The brown alga *Lobophora variegata*, a bioindicator species for surveying metal contamination in tropical marine environments

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**Abstract**

Uptake and depuration kinetics of Cd, Co, Cr, Mn, Ni and Zn were determined in the brown alga *Lobophora variegata* exposed to realistic concentrations of these metals, using highly sensitive radiotracer techniques. The experiments were designed to assess the possible influence of varying dissolved metal concentrations on the capacity of metal bioconcentration and retention in the alga. Results indicate that the alga takes up Cd, Co, Cr, Ni, and Zn in direct proportion to their ambient dissolved concentrations over the entire range of concentrations tested (three orders of magnitude). In contrast, Mn was taken up in proportion to its dissolved concentration only over a concentration range of 2 orders of magnitude (up to 250 ng Mn L⁻¹, i.e. 4.55 nM), then at higher concentrations its accumulation efficiency slightly decreased. Overall, *L. variegata* appears to be a reliable bioindicator species that shows a rapid response time in metal uptake (uptake rate constants ranging from 60 to 1,023 d⁻¹) and has a suitable potential to furnish valuable information on the bioavailable contamination levels occurring in New Caledonian areas affected by land-based mining activities. Furthermore, due to its wide geographical distribution, *L. variegata* could be considered as a useful bioindicator species for surveying metal contamination in many other tropical areas.

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1. Introduction

The main economic resources of New Caledonia are derived from nickel exploitation. The land-based open-cast mining activities inevitably result in direct and indirect metal contamination of the surrounding environment and in particular of the lagoon waters (Ambatsian et al., 1997; Labrosse et al., 2000). Although these metal inputs constitute a major threat to the local, highly diversified coastal marine ecosystems, little information is available regarding levels of contamination and possible impacts on the New Caledonian lagoon (Labrosse et al., 2000; Metian et al., 2005, 2008; Hédouin et al., 2006, 2007, in press).

Among the common approaches used to study environmental contamination, the use of bioindicator species has proved to be a valuable and informative tool. The main advantage of bio-monitoring approach compared to direct measurement in water or sediment is to provide a direct and time-integrated assessment of the metal fraction that is actually available to the organisms (bioavailable fraction) (e.g., Phillips, 1990, 1994; Coteur et al., 2003; Danis et al., 2004). In the marine environment, bivalves are the most widely used bioindicator species and have been successfully used in the implementation of large scale monitoring programmes such as the US and European “Mussel Watches” (e.g., Goldberg et al., 1983; Warnau and Acuña, 2007). Although less commonly used, marine macrophytes and in particular seaweeds are well documented for their ability to concentrate contaminants from the surrounding waters (e.g., Bryan and Hummerstone, 1973; Burdon-Jones et al., 1982; Phillips, 1994; Warnau et al., 1996).

According to recent studies, the brown alga *Lobophora variegata* has been identified as a potential bioindicator species for use in the New Caledonian lagoon (Breau, 2003; Hédouin, 2006). Indeed, this alga meets the criteria that a bioindicator species should fulfill (e.g., Phillips, 1994; Warnau et al., 2006), namely it shows a good capacity to bioconcentrate metals from its surrounding environment, is abundant, widely distributed and easy to collect (e.g., Coen and Tanner, 1989; Breau, 2003; Hédouin, 2006). However, no information is available on one of the most important pre-requisites to be met by a bioindicator species: the existence of a simple relationship between metal concentrations in organisms and those in seawater (e.g., Warnau et al., 1997). This is indeed the very criterion that allows the use of biota as reliable indicator of the metal contamination levels occurring in the environment. For this reason, the influence of ambient concentration of metals of local concern (Cd, Co, Cr, Mn, Ni and Zn) on their bioconcentration and depuration in *L. variegata* was investigated using the unique advantages of radiotracer techniques (Warnau and Bustamante, 2007) in order to further assess its value as a reliable sentinel species.
2. Materials and Methods

2.1. Sampling

Brown algae, *Lobophora variegata* (Lamouroux) Womersley, were collected by SCUBA diving in the south-western lagoon of New Caledonia (Maa Bay) in October 2003. This zone is considered as a relatively clean site in terms of metal contamination (Hédouin, 2006; Hédouin et al., in press). Three different morphological forms of *L. variegata* (ruffled, decumbent, and encrusting) have been described in the Caribbean; their occurrence depends on the habitat and intensity of herbivore predation (Coen and Tanner, 1989). To the best of our knowledge, no similar information is reported in the open literature for the New Caledonian populations but we (MW and LH) have personally observed two out of the three morpho-types in the field (i.e. ruffled and decumbent forms). In the present study, the algae collected in Maa Bay were from one single type, i.e. decumbent. Algae were then shipped to the IAEA-MEL premises in Monaco and were acclimated for 1 month to laboratory conditions before experiments (open circuit aquarium; seawater renewal: 50% h−1; salinity: 36 p.s.u.; T°: 25±1 °C; pH: 8.0±0.1; 12-h photoperiod at an irradiance of light intensity 300 µmol photons m−2 s−1).

2.2. Stable elements and radiotracers

Selected metals (Cd, Co, Cu, Mn, Ni and Zn) were introduced into the experimental microcosms both as stable elements and their corresponding radiotracers (five γ-emitting tracers: 51Cr, 54Mn, 57Co, 65Zn 109Cd, and one β-emitting isotope: 54Ni) to allow tracking the biokinetics of these elements with high sensitivity (Warnau et al., 1996, 1997, 1999). Stable elements were introduced as HNO3 salts biokinetics of these elements with high sensitivity (Warnau et al., 1996, 1997, 1999). Stable elements were introduced as HNO3 salts (open circuit aquarium; seawater renewal: 50% h−1; salinity: 36 p.s.u.; T°: 25±1 °C; pH: 8.0±0.1; 12-h photoperiod at an irradiance of light intensity 300 µmol photons m−2 s−1).

2.3. Experimental procedure

For each concentration of each element tested, one different batch of *L. variegata* thalli was placed in an aquarium containing 20 L of natural seawater (closed circuit aquarium; salinity: 36 p.s.u.; T°: 25±1 °C; pH: 8.0±0.1; 12-h photoperiod at an irradiance of 300 µmol photons m−2 s−1). Batches of *L. variegata* thalli (average weight=0.94±0.32 g wet wt; 0.28±0.10 g dry wt) were composed of n=5 for Cd, Co, Cr, Mn and Zn, and of n=48 for Ni (the destructive analysis of β-emitting radiotracers requires a higher number of individuals than for γ-spectrometry; see below). In order to identify the algae easily during the experiments, each individual seaweed thallus was held in one cylindrical PVC container (80 mm×50 mm) covered with 300-μm size mesh net (to allow for free water circulation). Algae were then exposed for 14 d to one out of five different added concentrations of a given element (one different batch of algae was used for each different concentration of each different element tested). Concentrations tested were up to 250 ng Cd L−1 (2.2 mM), 998 ng Co L−1 (17 mM), 1,250 ng Cr L−1 (24 nM), 1,250 ng Mn L−1 (23 nM), 1,400 ng Ni L−1 (24 nM) and 1,750 ng Zn L−1 (27 nM) (Table 1).

The concentration ranges were selected in order to cover the whole ranges of those actually encountered in the New Caledonia lagoon waters (Fernandez et al., 2002). The added concentrations and radioactive media were prepared using increasing amount of the stable element and a fixed activity of the corresponding radiotracer: 51Cr (1.5 kBq L−1), 54Mn (0.5 kBq L−1), 57Co (0.5 kBq L−1), 65Ni (1 kBq L−1), 65Zn (0.5 kBq L−1) and 109Cd (1 kBq L−1). In terms of stable element additions, these radioactive levels corresponded to Cr (0.14 ng L−1), Mn (0.36 ng L−1), Co (0.028 ng L−1), Ni (4.2 ng L−1), Zn (5.99 ng L−1) and Cd (0.048 ng L−1).

Due to the methodological specificities of γ- and β-counting, the γ-emitting tracers (51Cr, 54Mn, 57Co, 65Zn, 109Cd) were radioanalyzed in algae on a wet wt basis whereas the β-emitting 54Ni was analysed on a dry wt basis. Therefore, in order to facilitate direct comparison with the other metals, all Ni-related results were also expressed on a wet wt basis using the measured wet: dry wt ratio of 3.3 for this alga. Radioactivity of 51Cr, 54Mn, 57Co, 65Zn and 109Cd was measured using a high-resolution γ-spectrometer system composed of 4 Germanium -N

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Kinetic parameters for Linear (L) and Exponential saturation (E) models: concentration factors at steady state (CFγ) and uptake rate constant (k0). ASE: asymptotic standard error; R2: determination coefficient of the model adjustment. Significance of the estimated parameters: * p<0.0001; ** 0.0001<p<0.001; *** 0.001<p<0.05; **** p>0.05.
or P type- detectors (EGNC 33-195-R, Canberra® and Euryxis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity of samples was determined by comparison with standards of known activities of appropriate geometry. All measurements were corrected for counting efficiency, background and radioactive decay. Counting time was adapted to obtain counting rates with propagated errors less than 5%.

Radioanalyses of 63Ni-exposed algae were performed using a 1600 TR Packard Liquid Scintillation Analyzer (destructive analyses). Seawater samples (2 mL) were directly transferred to 20 mL glass scintillation vials (Packard) and mixed with 10 mL of scintillation liquid (Ultima Gold®; Packard). Biota samples (thalli) were first dried at 60 °C until constant weight, then digested for one week at room temperature of 50 °C with 1 mL of Soluene® (Packard) for 100 mg dry wt tissues, and finally mixed with scintillation liquid (Hionic Fluor®, Packard) in the proportion 1 volume Soluene® : 5 volumes Hionic Fluor®. Counting time was adapted to obtain a propagated counting error less than 5% (maximum counting time was 2 h). The radioactivity was determined by comparison with standards of known activities and measurements were corrected for counting efficiency, physical radioactive decay and quenching effects.

2.5. Data Treatment

Uptake kinetics of the six radiotracers were expressed as change in concentration factor (CF, ratio between activity of the radiotracer in the organism -Bq g⁻¹ wet wt- and time-integrated activity of the radiotracer in seawater -Bq g⁻¹-), along with time. Radiotracer uptake kinetics were described using either a simple linear regression model (Eq. (1)) or, if the observed kinetics tended to reach a steady-state equilibrium, using a saturation exponential kinetic model (Eq. (2)):

\[
CF_t = k_u t
\]

\[
CF_S = CF_{SS} \left( 1 - \exp^{-k_u t} \right)
\]

where \( CF_t \) and \( CF_{SS} \) are the concentration factors at time \( t \) (d) and at steady state, and \( k_u \) and \( k_e \) the uptake and depuration rate constants (d⁻¹), respectively (Whicker and Schultz, 1982; Warnau et al., 1996). Linearity of the uptake kinetics was tested by a linearity test for regression with replication (Zar, 1996). Model constants (\( CF_{SS}, k_u \) and \( k_e \)) and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation using the non-linear curve-fitting routines in the Statistica® 6 software.

Depuration kinetics of the radiotracers were expressed as change in percentage of remaining radioactivity (radioactivity at time \( t \) divided by the initial radioactivity measured in the organism at the beginning of the depuration period * 100) along with time. The depuration kinetics of radiotracers were best fitted by either a single-component (Eq. (3)) or a double-component (Eq. (4)) exponential equation:

\[
A_t = A_0 \exp^{-k_{u,t}}
\]

\[
A_t = A_0 \exp^{-k_{u,t}+k_{l,t}} + A_0 \exp^{-k_{e,t}}
\]

where \( A_t \) and \( A_0 \) are the remaining activities (%) at time \( t \) (d) and 0, respectively, \( k_u \) is the depuration rate constant (d⁻¹) and ‘s’ and ‘l’ are the subscripts for the short-lived and long-lived component, respectively. The short-lived component is a model for the depuration of the portion of the radiotracer pool that is weakly associated to the organism, whereas the long-lived component is a model of the depuration of the fraction of the radiotracer pool that is tightly bound to the organism (Warnau et al., 1996, 1999). A biological half-life can be calculated for each exponential component (\( T_{1/2,s} \) and \( T_{1/2,l} \)), using the corresponding depuration rate constant (\( k_{ss} \) and \( k_{ll} \), respectively) according to the relation \( T_{1/2} = \ln 2 / k \) (Whicker and Schultz, 1982; Warnau et al., 1996).

In order to test the differences among exposure concentrations tested, the estimated kinetic parameters (\( k_u, CF_{SS}, k_{ss}, k_{ll} \)) were plotted against the exposure element concentration (stable+radioactive) in seawater and fitted using simple linear regression.

In the case of the γ-emitting tracers tested (51Cr, 54Mn, 57Co, 65Zn, 109Cd) for which there was no time independence, differences among uptake and depuration kinetics of each radiotracer over the concentration range were tested using the Linear Mixed Models (LMM) procedure.
implemented in SPSS v12.0 software. This procedure expands the general linear model so that the error terms and random effects are permitted to exhibit correlated and non-constant variability. The LMM, therefore, provides the flexibility to model not only the mean of a response variable, but its covariance structure as well. Time was specified as the repeated factor and the total dissolved concentrations (stable + radioactive) of Cd, Co, Cr, Mn and Zn as the fixed effect.

The level of significance for statistical analyses was always set at $\alpha=0.05$.

3. Results

In Fig. 1 we show the uptake kinetics of the six metals in Lobophora variegata over the tested concentration ranges. Cd, Co, Cr and Zn were taken up according to linear uptake kinetics ($R^2>0.65$) whereas Mn and Ni uptake were best described by a saturation model ($R^2>0.80$) for each concentration tested (Table 1). All metals were readily incorporated in L. variegata at each tested concentration, with uptake rate constants ranging from 60 to 1,023 d$^{-1}$.

Linear Mix Model (LMM) analysis indicated that uptake kinetics of $^{51}$Cr, $^{54}$Mn, $^{57}$Co and $^{65}$Zn were not significantly different over the range of concentrations tested ($p_{SPSS}=0.408, 0.070, 0.292, 0.256$, respectively). In contrast, for $^{109}$Cd, the exposure concentration of Cd significantly affected its uptake in algae ($p_{SPSS}=0.02$).

When examining separately the uptake kinetic parameters ($k_u$, CF$_{iad}$), no significant difference in uptake efficiency was observed among the different metal exposure concentrations in seawater except for Mn. For this metal, although the CF$_{iad}$ was not significantly affected by increasing exposure concentration, $k_u$ showed a slight but significant increase ($p=0.02$) over the Mn concentration range considered ($0$ to $1.250$ ng added Mn L$^{-1}$). This relationship was no longer significant (thus indicating independence of CF towards exposure concentration) when the range of Mn concentration considered was restricted up to $250$ ng added Mn L$^{-1}$.

At the end of the exposure period, seaweeds were maintained for 21 d ($28$ d for Ni) in non contaminated seawater to follow depuration of the radiotracers. Depuration of $^{51}$Cr, $^{54}$Mn and $^{65}$Zn was best described by a single exponential model (SEM) for all concentrations tested ($R^2>0.56$; Fig. 2, Table 2). For the three other radiotracers ($^{57}$Co, $^{63}$Ni and $^{109}$Cd), depuration kinetics were best described by a two-component exponential model (TEM). Retention capacity of the alga was metal-dependent and can be ranked as follows: Cd > Cr = Zn = Mn = Co > Ni.

LMM analysis indicated that for all metals no significant difference could be observed among depuration kinetics determined over the concentration ranges examined ($p_{SPSS} >0.05$). Simple linear regressions between kinetic parameters ($A_0$, $k_e$, $T_{b1/2}$ for SEM and $A_{0u}$, $k_{el}$, $T_{b1/2l}$ for TEM) and exposure concentrations indicated that none of these parameters varied significantly with ambient metal levels over the entire concentration range tested ($p_{loge} >0.05$).

The long-lived component of $^{51}$Cr, $^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{109}$Cd depuration kinetics represented the loss of the major proportion of the radioactivity (92 to 100%, 95 to 99%, 70 to 77%, 93 to 97% and 85 to 96%, respectively) and $^{109}$Cd kinetics were characterized by a long $T_{b1/2l}$ ranging from 85 d to infinity ($k_{el}$ not significantly different from 0). $^{63}$Ni was the only metal for which the major fraction of incorporated activity ($A_{0u}$ $31$–$69\%$) was rapidly lost ($T_{b1/2l}$ from 0.03 to 0.62 d; data not shown). In addition, $T_{b1/2l}$ of the long lived component of $^{63}$Ni depuration were short (7–8 d) compared to the $T_{b1/2l}$ of all other metals studied.

4. Discussion

Among laboratory and field studies on accumulation capacities of Phaeophyceae representatives (e.g., Bryan and Hummerstone, 1973; Foster, 1975; Burdon-Jones et al., 1982) as well as their use in metal contamination biomonitoring programmes (e.g., Phillips, 1990; Amado Filho et al., 1999; Paez-Osuna et al., 2000), few studies have actually investigated the relationships between metal concentrations in brown algae and those in their environment. To the best of our knowledge, only Bryan (1969) has reported data on this crucial information in brown algae; he observed that the CF of Zn in Laminaria digitata decreased with increasing surrounding concentrations.

The present study highlights the enhanced capacity of Lobophora variegata to concentrate dissolved metals and, consequently, to
permit detection of low metal levels in seawater. Indeed, the alga accumulated Ni and Zn up to 120 and 7,800 times higher, respectively, than the concentrations present in dissolved phase.

The results have also shown that, over the range of concentrations encountered in the New Caledonia lagoon, the relative efficiency of metal uptake and retention was not affected by the dissolved concentrations of Cr, Co, Ni and Zn. Indeed, uptake and depuration rate constants and Cf for these metals were constant over the concentration range examined. In term of metal bioaccumulation, the independency of these relative parameters towards exposure concentrations indicates that the increase in Cr, Co, Ni and Zn concentrations in algal tissues is directly proportional to the concentrations occurring in the surrounding seawater. In contrast, uptake kinetics of Cd and Mn in *L. variegata* were affected by the element concentration in seawater. However, in the case of Cd, this effect was not obvious when the kinetic parameters were tested individually. On the other hand, although the Cf of Mn in alga showed no significant difference over the range of tested concentrations, the uptake rate constant (k_u) was affected by the highest Mn exposure concentration (1,250 ng added Mn L\(^{-1}\)). However, this reduction would mainly affect the initial phase of the uptake behaviour in the alga, since the estimated CF\(\text{Mn}\) at 1,250 ng added Mn L\(^{-1}\) was found to be not significantly different from those assessed at the lower concentrations.

These observations indicate that the alga could be used as a bio-indicator for Mn contamination over a range of concentrations restricted from 0 to 250 ng added Mn L\(^{-1}\) since k_u was affected by added Mn concentrations greater than 250 ng L\(^{-1}\). However, as CF\(\text{Mn}\) has been shown not to be affected by the metal exposure concentrations, if algal remain in the field for more than 1 month (time needed to reach the CF\(\text{Mn}\)) and no major variation in contamination levels occur during that period (which could affect k_u), *L. variegata* could also be used to identify areas where higher Mn concentrations occur (>250 ng L\(^{-1}\)). Nevertheless, one has to keep in mind that the limitations stated here do concern only very particular areas (viz. the coastal areas in the immediate vicinity of the biggest mining facilities; Fernandez et al., 2002). Indeed, a dissolved concentration of 250 ng Mn L\(^{-1}\) is a very high concentration that is not commonly found in coastal waters.

With the exception of Ni, all elements were efficiently retained by the alga (T\(1/2\)) higher than two weeks), indicating that it would be able to preserve information regarding contamination over a relatively long period of time. Furthermore, retention of the six tested metals in *L. variegata* was similar regardless of the previous concentration to dissolved metals to which the algae were exposed experimentally. A similar observation has been reported for temperate brown algae with Zn (Bryan, 1969).

Several studies have investigated metal accumulation mechanisms (adsorption and absorption) in brown algae species (see Phillips, 1990). It was demonstrated that metals in solution bind to the cell walls of the macroalgae through a process approximating ion exchange, and several authors have reported the high affinity of trace elements for polysaccharides such as alginates which are present in the cell walls (e.g., Ragan and Jensen, 1979; Davis et al., 2003). Besides these adsorption-related processes, mechanisms of absorption were demonstrated in Phaeophyceae, viz. metals crossing the cell and binding strongly to intra-cellular macromolecules with high affinity for metals, such as polyphenols, phytocelatins and metallothioneins (e.g., Ragan et al., 1979; Phillips, 1994; Morris et al., 1999; Cobbett and Goldbrough, 2002).

From our results, it is not obvious to invoke the involvement of one or several of these mechanisms in metal accumulation in *L. variegata*. Nevertheless, Ni has a contrasting behaviour in the alga compared to the other metals investigated. Indeed, Ni CFs were substantially lower (including at steady state) than those of all other metals tested. Moreover, depuration phase revealed much lower retention of Ni by *L. variegata* compared to the other metals. All these characteristics strongly suggest that *L. variegata* has a low affinity for Ni, and that Ni forms weak binding with algal intra- and/or extra-cellular components. Hence, the mechanisms governing Ni bioconcentration in the alga are most probably related to adsorption processes.

In contrast to Ni, all other metals showed high bioconcentration and efficient retention in the alga. It is thus suggested that bioconcentration of these elements results from a combination of adsorption and absorption processes. Brown algae are known to contain sometimes high levels of polyphenolic substances (≥2% dry wt; Targett et al., 1992). Among this group, *L. variegata* has been shown to display very high concentrations in polyphenols in all three morphological forms of the alga (8.3–13.4% on a dry wt basis; Targett et al., 1992). Hence, due to the metal binding properties of these compounds (Ragan et al., 1979; Cobbett and Goldbrough, 2002) and their particular abundance in *L. variegata*, it is most likely that polyphenols would play a central role in metal sequestration in this alga.
It is important to stress that the present study was carried out in the laboratory. Thus, although care was taken to simulate some key parameters realistically as in field conditions for (e.g., temperature and light), others were not (e.g., suspended particle load and co-occurrence of several metals in different concentrations as well as variability and combination of these parameters). This is especially relevant, since several physico-chemical and biological factors can influence metal bioavailability in the field and thereby alter the way in which ambient metal concentrations are reflected by those measured in the organisms (e.g., Phillips, 1990; Temara et al., 1997; Warnau et al., 1998; Hédouin et al., in press). In addition, in the case of L. variegata, the occurrence of different morpho-types could also affect bioaccumulation to some extent. Although the three morphological types show similar contents in polyphenols (Targett et al., 1992), which are likely to drive metal bioaccumulation in this species, other factors such as differences in algal wall structure (de Ruyter van Steveninck et al., 1988) or in surface:volume ratios could affect metal adsorption and/or absorption processes. This in turn could possibly imply that the information obtained through the alga on its environment could be dependent on the habitat where the collection was carried.

Prior to extrapolating our results to field situations, it is therefore necessary to conduct further field studies to confirm the bioaccumulation behaviour observed in the laboratory.

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