et al., 2003; VonDamm, 1995.

Site	Menez Gwen	Lucky Strike	Rainbow	EPR 13°N	Guaymas
T (°C)	265 - 284	152 - 333	360 - 365	230-359	100-315
Fe (mM)	0.024 - 0.028	0.03 - 0.86	24	0.6- 10.8	0.017 - 0.18
Cu (μ M)	0.6 - 2.9	1 - 26	140		
Zn (μ M)	2.4-5.1	5-58	160	2-102	
Cd (nM)	9 - 12	18 - 79	130	55-70	
Pb (nM)	21 - 56	35 - 130	148	14-135	

Figure 1: Location of the vent fields on the Mid-Atlantic Ridge (adapted from Desbruyères et al., 2006).

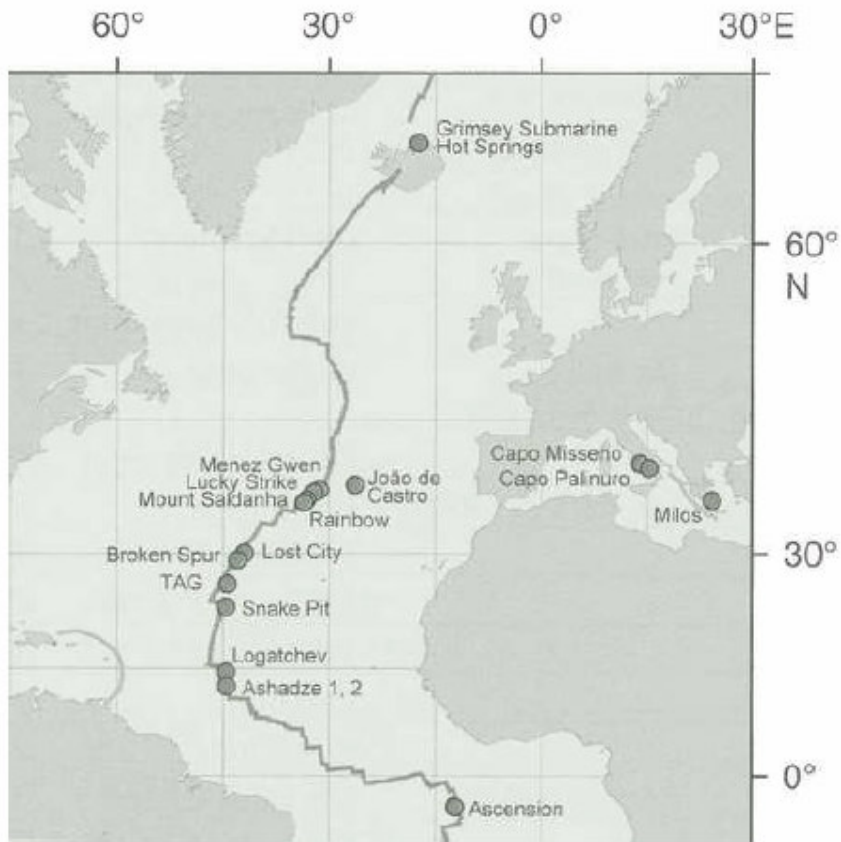
Figure 2 : First plane principal component analysis (PCA) of mussels based on metal levels in soluble and insoluble fractions of the gills. (A) individual scores. (B) descriptor scores. MG-A8 and MG-A10 (Menez Gwen field), BA and ET (Bairro Alto and Eiffel Tower - Lucky Strike field), Rb (Rainbow field).

Figure 3 : First plane principal component analysis (PCA) of mussels based on metal levels in soluble and insoluble fractions of the mantle. (A) individual scores. (B) descriptor scores. MG-A8 and MG-A10 (Menez Gwen field), BA and ET (Bairro Alto and Eiffel Tower - Lucky Strike field), Rb (Rainbow field).

Figure 4 : First plane principal component analysis (PCA) of mussels based on metal levels in soluble and insoluble fractions of the digestive gland. (A) individual scores. (B) descriptor scores. MG-A8 and MG-A10 (Menez Gwen field), BA and ET (Bairro Alto and Eiffel Tower - Lucky Strike field), Rb (Rainbow field).

Figure 5 : Comparison of *Bathymodiolus azoricus* gill and mantle metal level range with the average levels of metals observed in corresponding organs within bivalves from the East Pacific Rise hydrothermal vents. **A:** Ag, Cd, Cu, Mn levels, scale 0-220 $\mu\text{g}\cdot\text{g}^{-1}$ dw. **B:** Fe, Zn levels, scale 0-2200 $\mu\text{g}\cdot\text{g}^{-1}$ dw. *Calyptogena magnifica* (Roesijadi and Crecelius, 1984; Roesijadi et al., 1985; Cosson-Mannevy et al., 1988); *Vesicomya gigas* (Ruelas-Inzunza et al., 2003); *Bathymodiolus thermophilus* (Rousse, 1999).

Figure 1



ACCEPTED

SCRIPT

Figure 2

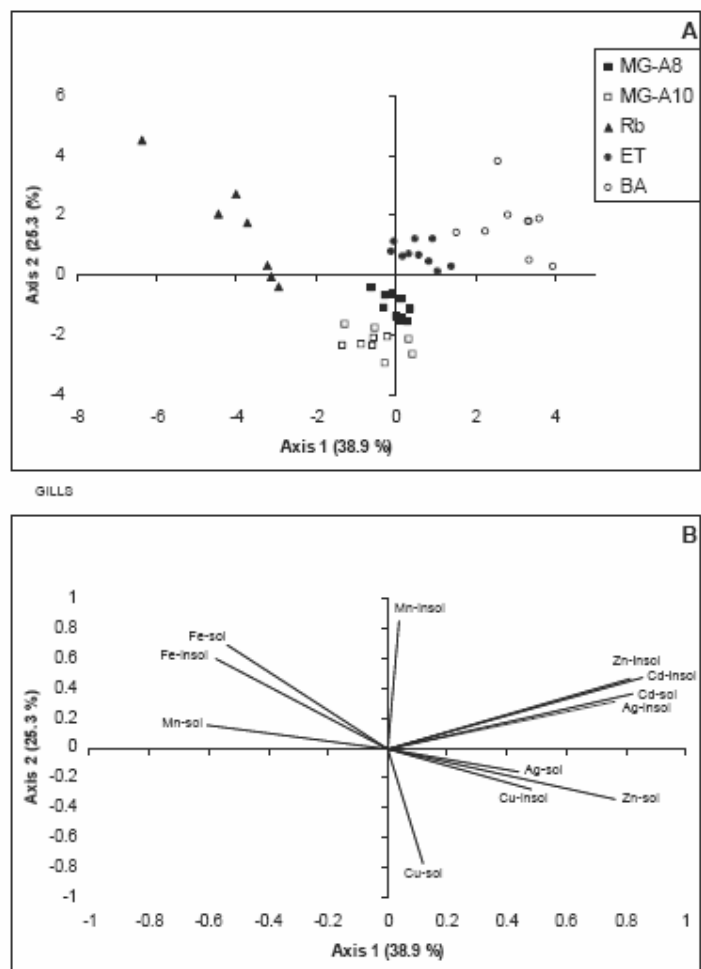


Figure 3

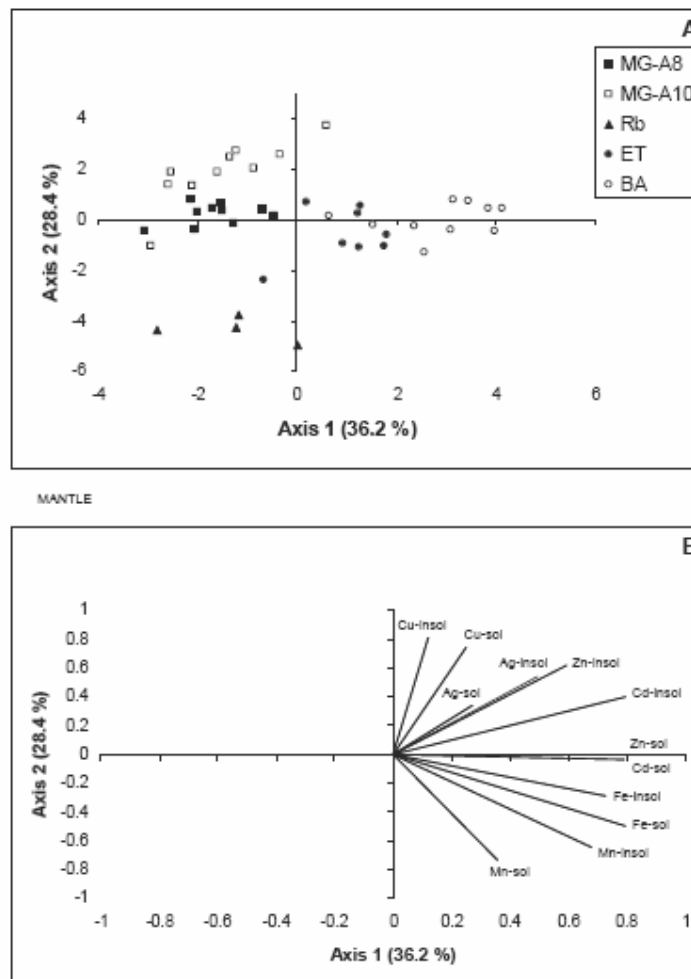
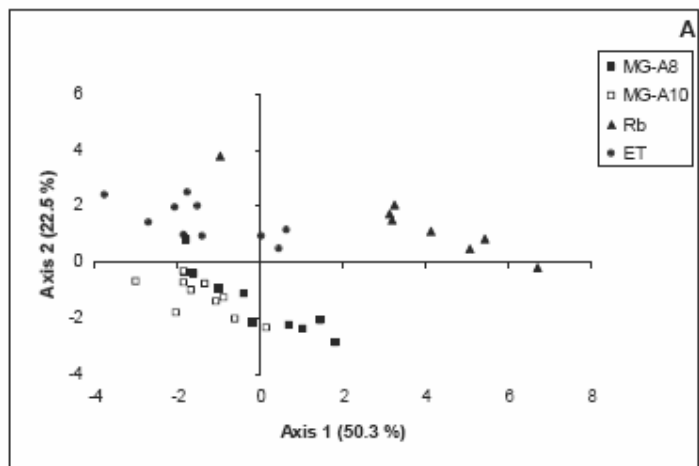


Figure 4



DIGESTIVE GLAND

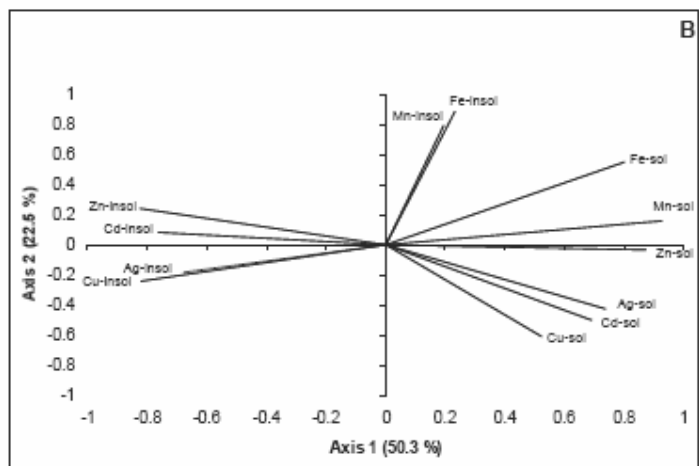
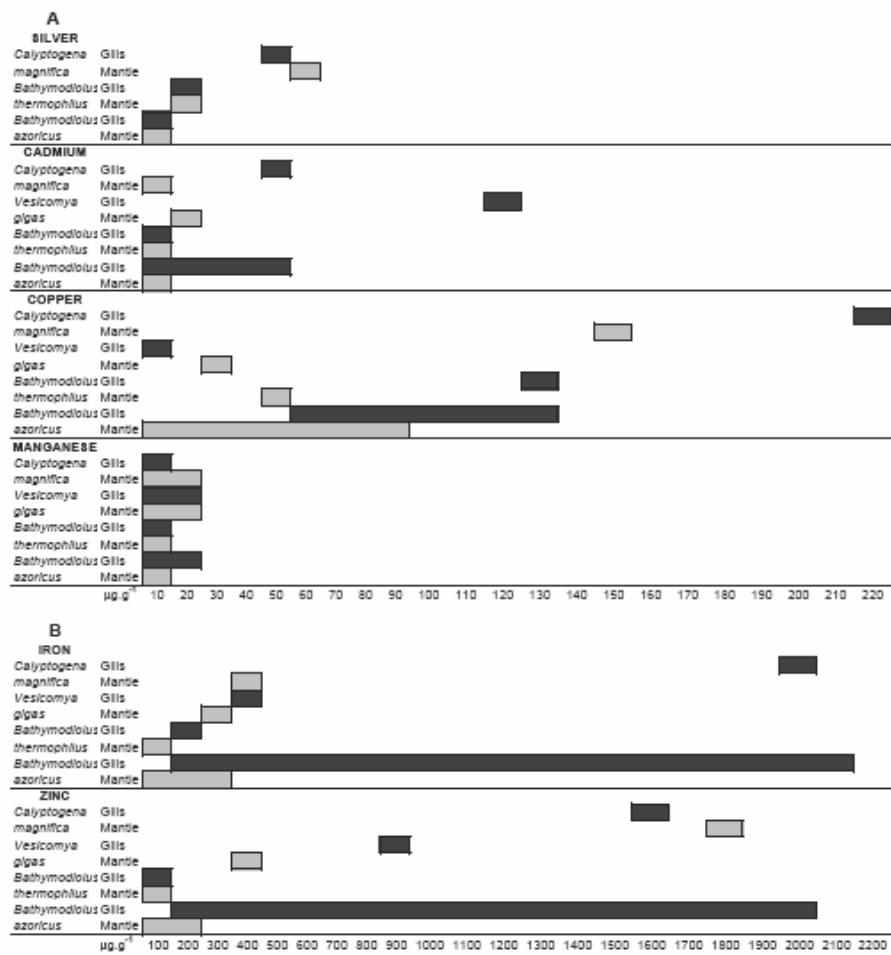


Figure 5



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