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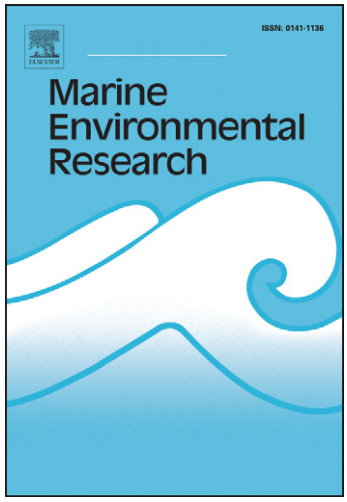

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The tropical brown alga *Lobophora variegata* as a bioindicator of mining contamination in the New Caledonia lagoon: a field transplantation study

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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

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) µg g^{-1} dry wt for ICP-OES
140 and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) µg 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 $C_t = C_0 + k_u t$ (eq. 1)
148
149 $C_t = C_0 - k_e t$ (eq. 2)
150
151 where C_t and C_0 are the element concentration (µg 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 (µg 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

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 ($p_{Tukey} < 0.0006$ 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 ($p_{regression\ slope} = 0.5$) (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

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 µg g^{-1} dry wt at the beginning of the experiment up to 192,
189 516 and 280 µg 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 µg 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 µg 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 µg 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 µg 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

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 ($T_{b½}$) 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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References

Amado Filho, G.M., Andrade, L.R., Karez, C.S., Farina, M., Pfeiffer, W.C. 1999. Brown algae species as biomonitors of Zn and Cd at Sepetiba Bay, Rio de Janeiro, Brazil. Marine Environmental Research 48, 213-224.

Ambatsian, P., Fernex, F., Bernat, M., Parron, C., Lecolle, J. 1997. High metal inputs to closed seas: the New Caledonia lagoon. Journal of Geochemical Exploration 59, 59-74.

Bird, E.C.F., Dubois, J.P., Iltis, J.A. 1984. The impacts of opencast mining on the rivers and coasts of New Caledonia. United Nations University Press, Tokyo.

Bryan, G.W. 1983. Brown seaweed, *Fucus vesiculosus*, and the gastropod, *Littorina littoralis*, as indicators of trace-metal availability in estuaries. Science of the Total Environment 28, 91-104.

Bryan, G.W. 1984. Pollution due to heavy metals and their compounds. Marine Ecology 5, 1290-1331.

Burrows, E.M. 1971. Assessment of pollution effects by the use of algae. Proceedings of the Royal Society of London B Biological Sciences 177, 295-306.

Cobbett, C., Goldsbrough, P. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annual Review of Plant Biology 53, 159-182.

Coteur G., Gosselin P., Wantier P., Chambost-Manciet Y., Danis B., Pernet P., Warnau M., Dubois P. 2003. Echinoderms as bioindicators, bioassays, and impact assessment tools of

354 sediment-associated metals and PCBs in the North Sea. Archives of Environmental
355 Contamination and Toxicology 45, 190-202.
356 Fichez, R., Adjeroud, M., Bozec, Y.M., Breau, L., Chancerelle, Y., Chevillon, C., Douillet,
357 P., Fernandez, J.M., Frouin, P., Kulbicki, M., Moreton, B., Ouillon, S., Payri, C., Perez,
358 T., Sasal, P., Thébault, J. 2005. A review of selected indicators of particle, nutrient and
359 metal inputs in coral reef lagoon systems. Aquatic Living Resources 18, 125-147.
360 Försberg, A., Söderlung, S., Frand, A., Petersson, L.R., Pedresén, M. 1988. Studies on metal
361 content in the brown seaweed, *Fucus vesiculosus*, from the Archipelago of Stockholm.
362 Environmental Pollution 49, 245-263.
363 Hédouin, L., Metian, M., Cotret, O., Teyssié, J.L., Fowler, S.W., Fichez, R., Warnau, M.
364 2006. Allometric relationships in the bioconcentration of heavy metals by the edible
365 tropical clam *Gafrarium tumidum.* Science of the Total Environment 366, 154-163.
366 Hédouin, L., Pringault, O., Metian, M., Bustamante, P., Warnau, M. 2007. Nickel
367 bioaccumulation in bivalves from the New Caledonia lagoon: Seawater and food
368 exposure. Chemosphere 66, 1449-1457.
369 Hédouin, L., Bustamante, P., Churlaud, C., Pringault, O., Fichez, R., Warnau, M. 2008.
370 Trends in concentrations of selected metalloid and metals in two bivalves from the SW
371 lagoon of New Caledonia. Ecotoxicology and Environmental Safety (in press,
372 doi:10.1016/j.ecoenv.2008.04.004).
373 Hédouin, L. 2006. Caractérisation d'espèces bioindicatrices pour la surveillance des activités
374 minières et la gestion de l'environnement en milieu récifal et lagonaire: application au
375 lagon de Nouvelle-Calédonie. Ph.D. Thesis, Océanologie Biologique et Environnement
376 Marin, University of La Rochelle, France.
377 Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's
378 coral reefs. Marine and Freshwater Research 50, 839-866.

379 Karez, C.S., Magalhaes, V.F., Pfeiffer, W.C., Amado Filho, G.M. 1994. Trace metal
380 accumulation by algae in Sepetiba Bay, Brazil. Environmental Pollution 83, 351-356.
381 Klerks, P.L., Weis, J.S. 1987. Genetic adaptation to heavy metals in aquatic organisms: a
382 review. Environmental Pollution 45, 173-205.
383 Labrosse, P., Fichez, R., Farman, R., Adams, T. 2000. New Caledonia, in: Sheppard, C.R.C.
384 (Ed.), Seas at the Millenium: an environmental evaluation. Pergamon, Amsterdam, pp.
385 723-736.
386 Laganier, R. 1991. Erosion, transport and sedimentation processes associated with opencast
387 mining in New Caledonia: interactions with weather and climate, in: ORSTOM (Ed.),
388 South Pacific environments: interactions with weather and climate. New Zealand, pp. 83-
389 85.
390 Ma, X.L., Cowles, D.L., Carter, R.L. 2000. Effect of pollution on genetic diversity in the bay
391 mussel *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. Marine
392 Environmental Research 50, 559-563.
393 Metian, M., Hédouin, L., Barbot, C., Teyssié, J.L., Fowler, S.W., Goudard, F., Bustamante,
394 P., Durand, J.P., Piéri, J., Warnau, M, 2005. Use of radiotracer techniques to study
395 subcellular distribution of metals and radionuclides in bivalves from the Noumea Lagoon,
396 New Caledonia. Bulletin of Environmental Contamination and Toxicology 75, 89-93.
397 Metian, M., Hédouin, L., Giron, E., Buschiazzo, E., Borne, V., Paganelli, J., Boudjenoun, R.,
398 Cotret, O., Teyssié, J.L., Fowler, S.W., Warnau, M. 2006. Bioaccumulation of selected
399 heavy metals in the brown alga *Lobophora variegata*: a radiotracer study, in: Isotopes in
400 Environmental Studies, IAEA-CSP-26. IAEA, Vienna, pp. 323-324.
401 Metian, M., Bustamante, P., Hédouin, L., Warnau, M. 2008a. Accumulation of trace elements
402 in the tropical scallop *Comptopallium radula* from coral reefs in New Caledonia.
403 Environmental Pollution 152, 543-552.

Metian, M., Giron, E., Borne, V., Hédouin, L., Teyssié, J.L., Warnau, M. 2008b. The brown alga *Lobophora variegata*, a bioindicator species for surveying metal contamination in tropical marine environments. Journal of Experimental Marine Biology and Ecology 362, 49-54.

Morris, C.A., Nicolaus, B., Sampson, V., Harwood, J.L., Kille, P. 1999. Identification and characterization of a recombinant metallothionein protein from a marine alga, *Fucus vesiculosus*. The Biochemical Journal 338, 553-560.

O'Connor, T.P. 1998. Mussel Watch results from 1986 to 1996. Marine Pollution Bulletin 37, 14-19.

Phillips, D.J.H. 1990. Use of macroalgae and invertebrates as monitors of metal levels in estuaries and coastal waters, in: Furness, R.W., Rainbow, P.S. (Eds.), Heavy metals in the marine environment. CRC Press, Boca Raton, pp. 81-99.

Ragan, M.A., Smidsrod, O., Larsen, B. 1979. Chelation of divalent metal ions by brown algal polyphenols. Marine Chemistry 7, 265-271.

Rainbow, P.S. 2002. Trace metal accumulation in aquatic invertebrates: why and so what? Environmental Pollution 120, 497-507.

Söderlund, S., Forsberg, A., Pedersen, M. 1988. Concentrations of cadmium and other metals in *Fucus vesiculosus* L. and *Fontinalis dalecarlica* Br. Eur. from the northern Baltic Sea and the southern Bothnian Sea. Environmental Pollution 51, 197-212.

Targett, N.M., Coen, L.C., Boettcher, A.A., Tanner, C.E. 1992. Biogeographic comparisons of marine algal polyphenolics: evidence against a latitudinal trend. Oecologia 89, 464-470.

Temara A., Aboutboul P., Warnau M., Jangoux M., Dubois P. 1998. Uptake and fate of lead in the common asteroid *Asterias rubens* (Echinoderm). Water, Air, and Soil Pollution 102, 201-208.

429 Wallner-Kersanach, M., Theede, H., Eversberg, U., Lobo, S. 2000. Accumulation and
430 elimination of trace metals in a transplantation experiment with *Crassostrea rhizophorae*.
431 Archives of Environmental Contamination and Toxicology 38, 40-45.
432 Warnau, M., Bustamante, P. 2007. Radiotracer techniques: a unique tool in marine
433 ecotoxicological studies. Environmental Bioindicators 2, 217-218.
434 Warnau, M., Teyssié, J.L., Fowler, S.W. 1995. Effect of feeding on cadmium
435 bioaccumulation in the echinoid *Paracentrotus lividus* (Echinodermata). Marine Ecology
436 Progress Series 126, 305-309.
437 Warnau, M., Teyssié, J.L., Fowler, S.W. 1996. Biokinetics of selected heavy metals and
438 radionuclides in two marine macrophytes: the seagrass *Posidonia oceanica* and the alga
439 *Caulerpa taxifolia*. Marine Environmental Research 41, 343-362.
440 Warnau, M., Teyssié, J.L., Fowler, S.W. 1997. Cadmium bioconcentration in the echinoid
441 *Paracentrotus lividus:* influence of the cadmium concentration in sea water. Marine
442 Environmental Research 43, 303-314.
443 Zar, J.H. 1996. Biostatistical analysis. Prentice-Hall, Upper Saddle River, New Jersey.
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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 (µg g^{-1} dry wt; mean ± 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 (µg g^{-1} dry wt; mean ± 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).

465

Table 1. ICP-OES (Inductively coupled plasma – optical emission spectrometry) and ICP-MS (Inductively coupled plasma – mass spectrometry) analyses of two certified reference materials: certified and measured values (mean ± SD; μg 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 ±	1.20 ± 0.07	89
As	**ICP-OES**	22.3 ± 2.2	21.6 ± 1.8	103	9.45 ± 0.97	10.20 ± 0.50	93
Cd	**ICP-MS**	26.4 ± 3.8	26.7 ± 0.6	99	17.0 ± 3.1	19.4 ± 0.6	88
Co	**ICP-MS**	0.52 ± 0.09	0.51 ± 0.09	102			
Cr	**ICP-OES**	0.66 ± 0.19	0.77 ± 0.15	85			
Cu	**ICP-OES**	98.4 ± 11.2	106.0 ± 10.0	93	31.2 ± 2.4	31.2 ± 1.0	100
Mn	**ICP-OES**	12.5 ± 1.2	13.6 ± 1.2	92			
Ni	**ICP-OES**	2.02 ± 0.35	2.50 ± 0.19	81	3.05 ± 0.76	2.72 ± 0.35	112
Zn	**ICP-OES**	188 ± 20	180 ± 6	104	97.7 ± 7.0	86.6 ± 2.4	113

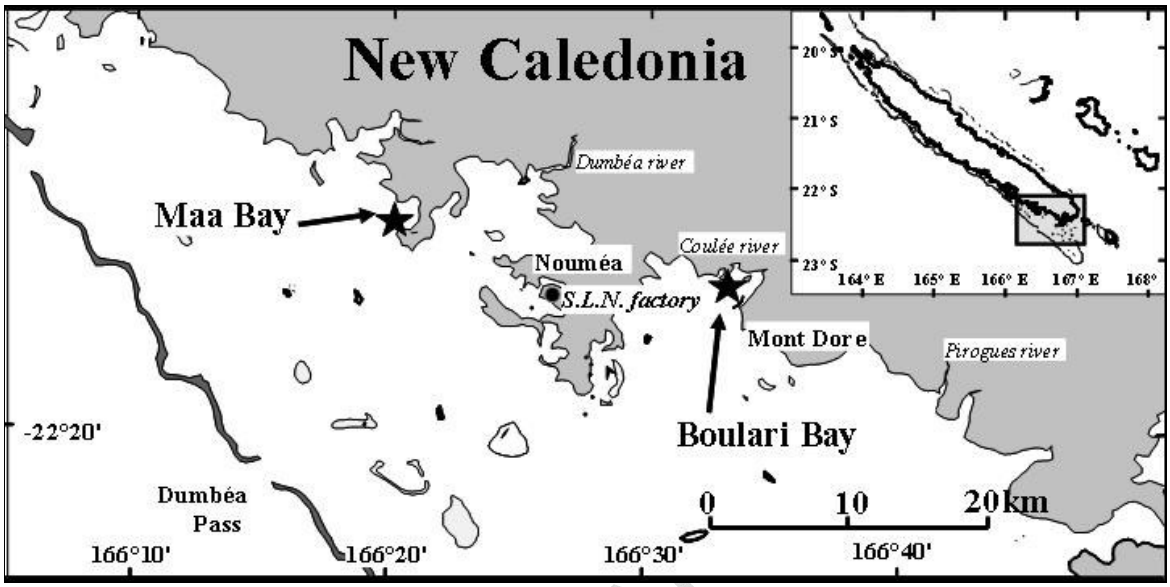

Figure 1

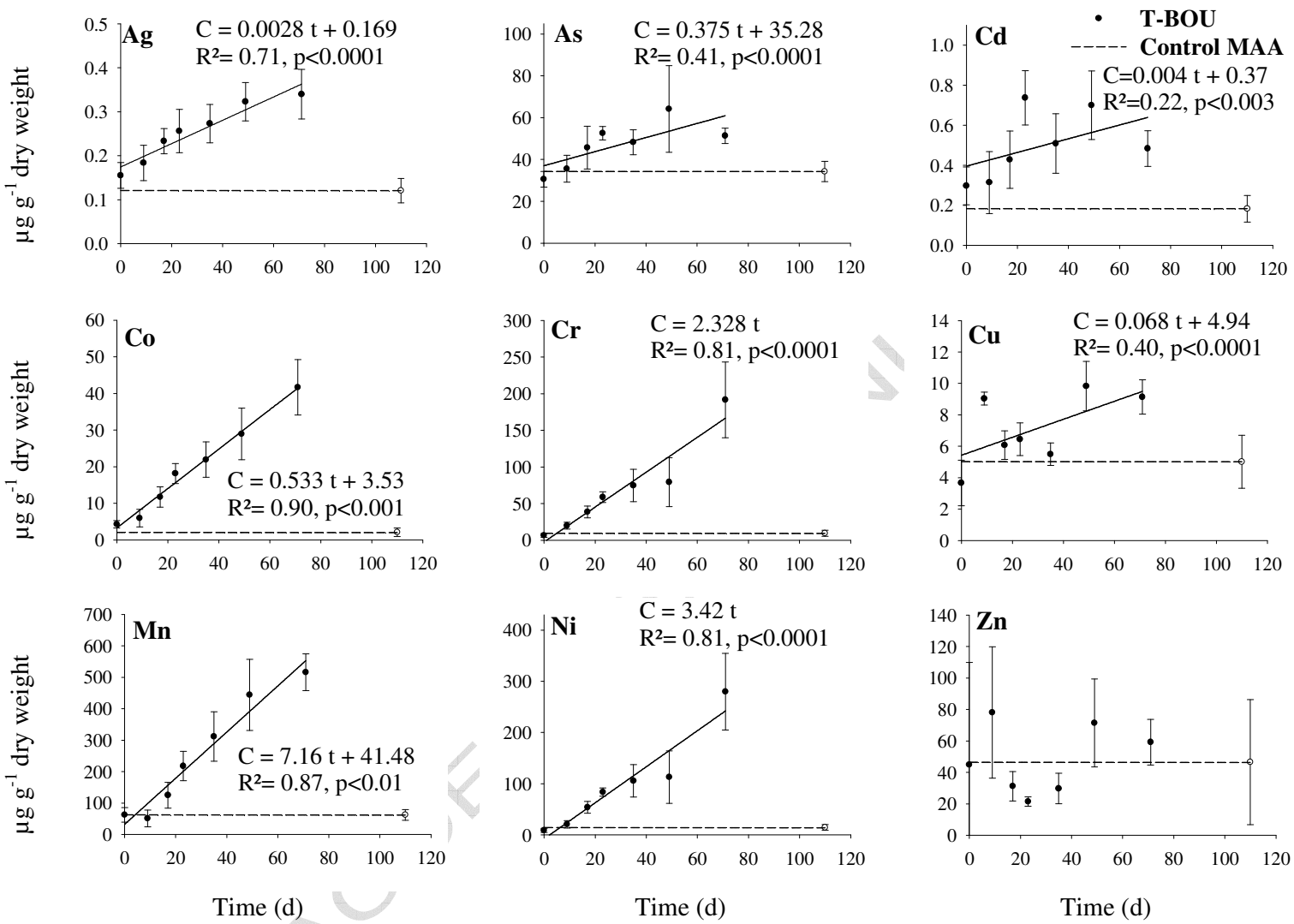
Ag
C = 0.0028 t + 0.169
R²= 0.71, p<0.0001
As
C = 0.375 t + 35.28
R²= 0.41, p<0.0001
Cd
T-BOU
Control MAA
C=0.004 t + 0.37
R²=0.22, p<0.003
Co
C = 0.533 t + 3.53
R²= 0.90, p<0.001
Cr
C = 2.328 t
R²= 0.81, p<0.0001
Cu
C = 0.068 t + 4.94
R²= 0.40, p<0.0001
Mn
C = 7.16 t + 41.48
R²= 0.87, p<0.01
Ni
C = 3.42 t
R²= 0.81, p<0.0001
Zn
µg g-1 dry weight
Time (d)

Figure 2

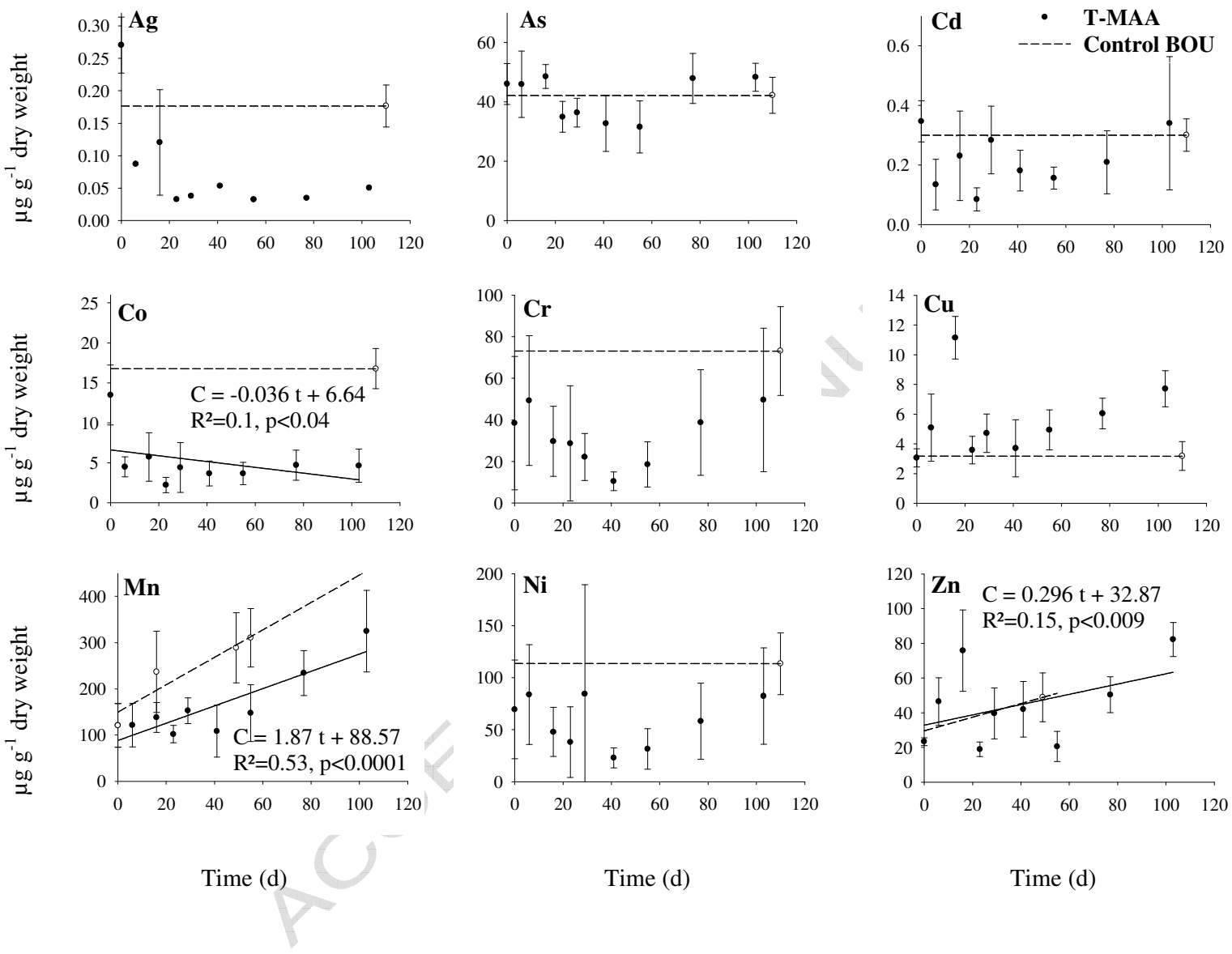
Ag
As
Cd
T-MAA
Control BOU
Co
C = -0.036 t + 6.64
R²=0.1, p<0.04
Cr
Cu
Mn
C= 1.87 t + 88.57
R²=0.53, p<0.0001
Ni
Zn
C = 0.296 t + 32.87
R²=0.15, p<0.009
µg g-1 dry weight
Time (d)

Figure 3