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The Tropical Brown Alga *Lobophora variegata* (Lamouroux) Womersley: A Prospective Bioindicator for Ag Contamination in Tropical Coastal Waters

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Abstract Uptake and depuration kinetics of dissolved silver were determined in the brown alga *Lobophora variegata*, using radiotracer techniques. Results indicate that this widely distributed alga could be a useful bioindicator species for surveying silver contamination in tropical environments. Indeed, results showed that the alga readily concentrates silver (algal concentration of silver was 7,000 times higher than in water after a 28-day exposure) and retains it efficiently within its tissues (biological half-life: 72 ± 4 days).

Keywords Silver · Bioconcentration · Coral reef ecosystems · Biomonitoring

One of the unique characteristics of silver relates to the diversity of its applications (Eisler 1996; Silver Institute 2003). On a global scale, 27,000 tons of silver are used every year (Silver Institute 2003), of which a minimum of 150 tons year^{-1} enters the aquatic environment from, for example, mine tailings, electroplating, or wastewater treatment plants (Silver Institute 2003).

Due to the Ag enrichment in sewage sludge from coastal cities, this metal is considered to be a good proxy for anthropogenic inputs in coastal waters (e.g., Sañudo-Wilhelmy and Flegal 1992). In addition, it has been shown both in the field and in the laboratory that several marine organisms can bioconcentrate Ag up to very high levels (see Ratte 1999; Metian et al. 2008). Hence, the use of Ag as a proxi for bioindicator species has been proposed as an efficient tool to survey and monitor urban contamination in the temperate coastal zones (Sañudo-Wilhelmy and Flegal 1992; Langston and Burt 1994). According to Rainbow and Phillips (1993), extending this concept to the tropical zones, using local bioindicators, would be most useful. However, although Ag^{+} is one of the most toxic ions to marine biota (e.g. Ratte 1999; Warnau et al. 1996b), studies on Ag dynamics in marine biota are relatively scarce and this holds particularly true for tropical environments (see e.g. Gorsuch et al. 2003).

The present work investigates Ag uptake and depuration biokinetics in the tropical brown alga *Lobophora variegata*, using radiotracer techniques. This species is widely distributed in the tropical zone (Coen and Tanner 1989) and is known to display quite elevated metal concentrations in the field (Hédouin et al. 2008); it is therefore a promising bioindicator species.

Materials and Methods

Specimens of *L. variegata* were collected by SCUBA diving in Maa Bay, SW lagoon of New Caledonia in October 2003. Algae were then shipped to IAEA-MEL, Principality of Monaco and acclimated to laboratory conditions (constantly aerated open circuit aquarium, 20% seawater renewal h^{-1}; salinity 37 p.s.u.; temperature 25.0 ± 0.5°C; 12-h photoperiod with a light irradiance of 300 µmol photons m^{-2} s^{-1}).

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Twelve *L. variegata* thallia (average wet weight = 1.7 ± 0.7 g) were placed in an closed-circuit aquarium containing 70 L of natural seawater (salinity, temperature and light conditions as described above). In order to allow for individual recognition, each alga was placed in a cylindrical 200-mL PST container covered above and below with a 300-µm mesh net. Algae were then exposed for 28 days in seawater spiked with 0.5 kBq L^{-1} of ^{110m}Ag (as $^{110m}AgNO_3$; CERCA, France). Due to the specific activity of the radiotracer, this spike corresponds to an addition of 21 ng Ag L^{-1}, viz. a concentration that is actually found in contaminated environments (Smith and Flegal 1993). The seawater was changed and the radiotracer spike was renewed daily to maintain ^{110m}Ag activity constant. Radioactivity in the water was measured before and after each seawater renewal in order to determine the time-integrated radiotracer activity (Rodriguez y Baena et al. 2006). *L. variegata* thallia were regularly γ-counted to determine the ^{110m}Ag uptake kinetics. Radioactivity was measured using a high-resolution γ-spectrometry system consisting of four coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). Activities of the samples were determined by comparison with standards of known activities and appropriate geometry and were corrected for background and physical decay. Counting times were adapted to obtain counting rates with relative propagated errors <5%.

Following the exposure period, all algae were tranferred to new mesh-sided PST containers and placed in clean flowing seawater (open circuit; flux: 50 L h^{-1}; constantly aerated; salinity, temperature and light conditions as described above) for 62 days.These were then radioanalysed at different times (as described above) to determine ^{110m}Ag depuration biokinetics.

Uptake of ^{110m}Ag was expressed in terms of concentration factors (CF; *viz.* ratio between ^{110m}Ag activity in alga—Bq g^{-1} wet wt—and time-integrated activity in seawater—Bq g^{-1}) over time (Warnau et al. 1996a). Uptake kinetics were best fitted using the following saturation exponential equation:

$$CF = CF_{ss}\left(1 - e^{-k_e t}\right)$$

where CF and CF_{ss} are concentration factors at time t (d) and at steady state, respectively, and k_e is the depuration rate constant (d^{-1}).

Depuration of ^{110m}Ag was expressed as decrease in percentage of remaining activity (*viz.*, radioactivity at time t divided by the initial radioactivity measured in the alga at the beginning of the depuration period * 100) over time (Warnau et al. 1996a). Depuration kinetics were best fitted using the following exponential equation:

$$A_t = A_0 e^{-k_e t}$$

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively, and k_e is the depuration rate constant (d^{-1}). The biological half-life ($T_{b½}$) of ^{110m}Ag in *L. variegata* was calculated according to the relation $T_{b½} = \ln 2/k_e$ (Warnau et al. 1996a).

Constants of the models and their statistics were estimated by iterative adjustments of the model and Hessian matrix computation using the non-linear curve-fitting routines in the Statistica® 5.1 software. The level of significance for statistical analyses was always set at $\alpha = 0.05$.

Results and Discussion

Lobophora variegata concentrated readily ^{110m}Ag, reaching a mean concentration factor, CF, higher than 7,000 after 28 days of exposure. Uptake biokinetics were best described by a saturation model (Fig. 1), which was characterised by an estimated steady-state CF, CF_{ss}, of 12,900 ± 2,350 ($p < 0.0001$).

Subsequent depuration of incorporated ^{110m}Ag was followed over a 62-day period. Depuration kinetics were best fitted using a single exponential equation (Fig. 2), characterised by a relatively slow depuration rate constant ($k_e = 0.0097 \pm 0.0005$; $p < 0.0001$), indicating that

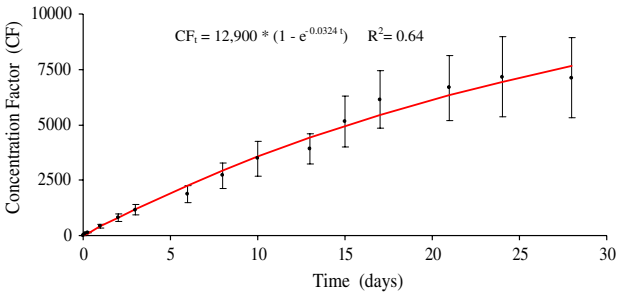

Fig. 1 Uptake kinetics of ^{110m}Ag in *Lobophora variegata* (mean concentration factor ± SD, n = 12)

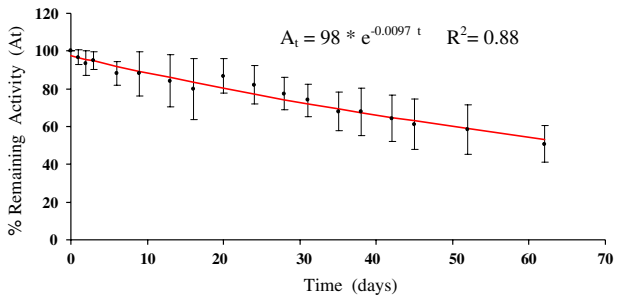

Fig. 2 Depuration kinetics of ^{110m}Ag in *Lobophora variegata* (% Remaining Activity ± SD, n = 12)

Table 1 Experimental ^{110m}Ag concentration factors (CF) in different organisms

Organisms	CF	References
Lobophora variegata (brown alga)	1.29 10^{4a}	Present study
Fucus vesiculosus (brown alga)	10^{3b}	Boisson et al. 1997
Caulerpa taxifolia (green alga)	2.5 10^{2c}	Warnau et al. 1996a
Posidonia oceanica (seagrass)	1.4 10^{2a}	Warnau et al. 1996a

[a] CF at steady state

[b] CF calculated after 12 days of exposure

[c] CF calculated after 15 days of exposure

^{110m}Ag was efficiently retained within the alga tissues, with a biological half-life of 72 ± 4 days.

Information on Ag bioaccumulation kinetics in macroflora representatives is scarce in the scientific literature, whether in the field or in the laboratory (Langston and Burt 1994; Warnau et al. 1996a; Boisson et al. 1997; Ratte 1999). Nevertheless, comparison with the data available from other marine macroalgae and macrophytes clearly shows the intensity of Ag bioaccumulation in *L. variegata* (Table 1).

The high CFs observed in *L. variegata* could result from (1) biosorption processes, where the metal in solution binds to the cell walls of the macroalgae (Schiewer and Wong 2000) and/or (2) bioconcentration and sequestration processes, where the metal enters within the cell and strongly binds to macromolecules such as polyphenols, phytochelatins and metallothioneins (Morris et al. 1999; Cobbett 2000).

According to the literature related to Ag marine geochemistry and algal bioaccumulation, the high Ag CF observed in *L. variegata* is likely driven by both adsorption and cell concentration processes. It is indeed documented that charged or polar Ag compounds can be adsorbed onto algae cell surfaces, and that lipophilic, non-polar Ag chloride complexes are directly incorporated into the cells (see e.g. Ratte 1999). The high CF observed in *L. variegata* suggests that bioconcentration should be particularly intense compared to adsorption in this species. This is also supported by the fact that *L. variegata* has a high content of polyphenolic compounds (up to 13.4% on a dry weight basis; Targett et al. 1992), for which Ag has a high affinity. This suggests that metal bioaccumulation and chelation with phlorotannins (viz. the brown algae polyphenols) could play a major role in Ag bioaccumulation in *L. Variegata*, as well as in the strong retention capacity shown by the alga for Ag.

Overall, our results indicate that *L. variegata* could be considered as a relevant bioindicator species for Ag contamination in tropical waters, as it shows a rapid response in metal uptake and strong retetention capacity. Therefore it has the potential to provide valuable information on the contamination levels occurring in its environment. Further research is needed to verify whether phlorotannins in *L. variegata* could also be proposed as a sensitive biomarker of Ag contamination in seawater.

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