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1 **The tropical brown alga *Lobophora variegata* as a bioindicator of mining**
2 **contamination in the New Caledonia lagoon: a field transplantation study**

3

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17

18

19 **ABSTRACT.**

20

21 Previous field and laboratory studies have identified the alga *Lobophora variegata* as a good
22 candidate for biomonitoring metal contamination in the New Caledonia lagoon which is
23 subjected to intensive and extensive metal inputs from land-based mining activities. The aim
24 of this work was to further assess the bioindicative potential of this species by investigating,
25 in the field, its bioaccumulation capacity for local key contaminants, i.e. Ag, As, Cd, Co, Cr,
26 Cu, Mn, Ni and Zn. Algae from clean and contaminated sites were cross-transplanted for a
27 period of three months in order to determine the *in situ* uptake and depuration kinetics of the
28 nine elements. Results indicate that algae transplanted to the contaminated site displayed a
29 significant linear increase in concentration with time for Ag, As, Cd, Co, Cr, Cu, Mn and Ni.
30 In contrast, algae transplanted to the clean site did not show major depuration of these
31 elements, except for Co. Overall, *L. variegata* showed a rapid temporal response in metal
32 uptake, especially for the elements intensively released into the coastal environment of New
33 Caledonia (viz., Co, Cr, Mn and Ni). This species appears therefore as an excellent
34 bioindicator species of metal contamination in this area. Our results also provide background
35 information necessary for using *L. variegata* under *in situ* experimental conditions so as to
36 provide better quantitative information on ambient metal contamination levels. The wide
37 distribution of *L. variegata* in tropical areas further enhances its potential as a bioindicator
38 species of metal contamination in other tropical coastal environments.

39

40

41 **Keywords:** Metals; Bioaccumulation; Depuration; Biomonitoring; Sentinel organism;

42 Tropical environment

43 **1. Introduction**

44

45 Most studies dealing with bioindicators in aquatic systems focus on contaminant
46 concentrations in various species, whereas very few have been conducted to actually test the
47 validity of the alleged bioindicator species (see e.g., Warnau et al., 1996; Rainbow, 2002;
48 Coteur et al., 2003). The lack of background scientific information necessary to derive proper
49 biological tools to be used in environmental monitoring programmes is even more
50 pronounced in coral reef ecosystems, despite their increasingly acknowledged sensitivity to
51 environmental stresses (e.g., Hoegh-Guldberg, 1999). In this respect, New Caledonia is a very
52 good example. This small SE Pacific tropical Island contains one of the major Ni ore deposits
53 worldwide and, for about a century, its economic development has been essentially based on
54 Ni mining activities. Its huge coral reef lagoon is naturally influenced by natural erosion of
55 the metal-rich soils and associated metal inputs (Labrosse et al., 2000). In addition, intense
56 local mining activities result in substantial anthropogenic inputs of metals into the lagoon and
57 thereby constitute a threat to the local coastal ecosystems (e.g., Bird et al., 1984; Laganier,
58 1991; Ambatsian et al., 1997). Despite these important metal contamination sources,
59 ecotoxicological information regarding the lagoon is very scarce (Labrosse et al., 2000;
60 Metian et al., 2005, 2008a,b; Fichez et al., 2005; Hédouin et al., 2006, 2007, 2008).

61 The usefulness of bioindicator species to monitor the extent of the contamination in the
62 marine environment is now well established (e.g., O'Connor, 1998; Warnau and Bustamante,
63 2007). Among marine organisms, brown macroalgae are known to efficiently accumulate
64 metals from their environment (e.g. Försberg et al., 1988; Phillips, 1990). Therefore, the
65 Phaeophyceae family has been used as indicators of metal contamination since the early
66 seventies (Burrows, 1971; Bryan, 1983; Söderlund et al., 1988). However, as the general rule
67 mentioned earlier, these studies were mainly conducted in temperate zones and far less

68 attention has been paid to the tropical and sub-tropical areas (e.g., Karez et al., 1994; Amado
69 Filho et al., 1999).

70 In New Caledonia, the brown alga *Lobophora variegata* has recently been suggested as a
71 possible bioindicator organism. Indeed, this species is of reasonable size, sessile, easily
72 collectable and displays high metal bioaccumulation capacity (Metian et al., 2006, 2008b). In
73 particular, Metian et al. (2006, 2008b) demonstrated that the alga concentrates Cd, Co, Cr,
74 Mn, Ni and Zn in direct proportion to the dissolved element concentrations in ambient
75 seawater, which is one of the most important pre-requisites for selecting a bioindicator species
76 (Phillips, 1990; Warnau et al., 1997). In this respect, *L. variegata* tissue concentrations were
77 thousands of times higher relative to those in seawater and metal retention efficiency was
78 shown to be independent of the exposure concentration (Metian et al., 2006, 2008b). The
79 latter laboratory study provided essential information regarding the excellent bioindicative
80 potential of *L. variegata*; however, information on the metal bioaccumulation behaviour of
81 the alga is still needed in the field.

82 The aim of the present work was therefore to determine the bioconcentration and
83 retention capacities of *L. variegata* for nine selected elements (Ag, As, Cd, Co, Cr Cu, Mn, Ni
84 and Zn) under field conditions, using cross-transplantation experiments in the New Caledonia
85 lagoon. These experiments were carried out in order to determine whether transplanted algae
86 could concentrate and eliminate the selected elements up to similar levels and at similar rates
87 as resident populations, as well as to assess the period of time necessary for them to reach
88 concentrations representative of the resident populations.

89

90 **2. Materials and methods**

91

92 2.1. Selection of the transplantation locations

93

94 Recent studies have provided data on element concentrations in different areas of the
95 lagoon of New Caledonia (Hédouin 2006; Hédouin et al. 2008; Metian et al. 2008a) and
96 allowed the selection of a “reference” site and a “contaminated” site where the cross-
97 transplantation experiments were carried out. Maa Bay was identified as a suitable
98 “reference” site because of the low element concentrations measured in algae as well as in
99 sediments, and Boulari Bay was identified as the “contaminated” site as it displays very high
100 concentrations in both the resident algae and sediments, due to terrigenous inputs from both
101 natural and mining-induced soil erosion (Fig. 1).

102

103 2.2. Experimental design

104

105 Fifty specimens of *L. variegata* were collected in February 2005 in Maa Bay. Ten
106 individuals were analysed for their content of Ag, As, Cd, Co, Cr Cu, Mn, Ni and Zn (see
107 below) in order to establish baseline concentrations at the beginning of the experiment (t_0).
108 The 40 remaining algae were transplanted for 103 d in Boulari Bay (contaminated site). The
109 same number of algae from Boulari Bay was transferred to Maa Bay at the same time in order
110 to follow the depuration of the contaminants from these algae.

111 From this time onwards, 5 individuals of the resident population and 5 transplanted
112 organisms (in both reference and contaminated sites) were collected at different time intervals

113 over a 103-d period in order to compare the change and variability of element concentrations
114 in transplanted and resident algae.

115 At t_0 , all organisms, including control resident specimens, were placed in plastic cages
116 ($100 \times 100 \times 50$ cm) anchored between 4 and 5 m depth. Cages were made of 1-cm mesh
117 plastic net to ensure free seawater circulation within the cage. Transplanted and control algae
118 were collected by SCUBA diving, transported to the laboratory in clean, acid-washed PET
119 bags, and processed for element analyses the same day (typically within 4 to 5 hrs).

120

121 2.3. Sample preparation and analyses

122

123 In the laboratory, the algae were cleansed of their epiphytes and any attached sediment
124 grains by gentle scrubbing and rinsing several times in seawater from their respective
125 sampling sites. The algae were then weighed (wet wt), dried at 60°C until constant weight,
126 and weighed again (dry wt) before being stored in acid-washed, hermetically sealed PET
127 containers until further analysis.

128 Algal samples (200-300 mg dry wt) were digested using 6 ml of 65 % HNO_3 , 2 ml of 30
129 % HCl and 0.5 ml of 40 % HF (Merck, suprapur quality). Acidic digestions were first carried
130 out overnight at room temperature, then using a MARS V microwave (30-min long linear
131 increase up to 115°C followed by 15 min at 115°C) to complete the mineralization. Each
132 sample volume was then adjusted to 50 ml with milli-Q quality water.

133 Elements were analysed using a Varian Vista-Pro ICP-OES (As, Cr, Cu, Mn, Ni, and Zn)
134 or a Varian ICP-MS Ultra Mass 700 (Ag, Cd and Co). Three control samples (two certified
135 reference materials –CRM– and one blank), treated and analysed in the same way as the
136 samples, were included in each analytical batch. The CRM were dogfish liver DOLT-3
137 (NRCC) and lobster hepatopancreas TORT-2 (NRCC). The results from CRM analysis

138 indicated a recovery ranging from 81 % (Ni) to 113 % (Zn) (Table 1). The detection limits
139 were 31 (As), 1.3 (Cr), 3.8 (Cu), 0.15 (Mn), 1.1 (Ni) and 2.4 (Zn) $\mu\text{g g}^{-1}$ dry wt for ICP-OES
140 and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) $\mu\text{g g}^{-1}$ dry wt for ICP-MS.

141

142 2.4. Statistical analyses

143

144 Uptake (eq. 1) and depuration (eq. 2) kinetics of the elements were determined using
145 simple linear regression equations:

146

$$147 \quad C_t = C_0 + k_u t \quad (\text{eq. 1})$$

148

$$149 \quad C_t = C_0 - k_e t \quad (\text{eq. 2})$$

150

151 where C_t and C_0 are the element concentration ($\mu\text{g g}^{-1}$ dry wt) in algae at time t (d) and 0,
152 respectively, and k is the uptake (k_u) or depuration (k_e) rate constant ($\mu\text{g g}^{-1}$ dry wt d^{-1})
153 (Temara et al. 1998). Constants of the equation and their statistics were estimated by iterative
154 adjustment of the model and Hessian matrix computation using the nonlinear curve-fitting
155 routines in the Statistica® 5.2.1 software.

156 On the first day (t_0) of the transplantations, element concentrations in algae from the
157 reference site were compared to those from contaminated stations using one-way analysis of
158 variance (ANOVA) followed by the multiple comparison test of Tukey (Zar 1996). Element
159 concentrations at the end of transplantation period were also compared to those of resident
160 algae from the transplanted stations (1-way ANOVA). In addition, when element
161 concentrations in resident algae showed a significant increase/decrease with time, the slope of

162 the regression (k_u or k_e) was compared with the slope of the regression for transplanted algae
163 (Zar 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

164

165 **3. Results**

166

167 3.1. Starting day of transplantation

168

169 At t_0 , the concentrations of Ag, As, Co, Cr, Mn and Ni were significantly higher in algae
170 from Boulari Bay than in those from Maa Bay ($p_{\text{Tukey}} < 0.0006$ for Ag, As, Co, < 0.008 for
171 Mn and Ni, < 0.02 for Cr), whereas no significant difference was found for Cd, Cu and Zn
172 concentrations between the two sites (see Figs 2 and 3).

173

174 3.2. Transplantation from Maa Bay (reference) to Boulari Bay (contaminated site) (Fig. 2)

175

176 Element concentrations in the resident *L. variegata* population from Maa Bay did not
177 vary significantly during the experiment for all elements, indicating that any variation in
178 concentrations in algae transplanted to Boulari Bay were actually related to changes in
179 environmental conditions. Since the cages in Boulari Bay were damaged after 71d, the
180 transplantation experiment had to stop at that time and could not last for the expected 103-d
181 period.

182 In algae transplanted to Boulari Bay, Zn was the only element which did not show a
183 statistically significant increase in concentrations during the transplantation period ($p_{\text{regression}}$
184 $\text{slope} = 0.5$) (Fig. 2). In contrast, Ag, As, Cd, Co, Cr, Cu, Mn and Ni increased linearly over the
185 observation period of 71 d ($R^2 = 0.71 - 0.90$ for Ag, Co, Cr, Mn and Ni, and $R^2 = 0.22 - 0.41$
186 for As, Cd and Cu). The uptake rate of Cr, Mn and Ni was higher by one to three orders of

187 magnitude than that for the other elements. The concentrations of Cr, Mn and Ni increased
188 respectively from 6.5, 63 and 9.0 $\mu\text{g g}^{-1}$ dry wt at the beginning of the experiment up to 192,
189 516 and 280 $\mu\text{g g}^{-1}$ dry wt after 71 d of transplantation.

190 At the end of the transplantation period, the concentrations of Ag, Cd, Co, Cr, Cu, Mn
191 and Ni in algae in Boulari Bay were significantly higher in transplanted algae than in the
192 resident population (from 1.6 to 2.9 fold; p_{Tukey} always < 0.003). No significant difference
193 was observed for As.

194

195 3.3. Transplantation from Boulari Bay (contaminated) to Maa Bay (reference site) (Fig. 3)

196

197 In the course of the transplantation from the contaminated site to the reference site, Co
198 concentrations decreased linearly (k_e : 0.036 $\mu\text{g g}^{-1}$ dry wt d^{-1} ; $R^2 = 0.1$) whereas Mn and Zn
199 concentrations increased linearly ($k_u = 1.87$ and 0.30 $\mu\text{g g}^{-1}$ dry wt d^{-1} , $R^2 = 0.53$ and 0.15,
200 respectively) (Fig. 3). With the exception of the measurements done at day 16, Ag
201 concentrations in algae were below the detection limit ($< 0.1 \mu\text{g g}^{-1}$ dry wt), precluding any
202 regression fit calculation. No significant linear regression could be calculated for all the other
203 elements.

204 No significant variation in element concentrations was found in the *L. variegata* resident
205 population in Boulari Bay during the observation period, except for Mn and Zn. For these two
206 latter metals, a significant increase in concentration was observed. The corresponding
207 estimated uptake rate constants ($k_u = 3.18$ and 0.39 $\mu\text{g g}^{-1}$ dry wt d^{-1} for Mn and Zn
208 respectively, $p < 0.04$) were not significantly different from those calculated for the Boulari
209 Bay algae that were transplanted in Maa Bay.

210 At the end of the experiment, Co and Mn concentrations were significantly higher (p_{Tukey}
211 = 0.03 and 0.0002, respectively) in transplanted algae than in the resident population (1.9 fold
212 higher for Co and 5.2 for Mn), whereas no significant difference was found for Zn.

213

214 **4. Discussion**

215

216 When transplanted from the reference (Maa Bay) to the contaminated site (Boulari Bay),
217 the tropical brown alga *L. variegata* readily took up Ag, As, Cd, Co, Cr, Cu, Mn and Ni,
218 which demonstrated that a significant proportion of the ambient trace metal contamination
219 was occurring in bioavailable chemical forms and confirmed that the target algal species has a
220 strong potential to accumulate these contaminants.

221 In a comparable one-month duration transplantation experiment in Sepatiba Bay, Brazil,
222 Amado Filho et al. (1999) observed that the brown alga *Padina gymnospora* was able to
223 rapidly accumulate Cd and Zn, which suggests that a strong bioaccumulation capacity is a
224 general feature among brown algae. This further supports the suggestion to consider this
225 taxonomic group as metal bioindicators in tropical areas.

226 After approximately one month *L. variegata* individuals transplanted to the contaminated
227 site reached Ag, As, Cd, Co, Cr, Cu, Mn and Ni concentrations similar to those measured in
228 resident algae from Boulari Bay (see Fig. 2). Surprisingly, beyond that period, concentrations
229 of Ag, Cd, Co, Cr, Cu, Mn and Ni continued to increase, reaching values significantly higher
230 than those measured in the resident population. These observations suggest that the resident
231 algae, subjected to chronic elevated metal exposure, could have developed adaptive
232 response(s) to handle high levels of contamination by, e.g., regulating the intake and/or
233 depuration rate of the contaminants, through either physiological or genetic adaptation
234 (Klerks and Weis 1987; Warnau et al. 1995; Ma et al. 2000). This is further supported by

235 laboratory experiments which demonstrated that during short-term exposures (14-d), viz. a
236 period of time insufficient to allow for any possible adaptation mechanisms to occur, uptake
237 of Ag, Cd, Co, Cr, Mn, Ni and Zn in *L. variegata* from a single population was linear over the
238 duration of the experiments and reached tissue concentrations that were directly proportional
239 to the metal concentrations in seawater (over 2 to 3 orders of magnitude) (Metian et al. 2006,
240 2008b).

241 The strong bioaccumulation capacity for Ag, As, Cd, Co, Cr, Cu, Mn and Ni and the
242 ability of the alga to provide quantitative information on contaminant levels in its
243 environment, as indicated by both previous laboratory studies and the present field work,
244 converge in demonstrating the usefulness of *L. variegata* as a bioindicator species in the New
245 Caledonia lagoon.

246 For biomonitoring purposes, contaminant levels can be monitored in the marine
247 environment through the use of resident algae, since they were shown to be able to
248 discriminate different locations according to their level of contamination (Hédouin 2006,
249 present study). However, our study also indicates that the use of transplanted algae would
250 allow obtaining information that is more discriminating (since concentrations of several
251 contaminants are higher in transplanted algae than in the resident ones) and that reflects more
252 quantitatively the contaminant concentrations in the ambient seawater. Indeed a proportional
253 relationship between contaminant concentrations in transplanted algae and that in the
254 environment exists (as has been shown experimentally, Metian et al. 2006, 2008b) but a
255 breakdown in this relationship may occur for resident algae, probably due to certain
256 adaptation mechanisms as discussed above. Hence, the use of transplanted algae could be a
257 more sensitive and discriminating tool than resident algae to assess the level of metal
258 contamination in the New Caledonia coastal zone, as it would avoid interference by such
259 possible adaptation mechanisms.

260 Whereas *L. variegata* showed a rapid and efficient response time when transplanted to a
261 contaminated environment, the case was very different when algae were transplanted from the
262 contaminated bay to the reference site. Indeed, except for Co where some significant
263 depuration occurred, our study showed that the concentrations of the other elements were
264 basically unchanged after 3 months of transplantation. These observations contrast somewhat
265 with the results from previous laboratory experiments on *L. variegata* which suggested that
266 some metals were characterized by relatively fast turnover rates in the alga, with short
267 biological half-lives ($T_{b/2}$) of about 1 month for Cr, Mn, and Zn and 1 week for Ni (Metian et
268 al. 2006, 2008b).

269 The Phaeophyceae, to which *L. variegata* belongs, are well known to strongly bind metal
270 ions (see e.g., Bryan 1984), both via cell wall adsorption (biosorption) and cell absorption
271 with subsequent strong binding to intracellular macromolecules such as polyphenols,
272 phytochelatins and metallothioneins (e.g. Ragan et al. 1979; Morris et al. 1999; Cobbett and
273 Goldsbrough 2002). Polyphenols are present in very large proportions in *L. variegata* (viz.,
274 typically from 8 to 13 % of the total algal dry wt; Targett et al. 1992). Therefore it is quite
275 likely that cell absorption would be the predominant accumulation process in this species.

276 Besides being responsible for the high bioconcentration efficiency of *L. variegata*, the
277 elevated content in these metal-binding macromolecules could also explain the virtual lack of
278 depuration of certain elements in the algae transplanted from the contaminated site to the
279 reference station during our experiment. Indeed, past exposure history may influence further
280 contaminant elimination, as has been reported for example in oysters (e.g., Wallner-
281 Kersanach et al. 2000). Therefore, if algae from the contaminated area have developed
282 efficient detoxification strategies based on metal sequestration (e.g. via their elevated content
283 in polyphenols), most of the tissue-associated metal pool would be strongly bound to
284 intracellular components, which would logically result in high initial metal retention when

285 transplanted into a less contaminated area. Nevertheless, in order to better understand
286 depuration and detoxification processes of metals in *L. variegata*, further experimental studies
287 should be conducted using long-term depuration experimental designs and with algae having
288 different metal exposure histories. This could be done by carrying out field depuration
289 experiments with (1) algae coming from a contaminated sites and (2) algae collected from a
290 reference site, then transplanted for a few months into a contaminated site prior to being
291 replaced in the reference site to follow metal depuration, in parallel with regular
292 measurements of the intracellular content of metal-binding macromolecules.

293 Regarding the particular case of Mn and Zn, an increase in concentration was observed in
294 the algae collected from the contaminated site (Boulari Bay) and transplanted in the reference
295 site (Maa Bay). However, a similar and concomitant increase in Mn and Zn concentrations
296 was measured in the resident algae from Boulari Bay. This observation strongly suggests that
297 this change in metal concentrations was due to some specific physiological parameters in the
298 algae rather than to an uptake of Mn and Zn in relation to the level of contamination in the
299 site of transplantation. This assumption is further supported by the fact that the Maa Bay
300 algae that were transplanted to Boulari Bay actually took up Mn very efficiently (tissue
301 concentrations increased by two orders of magnitude), thus indicating that, in terms of
302 bioavailable metal levels, Maa Bay is actually similarly (Zn) or much less (Mn) contaminated
303 than Boulari Bay.

304

305 **5. Conclusion**

306

307 The cross-transplantation experiments clearly demonstrated that the alga *L. variegata* is a
308 powerful and informative bioindicator of metal contamination in the New Caledonia lagoon.
309 It displays high bioconcentration capacities, especially for Co, Cr, Mn and Ni, which are the
310 main elements of concern in this region due to their worldwide importance in the context of
311 Ni-ore exploitation. The present *in situ* transplantation study has also provided essential data
312 regarding the relevance and usefulness of using this species for active biomonitoring, and
313 complements a former study on the use of resident *L. variegata* populations for surveying
314 metal contamination (passive biomonitoring) (Hédouin 2006). Hence this new information
315 allows extending monitoring studies to areas of the New Caledonia lagoon where *L. variegata*
316 does not occur naturally.

317 Finally, due to the wide distribution of the brown alga *L. variegata* in tropical areas
318 (Targett et al. 1992), our study further underscores the usefulness of this algal species as a
319 tool for biomonitoring metal contamination levels in other tropical environments where
320 adequate bioindicators may be lacking.

321

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323

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333

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445

446 **Captions to Figures**

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448

449 **Figure 1.** Map indicating the location of the transplantation sites: Maa Bay and Boulari Bay,
450 New Caledonia.

451

452

453 **Figure 2.** Element concentrations ($\mu\text{g g}^{-1}$ dry wt; mean \pm SD; n = 5) in *Lobophora variegata*
454 transplanted from Maa Bay (reference site) to Boulari Bay (contaminated site).

455 Solid lines indicate significant variation in element concentrations in transplanted algae (T-
456 BOU); dash lines indicate element concentrations in the resident algal population of Maa Bay
457 (Control MAA; n = 30).

458

459

460 **Figure 3.** Element concentrations ($\mu\text{g g}^{-1}$ dry wt; mean \pm SD; n = 5) in *Lobophora variegata*
461 transplanted from Boulari Bay (contaminated site) to Maa Bay (reference site).

462 Solid lines indicate significant variation in element concentrations in transplanted algae (T-
463 MAA); dash lines indicate element concentrations in the resident algal population of Boulari
464 Bay (Control BOU; n = 30).

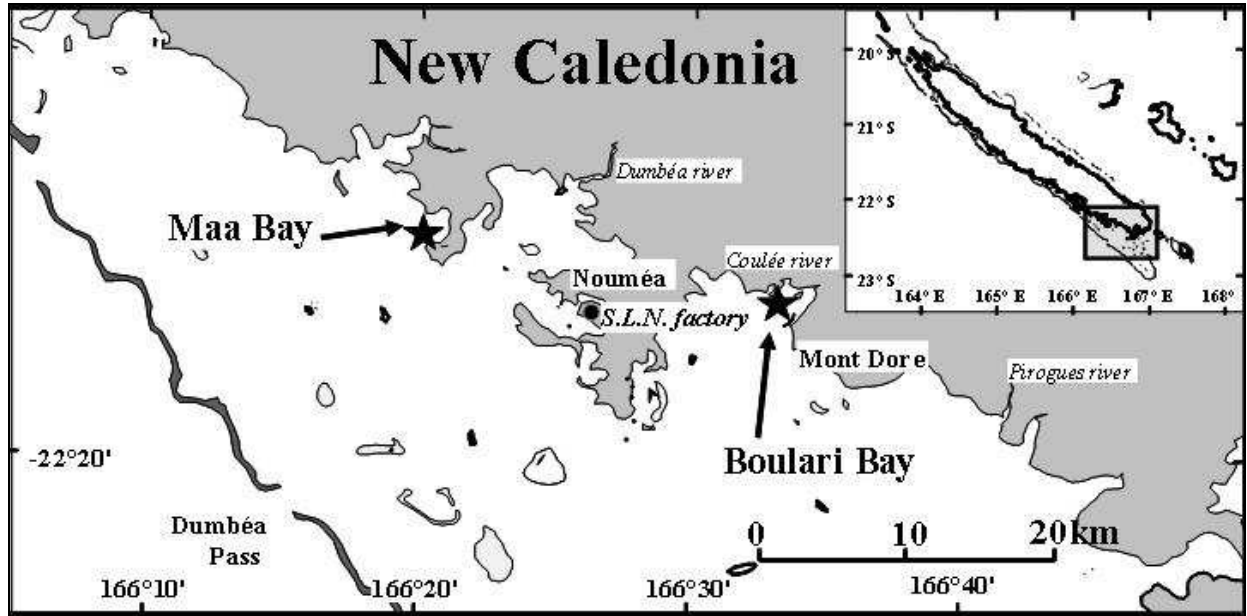
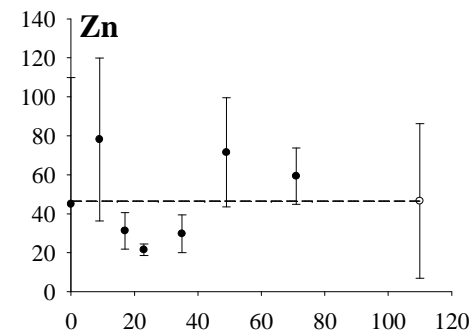
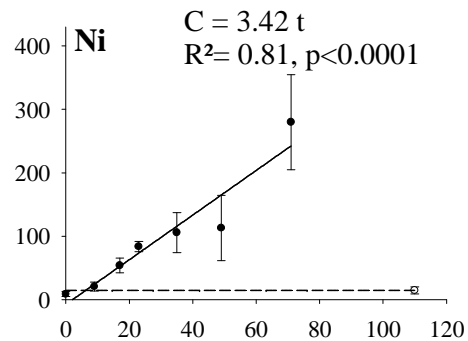
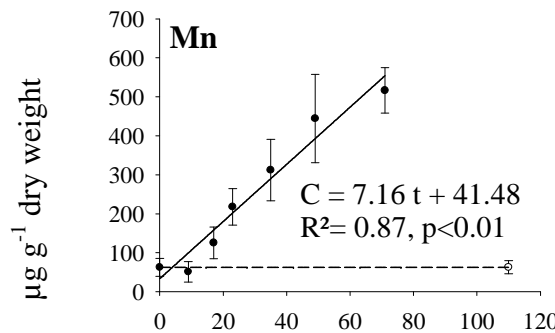
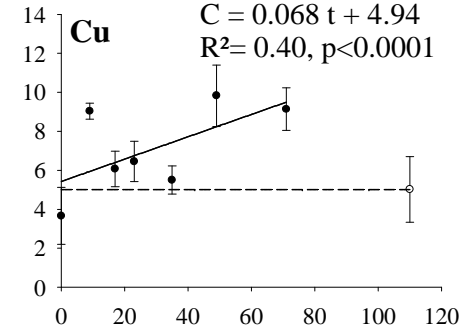
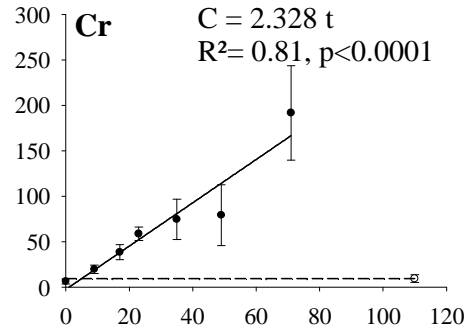
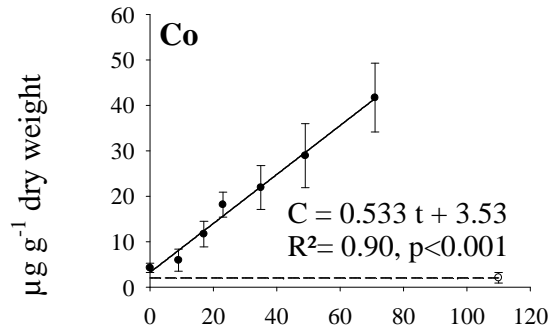
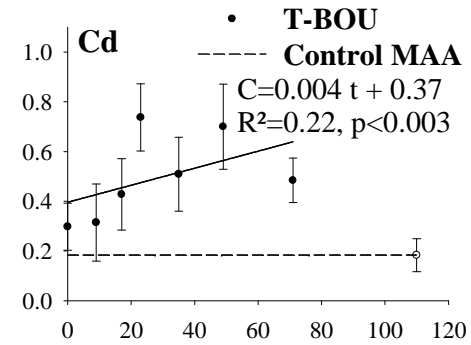
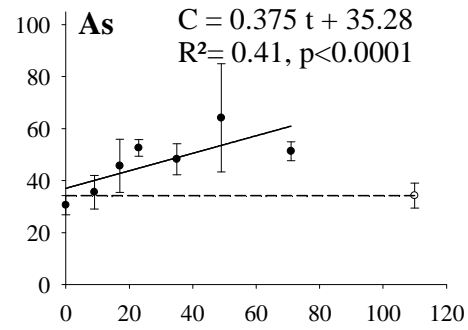
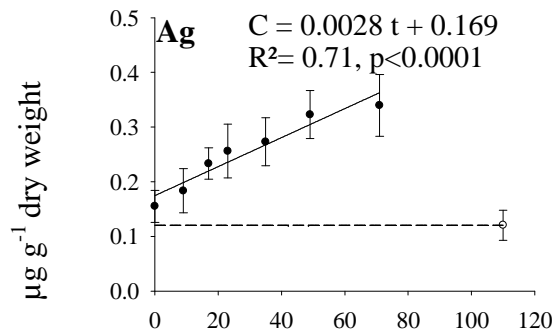


Figure 1

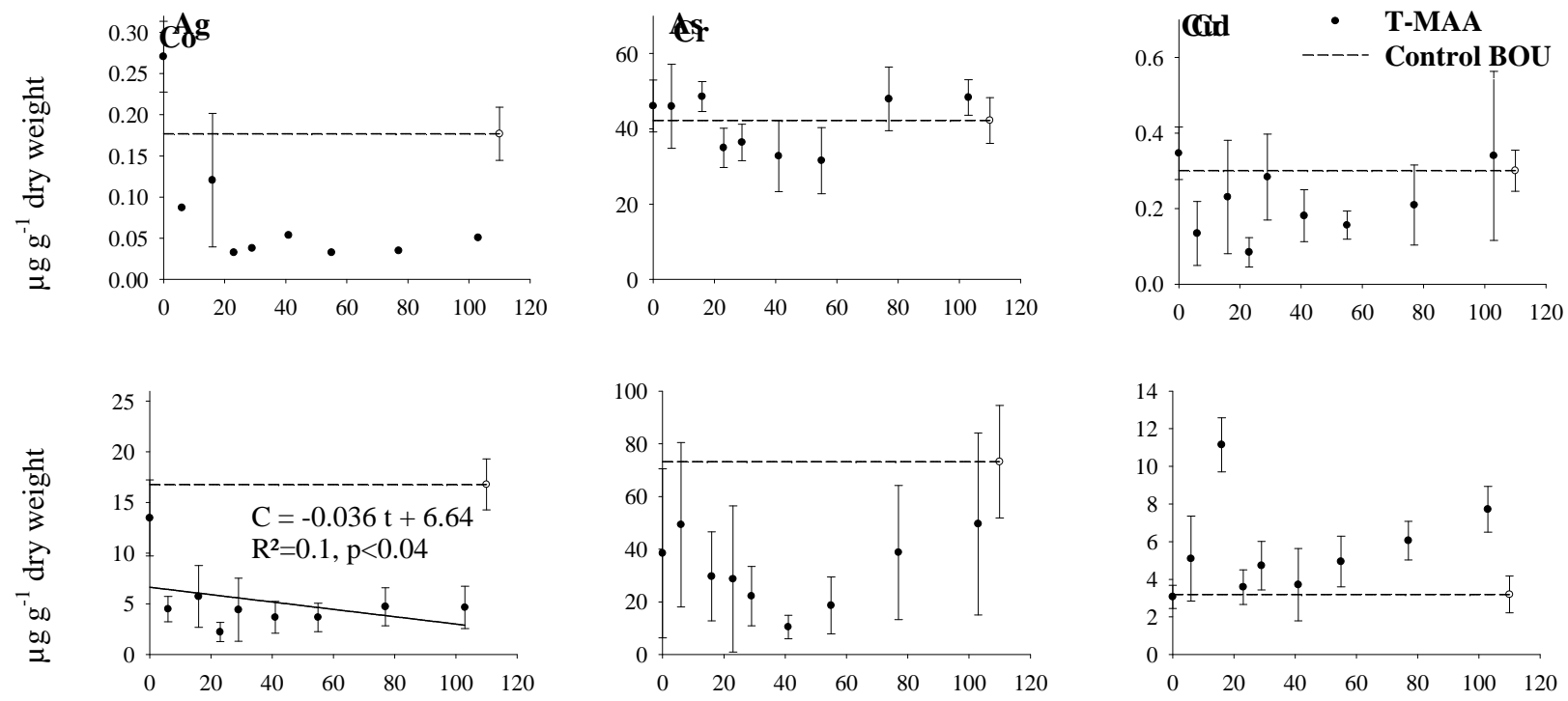


Time (d)

Time (d)

Time (d)

Figure 2



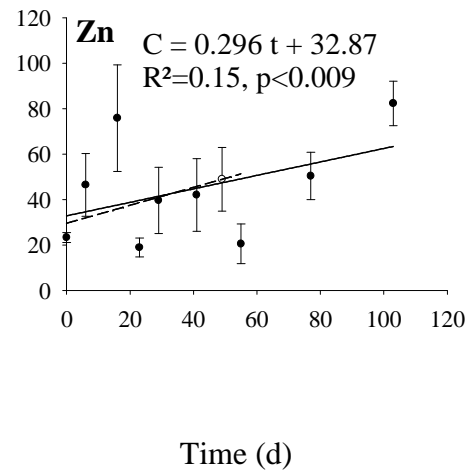
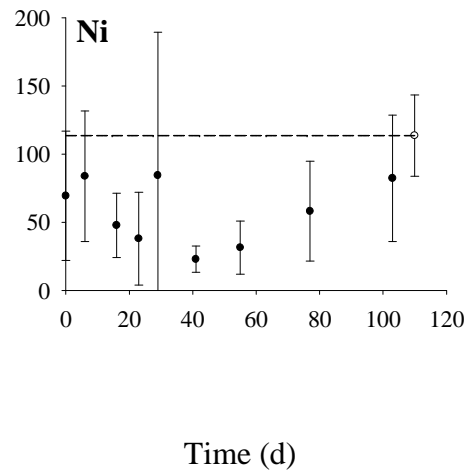
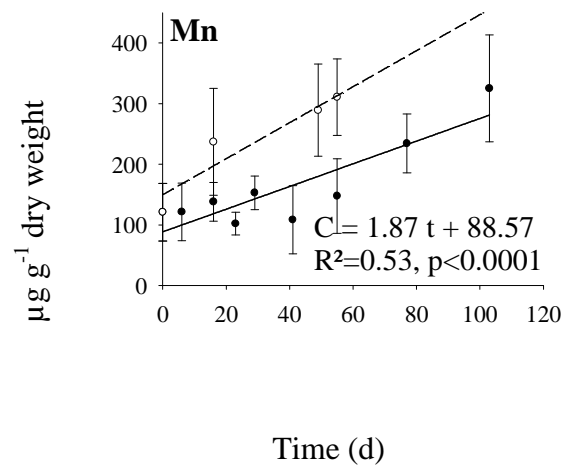


Figure 3

Table 1. ICP-OES and ICP-MS analyses of two certified reference materials: certified and measured values (mean \pm SD; $\mu\text{g g}^{-1}$ dry wt; n = 5) and recovery (%).

| Elements | Method | TORT-2 | | | DOLT-3 | | |
|----------|---------|-----------------|------------------|------------|-----------------|------------------|------------|
| | | Measured | Certified | % Recovery | Measured | Certified | % Recovery |
| Ag | ICP-MS | | | | 1.07 \pm | 1.20 \pm 0.07 | 89 |
| As | ICP-OES | 22.3 \pm 2.2 | 21.6 \pm 1.8 | 103 | 9.45 \pm 0.97 | 10.20 \pm 0.50 | 93 |
| Cd | ICP-MS | 26.4 \pm 3.8 | 26.7 \pm 0.6 | 99 | 17.0 \pm 3.1 | 19.4 \pm 0.6 | 88 |
| Co | ICP-MS | 0.52 \pm 0.09 | 0.51 \pm 0.09 | 102 | | | |
| Cr | ICP-OES | 0.66 \pm 0.19 | 0.77 \pm 0.15 | 85 | | | |
| Cu | ICP-OES | 98.4 \pm 11.2 | 106.0 \pm 10.0 | 93 | 31.2 \pm 2.4 | 31.2 \pm 1.0 | 100 |
| Mn | ICP-OES | 12.5 \pm 1.2 | 13.6 \pm 1.2 | 92 | | | |
| Ni | ICP-OES | 2.02 \pm 0.35 | 2.50 \pm 0.19 | 81 | 3.05 \pm 0.76 | 2.72 \pm 0.35 | 112 |
| Zn | ICP-OES | 188 \pm 20 | 180 \pm 6 | 104 | 97.7 \pm 7.0 | 86.6 \pm 2.4 | 113 |