Journal of the Marine Biological Association of the United Kingdom, 2014, 94(3), 435–442.
doi:10.1017/S0025315413001653

Metal mobility and bioaccumulation differences at lower trophic levels in marine ecosystems dominated by *Sargassum* species

PABLO HERNÁNDEZ-ALMARAZ[1], LÍA MÉNDEZ-RODRÍGUEZ[1], TANIA ZENTENO-SAVÍN[1], FEDERICO GARCÍA-DOMÍNGUEZ[2], ALFONSO VÁZQUEZ-BOTELLO[3] AND ELISA SERVIERE-ZARAGOZA[1]

[1]Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Instituto Politécnico Nacional 195, Col. Playa Palo de Santa Rita Sur, La Paz, B.C.S. 23096, Mexico, [2]Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional s/n, Col. Playa Palo de Santa Rita, La Paz, B.C.S. 23096, Mexico, [3]Instituto de Ciencias del Mar y Limnología-UNAM (ICMyL-UNAM), Circuito Exterior S/N, Ciudad Universitaria, Delegación Coyoacán, Mexico City, D.F. 04510, Mexico

Concentrations of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and iron (Fe) were measured in three macroalgal species, Codium simulans, Sargassum sinicola *and* Gracilaria pachydermatica*, and in the sea slug* Elysia diomedea*, living in marine ecosystems dominated by* Sargassum *species and located near exploited phosphorite deposits. Metal concentrations in macroalgae and sea slugs were significantly different among sites. The highest concentrations of Cd recorded in* C. simulans *and* S. sinicola*, as well as in* E. diomedea *were recorded at the site closest to the phosphorite deposit. In general, Cd and Zn concentrations in* E. diomedea *were higher than those recorded in specimens of* C. simulans, S. sinicola *and* G. pachydermatica. *In contrast, Pb, Cu, and Fe concentrations in* E. diomedea *were lower than or similar to those in macroalgae from all sampled sites. The information generated contributes to the knowledge about potential mobility and metal bioaccumulation at lower trophic levels in marine eco systems.*

Keywords: bioaccumulation, metals, *Sargassum* beds, Gulf of California, *Elysia diomedea*, macroalgae

Submitted 24 August 2013; accepted 24 October 2013; first published online 16 January 2014

INTRODUCTION

Marine macroalgae, as primary producers in coastal environments that accumulate metals, can be a source of metals for herbivores. Macroalgal species have different affinities for different metals related to their structural composition as green, brown and red algae, which may reflect competition among metals for binding or uptaking sites in the macroalgae, resulting in different bioaccumulation patterns (Sawidis *et al.*, 2001). Potential bioaccumulation of metals is related mainly to their concentration in the environment and their chemical presentation, which can be influenced by water effluents generated by human activities and environmental factors, such as upwelling.

An important source of metals in aquatic ecosystems is mineral deposits, including phosphorite (Mann & Ritchie, 1995). One of the largest phosphorite deposits in the world is located in the south-western region of the Gulf of California, about 50 km north-west of the city of La Paz, Baja California Sur (Riley & Chester, 1971). In phosphate rocks, common impurities are cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) (Voulgaropoulos *et al.*, 1991; Sabiha-Javied *et al.*, 2009). Although, phosphate rocks are not a common source of iron (Fe), they are important as a measure of re-suspension of sediments from the continental shelf (Elrod *et al.*, 2004). Among other factors, Cu, Zn and Fe can alter Cd and Pb bioavailability (Lönnerdal, 2000). All of these metals accumulate in marine biota in nearby coastal areas (Ray, 1984).

Sargassum species are the most conspicuous component of marine flora, forming extensive beds covering from a few square metres to several hectares throughout the Gulf of California (Hernández-Carmona *et al.*, 1990; Casas-Valdéz *et al.*, 1993), where numerous species of molluscs, crustaceans and fish inhabit this ecosystem (Foster *et al.*, 2007). The sea slug *Elysia diomedea* (Bergh, 1894) (Gastropoda, Sacoglossa) is one of the common invertebrate species in *Sargassum* beds in the Gulf of California (Bertsch, 2008), including Bahía de la Paz (Pacheco-Ruíz *et al.*, 2008). Elysiid sea slugs are suctorial feeders with distinct uniseriate radula. They are specialized herbivores, feeding on marine plants; the majority of species feed on siphonaceous green algae, such as the genera *Caulerpa*, *Codium* or *Halimeda*, among others (Bradley, 1984; Jensen, 1997; Gavagnin *et al.*, 2000; Curtis *et al.*, 2005; Pierce *et al.*, 2006; Trowbridge *et al.*, 2010, 2011). *Elysia diomedea* feeds on marine algae (Bertsch & Smith, 1973; Trowbridge, 2002; Hermosillo *et al.*, 2006; Bertsch, 2008), perhaps *Caulerpa* or *Codium* (Trench *et al.*, 1969, 1972).

The aim of our study was to compare the concentrations of Cd, Pb, Cu, Zn and Fe between primary producers and a herbivore, the sea slug *E. diomedea*, in marine ecosystems dominated by *Sargassum* species and located close to one of the largest exploited phosphorite deposits. The information

Corresponding author:
E. Serviere-Zaragoza
Email: serviere04@cibnor.mx

generated contributes to the knowledge about potential mobility and metal bioaccumulation at lower trophic levels in marine ecosystems.

MATERIALS AND METHODS

Field surveys were conducted at three sites in the south-western region of the Gulf of California: Las Animas (24°31′43.8″N 110°44′1.50″W), San Juan de la Costa (24°21′58.2″N 110°40′50.7″W) and El Sauzoso (24°18′38.6″N 110°38′28.8″W; Figure 1), which are characterized by rocky reefs where beds of *Sargassum* spp. predominate. At San Juan de la Costa there is a phosphorite deposit that has been exploited for nearly 30 years (Servicio Geológico Mexicano, 2008). Las Animas is 14.1 km to the north and El Sauzoso is 10.3 km to the south of San Juan de la Costa. There are no indications of human influences at either Las Animas or El Sauzoso (Méndez *et al.*, 2006).

At each site, 30 healthy fronds of three macroalgal species *Codium simulans* Setchell & Gardner (Chlorophyta), *Sargassum sinicola* Setchell & Gardner (Ochrophyta: Phaeophyceae) and *Gracilaria pachydermatica* Setchell & Gardner (Rhodophyta), and 18 adults of the sea slug *Elysia diomedea* (20.3 ± 7.1 mm) were randomly collected along *Sargassum* beds at a depth of 0.5–2 m in March 2011. Of the three macroalgae, *C. simulans* was considered the main food item of the sea slug (Trench *et al.*, 1969, 1972). The other two represent up to 70% of macroalgal biomass in coastal communities dominated by *Sargassum* spp. in the region. The taxonomic identification was performed with keys for macroalgae (Setchell & Gardner, 1924; Joly, 1967; Abbott & Hollenberg, 1976; Guiry & Guiry, 2013) and gastropods (Abbott, 1974; Hermosillo *et al.*, 2006).

Macroalgae were cleaned by hand to remove epiphytes. Concentrations of Cd, Pb, Cu, Zn and Fe were measured on three pooled samples (six specimens) of each species of macroalgae and of the sea slug at each site. The samples of macroalgae and sea slugs were dried in an oven at 75°C for 24–48 h and then ground in a grinder/mixer fitted with a steel vial and ball pestle to make a homogeneous mixture. Approximately, 0.5 g dry weight of each sample of macroalgae and *E. diomedea* were subjected to acid digestion with a 3:1 ratio of nitric acid and hydrogen peroxide (analytical grade; Mallinckrodt J.T. Baker, USA) in a microwave oven (Mars 5X, CEM; Matthews, USA). Levels of Cd, Cu, Fe, Pb and Zn in the digested samples were quantified by atomic absorption spectrophotometry (Avanta, GBC Scientific Equipment, Australia) with an air–acetylene flame (Matusiewicz, 2003). Reference standards TORT-2, DORM-2, and ALGAE (National Research Council Canada, Institute for Marine Biosciences, Certified Reference Materials Programme, Halifax, NS, Canada) were used to validate the accuracy of the analytical method. Recovery percentages were above 95%. Detection limits (in $\mu g\ g^{-1}$) for Cd, Pb, Cu, Zn and Fe were 0.017, 0.07, 0.02, 0.02 and 0.07, respectively.

Element concentration datasets were not normally distributed (Shapiro–Wilk test). To assess differences among metal concentrations among sites, nonparametric tests (Kruskal–

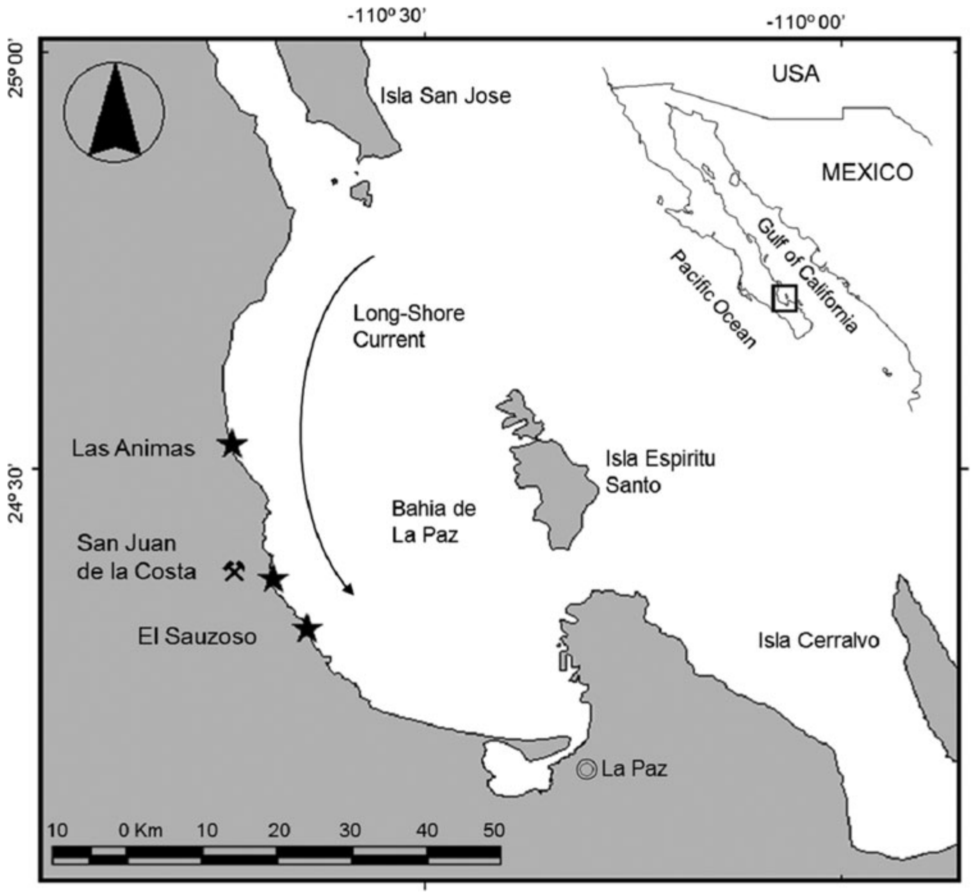

Fig. 1. Collection sites in the south-western region of the Gulf of California at Baja California Sur, Mexico. ★, collection sites; ⚒, mine at San Juan de la Costa.

Wallis test followed by Mann–Whitney *U*-test) were performed. Separately for each site, the species was considered as an independent variable and the element concentration as the dependent variable. Spearman rank correlation analyses between elements for each species and among species were also conducted (Zar, 2010). Analyses were carried out with the STATISTICA 8 software (Statsoft, 2007). For elements that were present in concentrations below the detection limit, the half value of the respective detection limit was used for statistical analyses (Farnham *et al.*, 2002). All results with a $P < 0.05$ were considered significant.

RESULTS

Macroalgae

Concentrations of Cd, Pb, Cu, Zn and Fe in the macroalgae were significantly different at the three sites (Table 1). Average Cd concentrations in *Codium simulans* and *Sargassum sinicola* were also significantly different among sites. For *C. simulans*, the average Cd concentrations in San Juan de la Costa and El Sauzoso were higher than the average in Las Animas (0.7 ± 0.3 μg g^{-1}). For *S. sinicola*, specimens from San Juan de la Costa had an average Cd concentration (9.6 ± 0.8 μg g^{-1}), 1.8 times higher than that from Las Animas and 1.4 times than that from El Sauzoso. For the red seaweed *Gracilaria pachydermatica*, no significant differences were found among collections sites, with mean values ranging from 5.1 to 6.0 μg g^{-1}. Cadmium concentrations in *G. pachydermatica* were significantly correlated ($r_s = 0.72$) with Fe concentration.

In *C. simulans*, detectable concentrations of Pb were found in specimens from Las Animas and San Juan de la Costa but not in specimens from El Sauzoso; average Pb concentration in Las Animas was 13 times higher than the average in San Juan de la Costa (0.3 ± 0.5 μg g^{-1}). Lead in the brown alga *S. sinicola* was only detected in specimens from San Juan de la Costa (2.8 ± 1.9 μg g^{-1}); it was below the detection limit in *G. pachydermatica* specimens from San Juan de la Costa, while levels in Las Animas (2.4 ± 0.7 μg g^{-1}) and El Sauzoso (2.2 ± 1.3 μg g^{-1}) were not significantly different. The Pb levels in *S. sinicola* were correlated with Cd ($r_s = 0.82$) and Zn ($r_s = 0.76$) concentrations; furthermore, Zn levels were correlated with Cu concentrations ($r_s = 0.72$).

Copper levels in the macroalgae *C. simulans* and *S. sinicola* showed significant differences among sites. The highest concentration of Cu in *C. simulans* specimens from Las Animas (3.2 ± 0.8 μg g^{-1}) was 1.9 times higher than the levels recorded in specimens from the other locations. For *S. sinicola*, mean Cu concentrations recorded in Las Animas and San Juan de la Costa were 2–3 times higher than those recorded in specimens from El Sauzoso (1.5 ± 0.4). Concentrations of Cu were significantly correlated ($r_s = 0.72$) with Zn concentration in *S. sinicola*. For the red seaweed *G. pachydermatica* no significant differences were found, with mean values ranging from 1.6 to 5.1 μg g^{-1}.

Zinc concentrations in the three macroalgal species were significantly different among sites; the highest levels were found in San Juan de la Costa. Zinc concentrations in *C. simulans* were correlated with concentrations in *S. sinicola* ($r_s = 0.83$). The average concentration in *G. pachydermatica* (18.7 ± 1.7 μg g^{-1}) was 1.7 times higher than the levels found in specimens from Las Animas and two times those at El Sauzoso.

Iron concentration in macroalgae was significantly different among sites only in *G. pachydermatica*. Levels recorded in specimens from San Juan de la Costa (197.0 ± 58.9 μg g^{-1}) were three times higher than those recorded in Las Animas and two times those at El Sauzoso.

Cadmium concentrations in the three macroalgae showed the same pattern (*C. simulans* < *G. pachydermatica* < *S. sinicola*) in all the three sites, as they were for Zn concentrations (*G. pachydermatica* < *C. simulans* ≈ *S. sinicola*) and Fe levels (*G. pachydermatica* ≈ *S. sinicola* < *C. simulans*).

Sea slug

Concentrations of Cd, Pb, Cu, Zn and Fe in the sea slug were significantly different between the three sites (Table 1). Detectable concentrations of Cd were found in organisms from the three sites with those from San Juan de la Costa

Table 1. Concentration of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and iron (Fe) (μg g^{-1} dry weight) in three macroalgal species and a sea slug collected at three coastal sites in the south-western region of the Gulf of California. Average value ±standard deviation; superscript letters denote significant differences by Kruskal–Wallis test followed by Mann–Whitney *U*-test ($P < 0.05$) among sites in concentrations of the elements in a species.

Group	Species	Site	Cd	Pb	Cu	Zn	Fe
Macroalgae							
Chlorophyta	*Codium simulans*	LA	0.7 ± 0.3^{a}	3.9 ± 2.4^{a}	3.2 ± 0.8^{a}	16.0 ± 1.8^{a}	333.0 ± 116.6
		SJ	2.4 ± 1.4^{b}	0.3 ± 0.5^{b}	1.7 ± 1.2^{b}	20.8 ± 2.7^{b}	372.3 ± 98.8
		ES	1.7 ± 0.4^{b}	<0.07^{b}	1.7 ± 0.4^{b}	16.6 ± 1.1^{a}	565.1 ± 297.3
Ochrophyta	*Sargassum sinicola*	LA	5.2 ± 1.0^{a}	<0.07^{a}	3.3 ± 0.8^{a}	17.1 ± 2.5^{a}	116.2 ± 13.9
		SJ	9.6 ± 0.8^{b}	2.8 ± 1.9^{b}	4.5 ± 1.5^{a}	23.0 ± 2.8^{b}	134.9 ± 26.4
		ES	6.7 ± 0.5^{c}	<0.07^{a}	1.5 ± 0.4^{b}	15.3 ± 0.4^{a}	123.4 ± 5.4
Rhodophyta	*Gracilaria pachydermatica*	LA	5.1 ± 0.4	2.4 ± 0.7^{a}	5.1 ± 2.0	11.1 ± 1.2^{a}	66.4 ± 7.2^{a}
		SJ	6.0 ± 0.8	<0.07^{b}	3.8 ± 0.8	18.7 ± 1.7^{b}	197.0 ± 58.9^{b}
		ES	5.4 ± 0.5	2.2 ± 1.2^{a}	1.6 ± 1.8	9.2 ± 2.4^{a}	98.1 ± 7.5^{c}
Gastropod	*Elysia diomedea*						
		LA	4.6 ± 0.4^{a}	<0.07^{a}	<0.02^{a}	24.7 ± 1.0^{a}	36.4 ± 1.4^{a}
		SJ	17.7 ± 4.2^{b}	<0.07^{a}	1.6 ± 1.2^{b}	29.7 ± 4.5^{ab}	47.6 ± 15.8^{a}
		ES	5.7 ± 0.8^{a}	3.7 ± 6.3^{b}	0.6 ± 1.0^{ab}	30.1 ± 4.4^{b}	117.5 ± 82.6^{b}

LA, Las Animas; SJ, San Juan de la Costa; ES, El Sauzoso.

(17.7 ± 4.2 µg g^{-1}) showing significantly higher values than those from Las Animas and El Sauzoso. Detectable levels of Pb were found only in El Sauzoso (3.7 ± 6.3 µg g^{-1}). No detectable levels of Cu were found in Las Animas. In addition, Cu concentrations were positively correlated ($r_s = 0.73$) with Cd values. Zinc concentrations showed significant differences between Las Animas and El Sauzoso but not with San Juan de la Costa (29.7 ± 4.5 µg g^{-1}). The average Fe concentration recorded in El Sauzoso (117.5 ± 82.6 µg g^{-1}) was 3.2 and 2.5 times higher than the average concentration recorded in Las Animas and San Juan de la Costa, respectively. Overall, concentrations of the five elements showed an ascending pattern: Pb $\approx$ Cu $<$ Cd $<$ Zn $<$ Fe across the three sites.

Sea slug vs macroalgae

The average Cd concentration in *Elysia diomedea* ranged from 3.4 to 7.4 times higher than the levels found in *C. simulans* from the three sampled sites, and it was from 1.8 to 3.0 times higher than the levels recorded in *S. sinicola* and *G. pachydermatica*, respectively, from San Juan de la Costa. The average Cd concentration in *E. diomedea* was lower than or similar to that found in *S. sinicola* and *G. pachydermatica* from Las Animas and El Sauzoso. The average Pb, Cu and Fe concentrations in *E. diomedea* were lower than or similar to those found in the three species of macroalgae, except for Pb in relation to *G. pachydermatica* from El Sauzoso, where the average Pb concentration in the sea slug was 1.7 times higher than the levels found in specimens of *G. pachydermatica*. Zinc concentrations in *E. diomedea* were between 1.3 times higher than those recorded in specimens of *S. sinicola* from San Juan de la Costa and 3.3 times higher than those recorded in specimens of *G. pachydermatica* from El Sauzoso.

DISCUSSION

Macroalgae

Metal concentrations in the three macroalga species (Table 1) were all within the ranges previously reported for these and other macroalgae in the Gulf of California and along the west coast of the Baja California Peninsula, Cd: 0.03–4.60 µg g^{-1}, Pb: 0.1–30 µg g^{-1}, Cu: 0.5–82 µg g^{-1}, Zn: 2–96 µg g^{-1} and Fe: 140–2898 µg g^{-1}) (Huerta-Díaz *et al.,* 2007; Jara-Marini *et al.*, 2009; Rodríguez-Figueroa *et al.*, 2009; Riosmena-Rodríguez *et al.*, 2010; Patrón-Prado *et al.*, 2011).

Cadmium levels in *Codium simulans* were similar to findings in *C. amplivesiculatum* Setchell & Gardner and *C. cuneatum* Setchell & Gardner at sites with no or little influence of human activity but subject to natural influences, such as upwelling (Huerta-Díaz *et al.*, 2007). Moderate upwelling has been reported in Bahía de la Paz (Jiménez-Illescas *et al.*, 1997). In *Sargassum sinicola*, Cd concentrations are similar to those reported in previous studies, 11 µg g^{-1}, at the same area of the Bahía de La Paz (Patrón-Prado *et al.*, 2011). However, higher mean levels of Cd were found in *Gracilaria pachydermatica* in relation to the range previously reported in this region for the red macroalgae *G. crispata* Setchell & Gardner, *G. textorii* Setchell & Gardner, and *G. vermiculophylla* (Ohmi) Papenfuss (0.6–4.6 µg g^{-1}) at sites with rare human activity but subject to upwelling (Talavera-Saenz *et al.*, 2007; Riosmena-Rodríguez *et al.*, 2010).

Levels of Pb in *C. simulans* (3.9 µg g^{-1}), *S. sinicola* (2.8 µg g^{-1}), and *G. pachydermatica* (2.4 µg g^{-1}) are lower than the concentration range found in sites with low anthropogenic influence compared with levels in algae from industrial areas, in which concentrations are reported to be 29.7 ± 3.3 µg g^{-1} in the green algae *Caulerpa serrulata* (Forsskål) J. Agardh, 25.8 ± 2.9 µg g^{-1} in the brown algae *Sargassum dentifolium* (Turner) C. Agardh, and 19.8 ± 1.8 µg g^{-1} in the red algae *Hypnea comuta* (Lamoroux) C. Agardh (Abdallah *et al.*, 2005). This element has been considered to have little potential to be bioaccumulated or biomagnified in lower trophic levels (Dietz *et al.*, 2000; Ruelas-Inzunza & Páez-Osuna, 2008), which is consistent with our results.

Copper, Zn and Fe levels in the species studied were lower than the concentrations recorded in the brown macroalgae *Padina durvillaei* Bory Saint-Vincent from sites with copper mining and smelting activities (Cu: 53 ± 38 µg g^{-1}, Zn: 63 ± 43 µg g^{-1} and Fe: 2243 ± 2325 µg g^{-1}) (Rodríguez-Figueroa *et al.*, 2009). However, Zn and Fe levels in *C. simulans* and *S. sinicola* tend to be higher than concentrations in species of the same genus from undisturbed sites with influence of upwelling, for example in *S. sinicola* (Zn: 9.3 µg g^{-1} and Fe: 186 µg g^{-1}) (Huerta-Díaz *et al.*, 2007) and *C. cuneatum* (Zn: 7.3 µg g^{-1} and Fe: 284 µg g^{-1}) (Riosmena-Rodríguez *et al.*, 2010).

Sea slug

The sea slug *Elysia diomedea* contained Cd concentrations similar to concentrations reported for the chocolate clam *Megapitaria squalida* (Sowerby, 1835) collected at the same study sites (Méndez-Rodríguez *et al.*, 2006). Low Pb levels ($<$0.07 µg g^{-1}) were found in the sea slug, but concentrations of 0.3 ± 0.2 µg g^{-1} were reported in the chocolate clam *M. squalida* at Las Animas, 4.8 ± 0.5 µg g^{-1} at San Juan de la Costa, and 7.8 ± 1.9 µg g^{-1} at El Sauzoso (Méndez-Rodríguez *et al.*, 2006). Such differences suggest that the ability of the sea slug (*E. diomedea*) to accumulate Cd from macroalgae is similar to that of the chocolate clam, but its ability to accumulate Pb is lower than that of molluscs in this region.

In our study, the levels of Cu, Zn and Fe found in *E. diomedea* ($<$0.02–1.6 µg g^{-1}, 24.7–30.1 µg g^{-1} and 36.4–70.0 µg g^{-1}, respectively) were lower than the amounts detected by Méndez *et al.* (2006) in the chocolate clam *M. squalida* at the same sites (Cu: 5–8 µg g^{-1}, Zn: 55–63 µg g^{-1} and Fe: 276–385 µg g^{-1}). The differences between *E. diomedea* and this filter-feeder bivalve seem to reflect differences not only in feeding habits between the two species, but also differential regulation of essential metals, depending on the specific metabolic functions they perform in each species. The lower Cu levels in *E. diomedea* (maximum 1.6 ± 1.2 µg g^{-1}), compared to those reported for other molluscs, such as the sea snail *Bembicium auratum* (Quoy & Gaimard, 1834) (88 ± 15 µg g^{-1}), as well as for Fe (70.0 ± 10.3 µg g^{-1}) (Barwick & Maher, 2003), may be associated with differences in respiratory pigments. *Elysia diomedea* seems to have haemoglobin (a Fe-based respiratory protein) as does the California sea hare *Aplysia* (Linnaeus, 1758) (Barnes, 1986), rather than hemocyanin (a Cu-based respiratory protein), as is the case for *B. auratum* (Barwick & Maher, 2003).

Organisms from the same site incorporate Cd and Pb based on differences in diet, as would be the case of planktivorous bivalve molluscs and herbivorous gastropods (Barwick & Maher, 2003; Jara-Marini *et al.*, 2009). For example, in a seagrass ecosystem, Barwick & Maher (2003) found the Sydney cockle *Anadara trapezia* (Deshayes, 1840) has lower Cd concentrations (0.030 $\pm$ 0.002 μg g^{-1}) but higher Pb concentrations (1.1 $\pm$ 0.1 μg g^{-1}) than *B. auratum* (0.5 $\pm$ 0.1 μg g^{-1} and 14 $\pm$ 1 μg g^{-1}, respectively). The authors state that these metals are accumulated by the animals from their food—in the bivalve by eating plankton and in the gastropod by eating the green macroalga *Ulva* sp. (referred to as *Enteromorpha* sp.). Those observations are consistent with our findings for *E. diomedea*, indicating that the fraction of metals that is accumulated depends not only on their concentration in food but also on their chemical presentation. Macroalgae contain proteins and various carbohydrates with which different metal ions can react, in turn, modifying their bioavailability. Polysaccharides are the main component in most algae, and vary between species, favouring some elements to be more easily accumulated than others, as well as being more or less bioavailable (Hu *et al.*, 1996; Chan *et al.*, 2003). Most brown algae contain alginates and fucoidin; red algae mainly contain carrageenans and agar, and green algae contain ulvan (Vera *et al.*, 2011). Polysaccharides in brown algae contain carboxyl groups, whereas those in green and red algae mostly contain sulphated groups. The balance between the different functional groups in each algae group will favour metals with similar properties to be absorbed. In our study, *C. simulans* had the highest Fe concentration, which is consistent with the results of Robledo & Freile (1997) for green algae, suggesting this species has more affinity for Fe than other metals.

The highest concentrations of Cd recorded in *E. diomedea*, as well as the highest levels of Cd and Zn in the three macroalgae species, were found at San Juan de la Costa where, unlike Las Animas and El Sauzoso, discharges occur in an environment enriched with phosphorite-associated elements, such as Cd and Zn (Méndez-Rodríguez *et al.*, 2006). Absorption of these metals by macroalgae is increased by the addition of ammonia and nitrate (Lee & Wang, 2001; Evans & Edwards, 2011). Nitrogen enrichment stimulates the synthesis of amino groups in macroalgae, in addition to indirectly increasing the synthesis of carboxyl and carbonyl groups, as photosynthesis is promoted (Lee & Wang, 2001). These functional groups facilitate binding of metals, such as Cd and Zn, promoting their accumulation in macroalgae. In addition, nitrogen enrichment facilitates growth in macroalgae, which increases the demand for essential elements, such as Zn. In the absence of selective Cd transport, its accumulation in different macroalga species will follow the same path as Zn (Chan *et al.*, 2003). The difference being that Zn is used for various metabolic functions, while Cd is not. Mechanisms used for element homeostasis can also be used for detoxification. Bound to various molecules, such as metallothioneins (in animal cells) or phytochelatins (in plant cells), metals such as Cd will be gradually eliminated from the body although the process might take years in some species (Hu *et al.*, 1996). For most organisms, Cd and Pb are not essential elements; Cu, Zn, and Fe are. These three metals are metabolically regulated (Rainbow, 2002). For example, planktivorous bivalves incorporate over twice as much Zn as do herbivores feeding on macroalgae, indicating that organisms from different groups have different mechanisms for accumulating this metal (Barwick & Maher, 2003; Ruelas-Inzunza & Páez-Osuna, 2008).

Fig. 2. Specimen of sea slug *Elysia diomedea* on thallus of *Codium* in Bahía de La Paz, Baja California Sur, Mexico.

Sea slug vs macroalgae

The ratio of metal concentrations between primary producers and their consumers is reported to be 0.7 for Pb, and up to 31 for Cd (Barwick & Maher, 2003; Ruelas-Inzunza & Páez-Osuna, 2008). The concentrations of Cd and Pb found in the three macroalgae species and its potential consumer, *E. diomedea*, in this study follow these patterns. The concentrations of Cd and Pb found in *E. diomedea* could be related to its potential feeding on *C. simulans*, as the genus *Elysia* (Risso, 1818) includes specialist herbivores (Trowbridge, 1991; Jensen, 1997; Gavagnin *et al.*, 2000; Curtis *et al.*, 2005; Pierce *et al.*, 2006; Trowbridge *et al.*, 2010, 2011), which look for specific siphonaceous green alga species to suck their cellular contents after piercing the cell walls (Jensen, 1997, 2009; Hermosillo *et al.*, 2006; Trowbridge *et al.*, 2010; Giménez-Casalduero *et al.*, 2011). In our study, specimens of *E. diomedea* sitting on *C. simulans* thalli were commonly observed during field surveys (Figure 2) and green algae chloroplasts in digestive diverticulae of sea slugs sampled were observed. Some *Elysia* species seek specific algae and sit on them to feed and lay eggs (Hermosillo *et al.*, 2006; Giménez-Casalduero *et al.*, 2011). The presence of chloroplasts from *Codium* sp., *Sargassum* sp., and an unidentified red alga species in the digestive diverticulae of *Elysia* cf. *furvacauda* (Burn, 1958) is evidence of consumption of several macroalgae species and, therefore, a wide variation in the diet of one of these gastropods (Bradley, 1984). These observations suggest that *S. sinicola* could constitute an alternative food source for *E. diomedea*, based on the correlations between Cd concentrations in the two species ($r_s = 0.86$) and its abundance in this environment. On the other hand, the lack of correlation with the concentration of metals in *G. pachydermatica* suggests that this red alga is not part of the gastropod's diet. However, further studies of *E. diomedea* feeding habits at different sites in the region are needed because feeding patterns might vary across its distribution range, as observed in other species of the same genus (Trowbridge, 1991; Jensen, 1997; Giménez-Casalduero *et al.*, 2011).

Like other herbivores that feed on macroalgae (Barwick & Maher, 2003; Ruelas-Inzunza & Páez-Osuna, 2008), concentrations of Zn, Cu and Fe in *E. diomedea* might be from feeding on some of the macroalgae, such as in the

Sargassum beds from El Sauzoso. At this site, the highest concentrations of Zn found in *E. diomedea* (30.1 ± 4.4 µg g^{-1}) compared to those recorded in *C. simulans* (16.6 ± 1.1 µg g^{-1}) and in *S. sinicola* (15.4 ± 0.4 µg g^{-1}), as well as the ratio between the concentrations observed in the slug and the macroalgae (1.8–3.3), are consistent with the proportions observed in other herbivores and their food (Barwick & Maher, 2003; Ruelas-Inzunza & Páez-Osuna, 2008). For example, Barwick & Maher (2003) reported that concentrations of Zn in the green alga *Ulva* sp. (53 µg g^{-1}) were lower than those recorded in its consumer, the gastropod *B. auratum* (87 µg g^{-1}), having a ratio of 1.6 between them. The low ratio for Cu concentrations (maximum values of 0.9) between the three macroalgae and *E. diomedea* in this study contrast with the highest ratio (27) reported between *Ulva* sp. and *B. auratum* (Barwick & Maher, 2003). There are no previous reports for Fe concentrations that could allow a comparison of the proportions found in *E. diomedea* and its potential food sources. Studying the dynamics of metals at different trophic levels is of potential interest because their concentrations can increase from lower to higher trophic levels, including species consumed by humans.

In general, Cd and Zn concentrations in *E. diomedea* were higher than those recorded in specimens of *C. simulans*, *G. pachydermatica*, and *S. sinicola*. In contrast, Pb, Cu, and Fe concentrations in the sea slug were lower than or similar to those in macroalgae from all sampled sites. Studying the accumulation of metals in marine organisms at different trophic levels provides important information about their potential mobility and accumulation. In some cases, incorporated metals may eventually reach high concentrations with potential increases between successive trophic levels and potentially affect economically-important species, sometimes affecting humans directly.

ACKNOWLEDGEMENTS

We thank Alejandra Mazariegos Villarreal, Alejandra Piñón Gimate, Baudilio Acosta Vargas, Griselda Peña Armenta, and Horacio Bervera León. Ira Fogel and Diana Dorantes of CIBNOR provided valuable editorial services.

FINANCIAL SUPPORT

This work was supported by the CIBNOR (PC 0.5), and Consejo Nacional de Ciencia y Tecnología of Mexico (CONACYT CB2012 179327). P.H.A. is a recipient of a doctoral fellowship (CONACYT 48345).

REFERENCES

Abbott I.A. and Hollenberg G.J. (1976) *Marine algae of California*. Stanford, CA: Stanford University Press.

Abbott R.T. (1974) *American seashells*. 2nd edition. New York: Van Nostrand.

Abdallah A.M.A., Abdallah M.A. and Beltagy A.I. (2005) Contents of heavy metals in marine seaweeds from the Egyptian coast of the Red Sea. *Chemistry Ecology* 21, 399–411.

Barnes R.D. (1986) *Invertebrate Zoology*. 5th edition. Philadelphia: Saunders College Publishing.

Barwick M. and Maher W. (2003) Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Marine Environmental Research* 56, 471–502.

Bertsch H. (2008) Opistobranquios. In Danemann G.D. and Ezcurra E. (eds) *Bahía de los Ángeles: recursos naturales y comunidad. Línea base 2007*. Mexico: Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Pronatura Noroeste and San Diego Natural History Museum, pp. 319–338.

Bertsch H. and Smith A.A. (1973) Observations on three opisthobranchs (Mollusca: Gastropoda) of the La Paz area, Baja California, Mexico. *The Southwestern Naturalist* 18, 165–176.

Bradley B.K. (1984) Aspects of the ecology and physiology of *Elysia* cf. *furvacauda* (Mollusca: Sacoglossa). *Bulletin of Marine Science* 34, 207–219.

Casas-Valdéz M., Sánchez I. and Hernández G. (1993) Evaluación de *Sargassum* spp. en la costa oeste de Bahía Concepción, BCS, México. *Investigaciones Marinas CICIMAR* 8, 61–68.

Chan S.M., Wang W.X. and Ni I.H. (2003) The uptake of Cd, Cr, and Zn by the macroalga *Enteromorpha crinita* and subsequent transfer to the marine herbivorous Rabbitfish, *Siganus canaliculatus*. *Archives of Environmental Contamination and Toxicology* 44, 298–306.

Curtis N.E., Massey S.E., Scwartz J.A., Maugel T.K. and Pierce S.K. (2005) The intracellular, functional chloroplasts in adult sea slugs (*Elysia crispata*) come from several algal species, and are also different from those in juvenile slugs. *Microscopy and Microanalysis* 11, 1194–1195.

Dietz F., Riget M., Cleemann M., Aarkrog A., Johansen P. and Hansen J.C. (2000) Comparison of contaminants from different trophic levels and ecosystems. *Science of the Total Environment* 245, 221–231.

Elrod V.A., Berelson W.M., Coale K.H. and Johnson K.S. (2004) The flux of iron from continental shelf sediments: a missing source for global budgets. *Geophysical Research Letters* 31, L12307, 1–4, doi: 10.1029/2004GL020216.

Evans L.K. and Edwards M.S. (2011) Bioaccumulation of copper and zinc by the giant kelp *Macrocystis pyrifera*. *Algae* 26, 265–275.

Farnham I.E., Singh A.K., Stetzenbach K.J. and Johannesson K.H. (2002) Treatment of nondetects in multivariate analysis of groundwater geochemistry data. *Chemometrics and Intelligent Laboratory Systems* 60, 265–281.

Foster M.S., McConnico L.M., Lundsten L., Wadsworth T., Kimball T., Brooks L.B., Medina-López M., Riosmena-Rodríguez R., Hernández-Carmona G., Vásquez-Elizondo R.M., Johnson S. and Steller D.L. (2007) Diversity and natural history of a *Lithothamnion muelleri–Sargassum horridum* community in the Gulf of California. *Ciencias Marinas* 33, 367–384.

Gavagnin M., Mollo E., Montanara D., Ortega J. and Cimino G. (2000) Chemical studies of Caribbean sacoglossans: dietary relationships with green algae and ecological implications. *Journal of Chemical Ecology* 26, 1563–1578.

Giménez-Casalduero F., Muniain C., González-Wangüemert M. and Garrote-Moreno A. (2011) *Elysia timida* (Risso, 1818) three decades of research. *Animal Biodiversity and Conservation* 34, 217–227.

Guiry M.D. and Guiry G.M. (2013) *AlgaeBase*. Galway: National University of Ireland. Available at: http://www.algaebase.org (accessed 5 November 2013).

Hermosillo A., Behrens D.W. and Ríos-Jara E. (2006) *Opistobranquios de México. Guía de babosas marinas del Pacífico, Golfo de California* *y las Islas oceánicas*. Mexico: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Hernández-Carmona G., Casas-Valdéz M., Fajardo-León C., Sánchez-Rodríguez I. and Rodríguez-Montesinos E. (1990) Evaluación de *Sargassum* spp. en la Bahía de La Paz, BCS, México. *Investigaciones Marinas CICIMAR* 5, 11–18.

Hu S., Tang C.H. and Wu M. (1996) Cadmium accumulation by several seaweeds. *Science of the Total Environment* 187, 65–71.

Huerta-Díaz M.A., De Leon-Chavira F., Lares M.L., Chee-Barragan A. and Siqueiros-Valencia A. (2007) Iron, manganese and trace metal concentrations in seaweeds from the central west coast of the Gulf of California. *Applied Geochemistry* 22, 1380–1392.

Jara-Marini M.E., Soto-Jiménez M.F. and Páez-Osuna F. (2009) Trophic relationships and transfer of cadmium, copper, lead and zinc in a subtropical coastal lagoon food web from SE Gulf of California. *Chemosphere* 77, 1366–1373.

Jensen K.R. (1997) Evolution of the Sacoglossa (Mollusca, Opisthobranchia) and the ecological associations with their food plants. *Evolutionary Ecology* 11, 301–335.

Jensen K.R. (2009) Sacoglossa (Mollusca: Gastropoda: Opisthobranchia) from Singapore. *Raffles Bulletin of Zoology* Supplement 22, 207–223.

Jiménez-Illescas A.R., Obeso-Nieblas M. and Salas-de León D. (1997) Oceanografía física de la Bahía de La Paz, BCS. In Urbán J.R. and Ramírez M.R. (eds) *La Bahía de La Paz, Investigación y Conservación*. Mexico: UABCS, CICIMAR and Scripps, pp. 31–41.

Joly A.B. (1967) *Generos de algas marinhas da costa Atlantica Latino-americana*. Sao Paulo: Universidad de Sao Paulo.

Lee W.Y. and Wang W.X. (2001) Metal accumulation in the green macroalga *Ulva fasciata*: effects of nitrate, ammonium and phosphate. *Science of the Total Environment* 278, 11–22.

Lönnerdal B. (2000) Dietary factors influencing zinc absorption. *The Journal of Nutrition* Supplement 130, 1378–1383.

Mann S.S. and Ritchie G.S.P. (1995) Forms of cadmium in sandy soils after amendment with soils of higher fixing capacity. *Environmental Pollution* 87, 23–29.

Matusiewicz H. (2003) Wet digestion methods. In Mester Z. and Sturgeon R. (eds) *Sample preparation for trace element analysis*. Amsterdam: Elsevier, pp. 193–233.

Méndez L., Palacios E., Acosta B., Monsalvo-Spencer P. and Álvarez-Castañeda T. (2006) Heavy metals in the clam *Megapitaria squalida* collected from wild and phosphorite mine-impacted sites in Baja California, Mexico. *Biological Trace Element Research* 110, 275–287.

Pacheco-Ruíz I., Zertuche-González J.A., Espinoza-Ávalos J., Riosmena-Rodríguez R., Galindo-Bect L., Gálvez-Télles A., Meling-López A.E. and Orduña-Rojas J. (2008) Macroalgas. In Danemann G.D. and Ezcurra E. (eds) *Bahía de Los Ángeles: Recursos naturales y comunidad. Línea base 2007*. Mexico: Secretaría de Medio Ambiente y Recursos Naturales, Instituto Nacional de Ecología, Pronatura Noroeste and San Diego Natural History Museum, pp. 181–214.

Patrón-Prado M., Casas-Valdéz M., Serviere-Zaragoza E., Zenteno-Savín T., Lluch-Cota D.B. and Méndez-Rodríguez L. (2011) Biosorption capacity for cadmium of brown seaweed *Sargassum sinicola* and *Sargassum lapazeanum* in the Gulf of California. *Water, Air, and Soil Pollution* 221, 137–144.

Pierce S.K., Curtis N.E., Massey S.E., Bass A.L., Karl S.A. and Finney C.M. (2006) A morphological and molecular comparison between *Elysia crispata* and a new species of kleptoplastic sacoglossan sea slug (Gastropoda: Opisthobranchia) from the Florida Keys, USA. *Molluscan Research* 26, 23–38.

Rainbow P.S. (2002) Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution* 120, 497–507.

Ray S. (1984) Bioaccumulation of cadmium in marine organisms. *Experientia* Supplement 40, 14–23.

Riley J.P. and Chester R. (1971) *Introduction to marine chemistry*. London: Academic Press.

Riosmena-Rodríguez R., Talavera-Sáenz A., Acosta-Vargas B. and Gardner S.C. (2010) Heavy metals dynamics in seaweeds and seagrasses in Bahía Magdalena, BCS, México. *Journal of Applied Phycology* 22, 283–291.

Robledo D. and Freile Y.P. (1997) Chemical and mineral composition of six potentially edible seaweeds species of Yucatán. *Botanica Marina* 40, 301–306.

Rodríguez-Figueroa G.M., Shumilin E. and Sánchez-Rodríguez I. (2009) Heavy metal pollution monitoring using the brown seaweed *Padina durvillaei* in the coastal zone of the Santa Rosalía mining region, Baja California Peninsula, México. *Journal of Applied Phycology* 21, 19–26.

Ruelas-Inzunza J.R. and Páez-Osuna F. (2008) Trophic distribution of Cd, Pb and Zn in a food web from Altata-Ensenada del Pabellón subtropical lagoon, SE Gulf of California. *Archives of Environmental Contamination and Toxicology* 54, 584–596.

Sabiha-Javied, Mehmood T., Chaudhry M.M., Tufail M. and Irfan N. (2009) Heavy metal pollution from phosphate rock used for the production of fertilizer in Pakistan. *Microchemical Journal* 91, 94–99.

Sawidis T., Brown M.T., Zachariadis G. and Sratis I. (2001) Trace metal concentrations in marine macroalgae from different biotopes in the Aegean Sea. *Environment International* 27, 43–47.

Servicio Geológico Mexicano (2008) *Panorama minero del Estado de Baja California Sur*. Mexico: Secretaria de Economía, Servicio Geológico Mexicano, Coordianción General de Minería and Secretaría de Gobernación.

Setchell W.A. and Gardner N.L. (1924) The marine algae: expedition of the California Academy of Sciences to the Gulf of California in 1921. *Procedings of the California Academy of Sciences* 12, 695–949.

StatSoft (2007) *STATISTICA (data analysis software system), version 8.0.* Tulsa, OK: StatSoft, Inc.

Talavera-Saenz A., Gardner S.C., Riosmena-Rodríguez R. and Acosta B.V. (2007) Metal profiles used as environmental markers of green turtle (*Chelonia mydas*) foraging resources. *Science of the Total Environment* 373, 94–102.

Trench R.K., Greene R.W. and Bystrom B.G. (1969) Chloroplasts as functional organelles in animal tissues. *Journal of Cell Biology* 42, 404–417.

Trench R.K., Trench M.E. and Muscatine L. (1972) Symbiotic chloroplasts: their photosynthetic products and contribution to mucus synthesis in two marine slugs. *Biological Bulletin, Marine Biological Laboratory, Woods Hole* 142, 335–349.

Trowbridge C.D. (1991) Diet specialization limits herbivorous sea slug's capacity to switch among food species. *Ecology* 72, 1880–1888.

Trowbridge C.D. (2002) Northeastern Pacific sacoglossan opisthobranchs: natural history review, bibliography, and prospectus. *The Veliger* 45, 1–24.

Trowbridge C.D., Hirano Y.M. and Hirano Y.J. (2011) Inventory of Japanese sacoglossan opisthobranchs: historical review, current records, and unresolved issues. *American Malacological Bulletin* 29, 1–22.

Trowbridge C.D., Hirano Y.M., Hirano Y.J., Sudo K., Shimadu Y., Watanabe T., Yorifuji M., Maeda T., Anetai Y. and Kumagai K.

(2010) Subtropical sacoglossans in Okinawa – at 'Special Risk' or 'Predictably Rare'? *American Malacological Bulletin* 28, 167–181.

Vera J., Castro J., Gonzalez A. and Moenne A. (2011) Seaweed polysaccharides and derived oligosaccharides stimulate defense responses and protection against pathogens in plants. *Marine Drugs* 9, 2515–2525.

Voulgaropoulos A., Paneli M., Papaefstathiou E. and Stavroulias S. (1991) Comparative determinations of cadmium and lead in phosphorites dissolved in nitric acid and aqua regia using differential pulse anodic stripping voltammetry and atomic absorption spectrophotometry. *Fresenius Journal of Analytical Chemistry* 341, 568–569.

and

Zar J.H. (2010) *Biotatistical analysis*. 5th edition. Upper Saddle River, NJ: Prentice Hall.

Correspondence should be addressed to:
E. Serviere-Zaragoza
Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Instituto Politécnico Nacional 195, Col. Playa Palo de Santa Rita Sur, La Paz, B.C.S. 23096, Mexico.
email: serviere04@cibnor.mx