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

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ABSTRACT.

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

Keywords: Metals; Bioaccumulation; Depuration; Biomonitoring; Sentinel organism; Tropical environment

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Selection of the transplantation locations

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

2.2. Experimental design

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

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

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

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

2.3. Sample preparation and analyses

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

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

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

indicated a recovery ranging from 81 % (Ni) to 113 % (Zn) (Table 1). The detection limits 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 and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) $\mu\text{g g}^{-1}$ dry wt for ICP-MS.

2.4. Statistical analyses

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

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

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

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, respectively, and k is the uptake (k_u) or depuration (k_e) rate constant ($\mu\text{g g}^{-1}$ dry wt d^{-1}) (Temara et al. 1998). Constants of the equation and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software.

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

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

3. Results

3.1. Starting day of transplantation

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

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

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

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

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

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

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

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

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

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

4. Discussion

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

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

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

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

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

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

Whereas *L. variegata* showed a rapid and efficient response time when transplanted to a contaminated environment, the case was very different when algae were transplanted from the contaminated bay to the reference site. Indeed, except for Co where some significant depuration occurred, our study showed that the concentrations of the other elements were basically unchanged after 3 months of transplantation. These observations contrast somewhat with the results from previous laboratory experiments on *L. variegata* which suggested that some metals were characterized by relatively fast turnover rates in the alga, with short biological half-lives ($T_{b1/2}$) of about 1 month for Cr, Mn, and Zn and 1 week for Ni (Metian et al. 2006, 2008b).

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

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

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

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

5. Conclusion

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

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

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Captions to Figures

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

Figure 2. Element concentrations ($\mu\text{g g}^{-1}$ dry wt; mean $\pm$ SD; $n = 5$) in *Lobophora variegata* transplanted from Maa Bay (reference site) to Boulari Bay (contaminated site). Solid lines indicate significant variation in element concentrations in transplanted algae (T-BOU); dash lines indicate element concentrations in the resident algal population of Maa Bay (Control MAA; $n = 30$).

Figure 3. Element concentrations ($\mu\text{g g}^{-1}$ dry wt; mean $\pm$ SD; $n = 5$) in *Lobophora variegata* transplanted from Boulari Bay (contaminated site) to Maa Bay (reference site). Solid lines indicate significant variation in element concentrations in transplanted algae (T-MAA); dash lines indicate element concentrations in the resident algal population of Boulari Bay (Control BOU; $n = 30$).

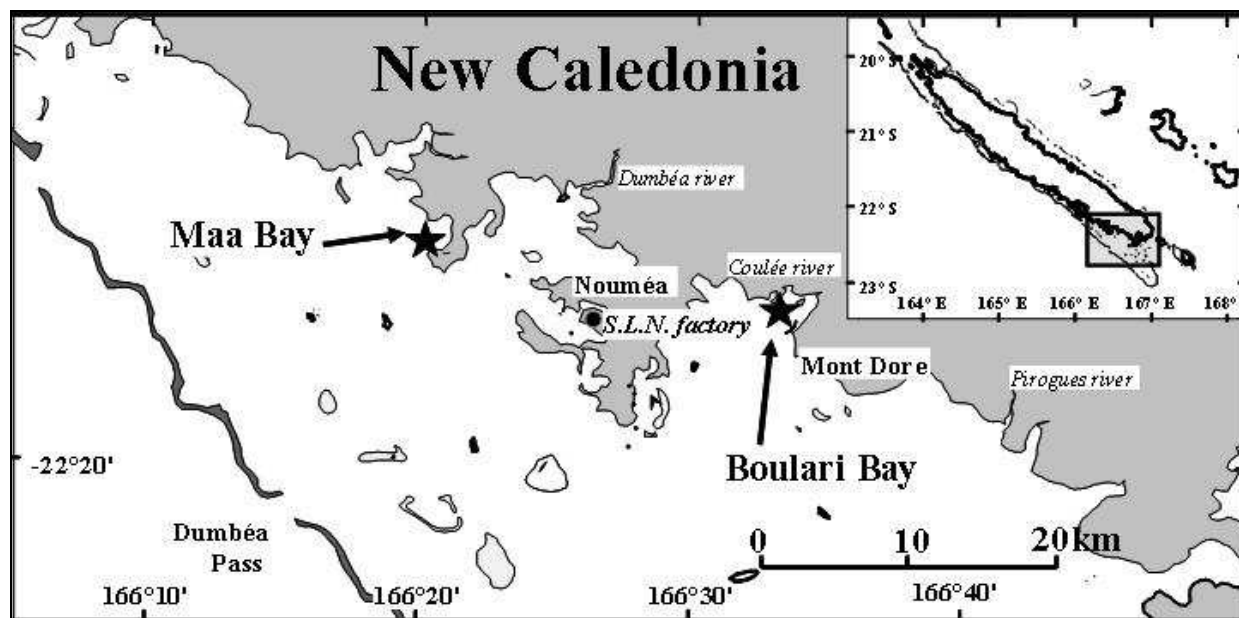


Figure 1

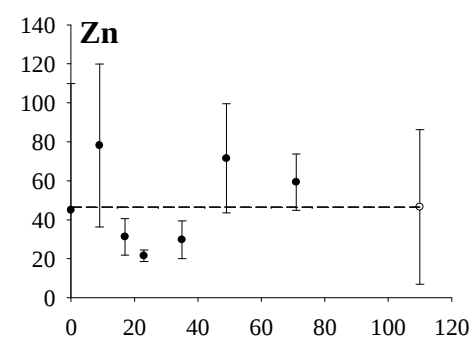
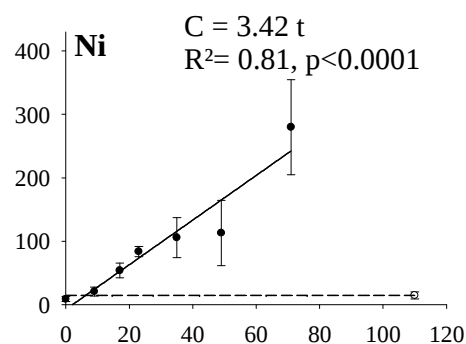
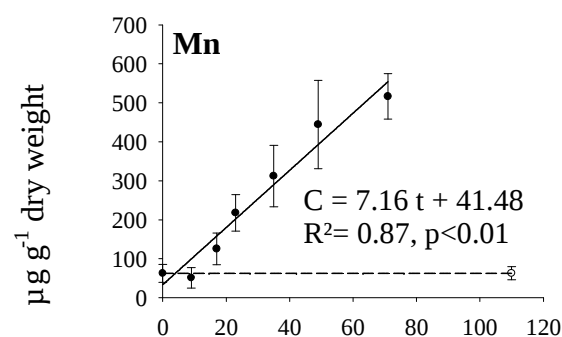
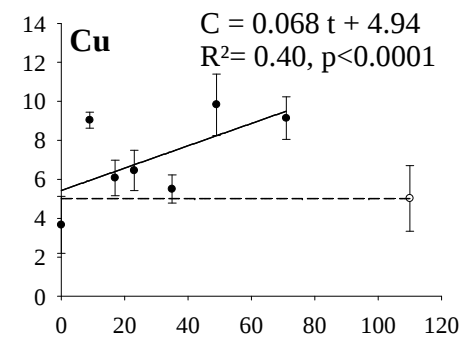
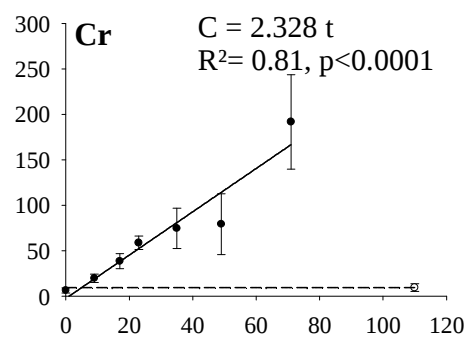
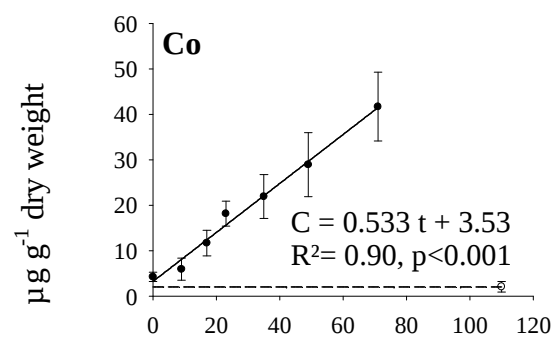
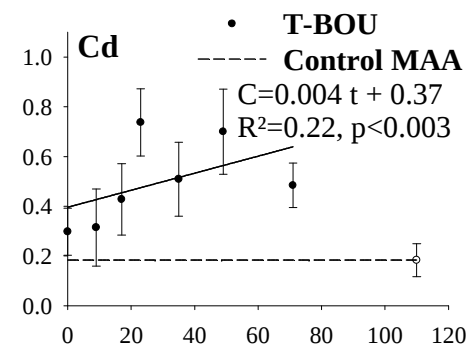
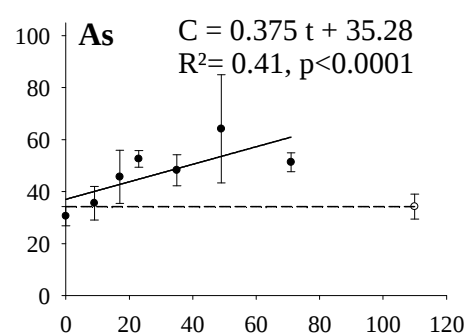
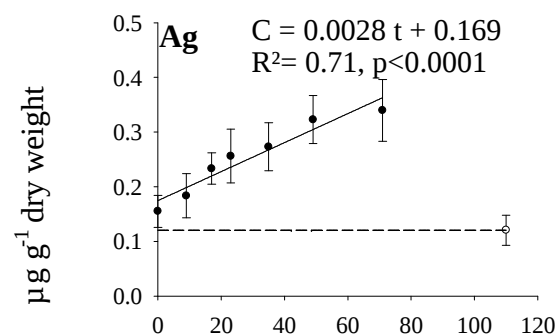
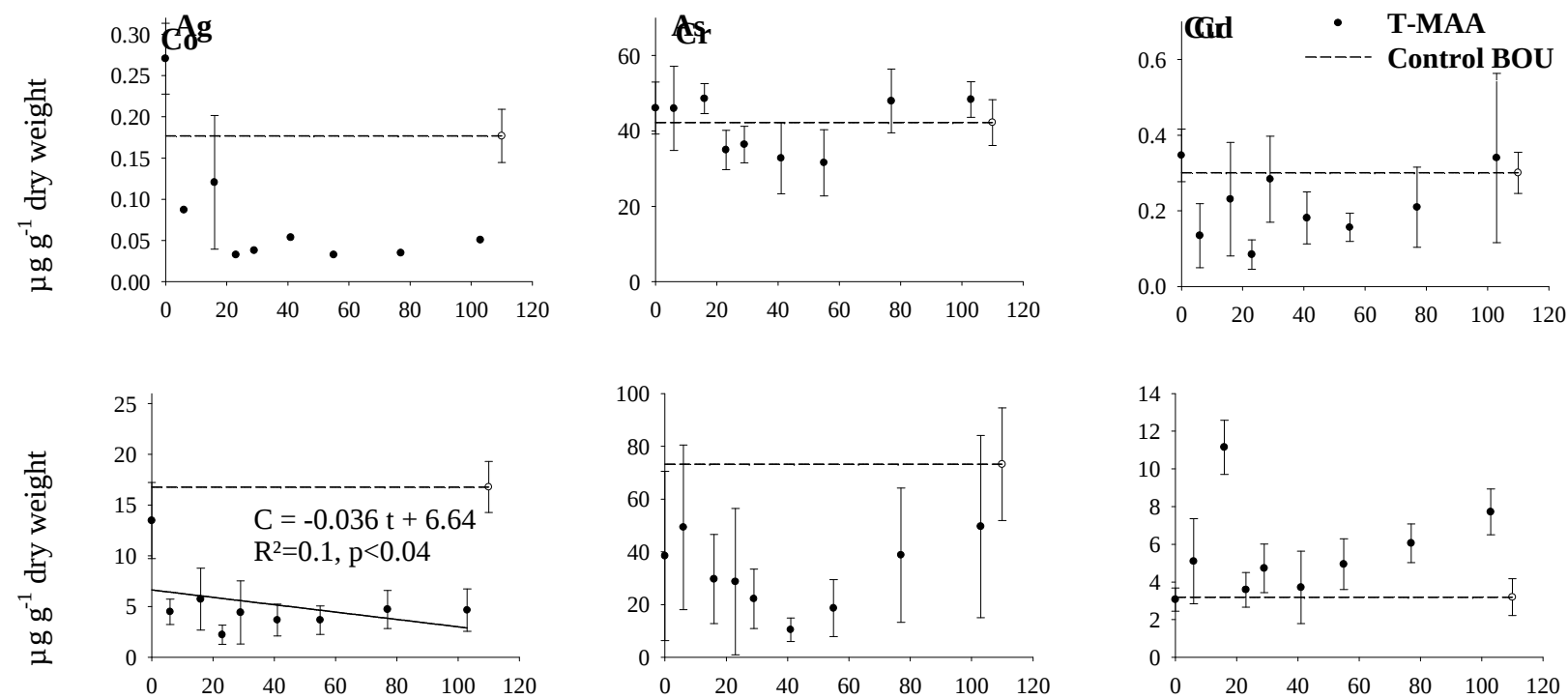


Figure 2



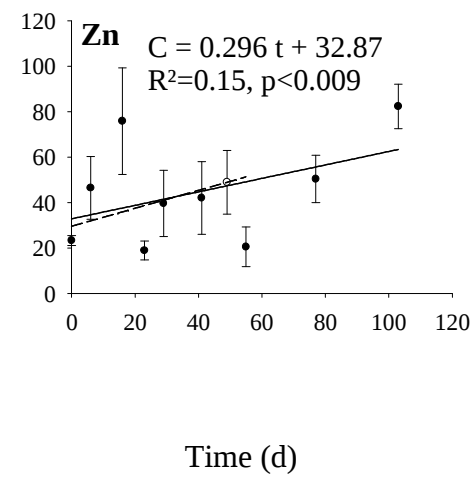
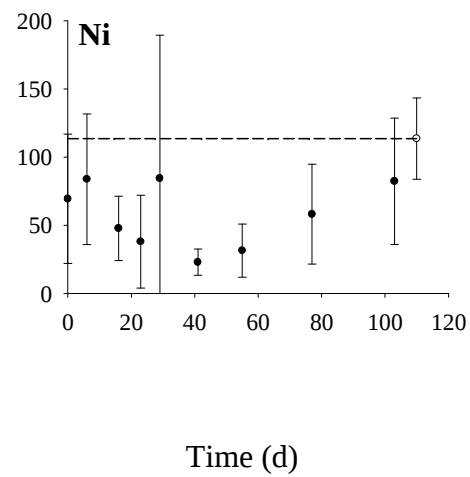
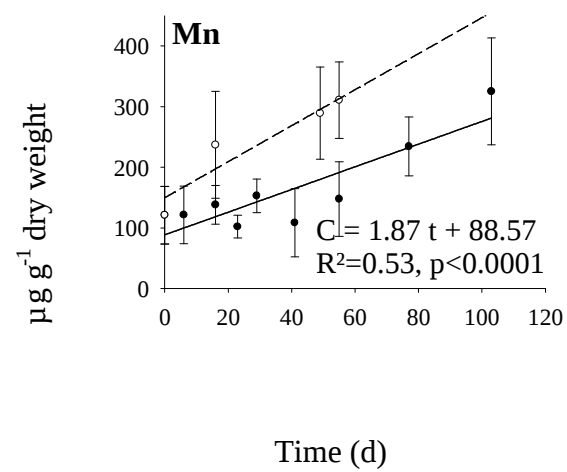


Figure 3

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