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Metal Content in *Ulva lactuca* (Linnaeus) from Navachiste Bay (Southeast Gulf of California) Sinaloa, Mexico

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Macroalgae are one of the most efficient and reliable indicator organisms to study trace metal pollution (Phillips, 1977). Seaweeds concentrate metals from solution and integrate short-term temporal fluctuations in concentrations of dissolved metals from the biologically available fraction (Lobban and Harrison, 1997). The use of seaweeds as biological monitors of marine pollution in coastal areas has been documented by several workers around the world by using Phaeophyta (Bryan and Hummerston, 1973; Muse, at al., 1995), Chlorophyta (Ho, 1990; Haritonidis and Malea, 1995, 1999; Brown, et al. 1999; Villares, et al. 2002) and Rhodophyta (Malea, at al. 1994; Muse et al. 1995) algae. The green algae *Ulva lactuca* has been considered as a good indicator for metal contaminations in tropical and subtropical regions due to its cosmopolitan distribution, its simple morphology, and its high capacity to accumulate metals (Ho, 1990). *U. lactuca* is widespread along the Gulf of California (Zertuche-Gonzalez et al. 1995), tolerate a wide range of salinities and grows well attached to any suitable solid substrate in habitats influenced by municipal and industrial wastewaters (Ho, 1990). Such habitats are also polluted by heavy metals. Since metals such as Cu, Zn, and Pb are associated with sewage, it is probable that *U. lactuca* growing along urban coastlines accumulates high levels of these metals.

Sinaloa State is located at the southeast Gulf of California, and is considered one of the most important agronomic areas of Mexico. Intensively agricultural practices are supported by eleven rivers and watering channels which are discharged without treatment into state coastal lagoons, in addition to municipal and industrial wastewaters. Heavy metal levels have been analyzed along the Sinaloa state coastal lagoons using different marine organisms of different taxonomic groups as biomonitors such as mollusks (Páez-Osuna, et al. 2002), crustaceans (Páez-Osuna and Ruíz-Fernandez, 1995), and algae (Páez-Osuna, et al. 2000), nevertheless, there is not information about seasonal changes in metal content in seaweeds. Navachiste Bay, a coastal lagoon located in north Sinaloa receives freshwater inputs from land runoff during the rainy season and untreated domestic effluents from populations of the city of Guasave and the village of Juan José Rios (300 000 inhabitants approximately) throughout main watering channels. The aim of the present work was to assess the seasonal levels of Cd, Cu, Fe, Pb, and Zn in the green algae *U. lactuca* from the Navachiste Bay in southern Gulf of California.

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MATERIALS AND METHODS

Navachiste Bay is a semi-closed lagoon *ca.* 260 Km^2 in area situated between 25° 21' and 25° 35' N and 108° 40' and 109° 00' W located in the southeast coast of the Gulf of California. This bay is of great ecological and economic importance since fisheries and aquaculture activities, specifically shrimp farms, are carried out by more than 1850 fishermen.

The green alga *U. lactuca* was hand picked (0.5 to 1 Kg wet weight) every two months from January through December 2002 from natural population at 1 to 1.5 m depth using skin dive. The collection site receives freshwater inputs from land runoff and untreated domestic effluents from populations of the city Guasave, the village of Juan José Rios and inputs from land runoff during the rainy season throughout one of the main watering channels (named "dren Batamote"). Samples were placed in plastic bags containing seawater from the collection site and returned in coolers to the laboratory. Upon arrival, plants were rinsed first with seawater from the collection site and then with distilled water in order to remove epiphytes and sediment particles. Plants were freeze-dried (- 45 °C and 90 x 10^{-3} mBar) over 72 h using a Labconco freeze dry system / Freezone 4.5. Dried samples were ground and the powder screened through a plastic mesh (100 µm) to ensure proper homogenization of the tissue to enhance the efficiency of acid digestion. Samples of *U. lactuca* (0.2 g dry weight) (n=6) were mixed in a plastic cap test tube containing 5 ml of concentrated Nitric acid (Suprapure grade from Aldrich) and allowed to stand for 2 h at room temperature. Tubes were then heated in a water bath at 95 °C for three hours to digest the samples. When pressure in the tubes come down, the acid was filtered through a Whatman GF/F filter paper (0.7 µm), and transferred to 25 ml volumetric flask. Then, deionized water was added up to the mark. Reference standard seaweed material (IRMM, CRM- 279 *Ulva lactuca*) certified for Cd, Cu, Pb and Zn and with indicative value for Fe was digested (n=6) in the same way and concurrently analyzed with the algae samples to verify the accuracy of the digestion and analysis procedure.

Acid-washed glassware was used throughout the procedure. The digested samples of algae were assayed for Cd, Cu, Fe, Pb and Zn in an Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP) (Perkin Elmer Optima 3100 RL). Calibration of the spectrophotometer was performed by using standard solutions of the different metals prepared from commercial stock solutions (atomic spectroscopy grade from Perkin Elmer).

RESULTS AND DISCUSSION

Results of the metal analysis carried out with the standard reference material are showed in Table 1. The percentages of recovery were between 95 and 99, and only Cd concentration was significantly different from expected value (t-test, $p < 0.05$).

Concentrations of metals in *U. lactuca* varied appreciably during the sampling period (Figure 1). The relative seasonal variation was higher for Fe and Zn, in which the highest concentrations were about five times higher than the lowest concentration. On the other hand, the relative variation for Cu, Cd and Pb was the lowest, since the highest concentration was only two times higher than the lowest concentration. In general, concentrations of all metals were minimum in summer, showed and increasing in October, and reached a maximum in winter, with the exception of Fe.

Non-parametric one way analysis of variance (Kruskal-Wallis test, $p < 0.05$) performed to assess the differences between mean metal concentrations showed significant variations between seasons in the mean concentration of all metals analyzed. The highest Fe concentrations were found in August (3652 µg g^{-1}), whereas the rest of the year concentrations ranged between *ca.* 500 µg g^{-1} to 1500 µg g^{-1}. Copper concentrations ranged from a minimum of 23 µg g^{-1} in the period June-August (summer) to a maximum of 44 µg g^{-1} in December. Cadmium concentrations were low relatively to the other metals and showed no strong variations during the seasons analyzed.

Table 1. Concentrations of elements in reference material.

Metal	Certified value	Concentration found	Recovery (%)
Fe*	2.43 (mg g^{-1})	2.39 (mg g^{-1})	98.35
Cu	13.14	13.02	99.08
Zn	51.3	50.01	97.48
Cd	13.48	13.22**	98.07
Pb	2.192	2.10	95.80

(Values in µg g^{-1} dry weight. *Ulva lactuca* (IRMM, CRM- 279)).* Non certified value in reference material. **Significantly different ($p < 0.05$) from expected value.

The lowest Cadmium concentrations (1.3-1.5 µg g^{-1}) were detected in summer, whereas maximum values were found in winter (2.6 µg g^{-1}). The lowest concentrations of Zinc was found in August (98 µg g^{-1}) and the highest in April (547 µg g^{-1}). On the other seasons analyzed Zn concentrations fluctuated between 350 and 500 µg g^{-1}. *U. lactuca* Pb concentrations varied from 94 µg g^{-1} in August to 259 µg g^{-1} in February. The relative abundance of metals in *U. lactuca* decreased in the order: Fe> Zn> Pb> Cu> Cd.

Among the five metals examined in this study, concentrations of iron in *Ulva lactuca* were similar or lower to previously report in the same species (Hornung et al. 1992; Buo-Olayan and Subrahmanyam, 1996; Villares, et al. 2002). High concentration of iron in summer has been observed in algae (Haritonidis and Malea, 1999) and seagrasses (Wahbeh, 1984; Malea and Haritonidis, 1995), and explained as a result of an enhancement in photosynthesis and respiration, which increase the metal uptake within the plant. Maximum concentration of Fe in algae occurred in August when irradiance and temperature of seawater are maximal in this subtropical area were observed in this work, which concords with the previous work mentioned above, which suggests that the same processes operate in *U. lactuca* from Navachiste during this season.

Cu concentrations reported for *U. lactuca* species around the world are extremely variable, and range from less than 10 μg g^{-1} (Ho, 1990; Hornung et al. 1992) to 445 μg g^{-1} (Buo-Olayan and Subrahmanyam, 1996). This variability could be explained as a consequence of this metal availability in the seawater due to differences in the terrestrial inputs during the different seasons of the year. Although local focus of contamination by this metal and the difference in collection season, may also account for such variability. According to Brown et al. (1999) the range of values for Cu in *U. lactuca* from uncontaminated sites was 0.1- 3.0 μg g^{-1} dry weight, whereas values from highly contaminated sites range between 14 and 134 μg g^{-1}. Then, according to this author, the range of copper concentrations obtained in the algae from Navachiste Bay (23-44 μg g^{-1}) indicates that this area is contaminated with respect to this metal.
Concentrations of Cd found in *U. lactuca* were relatively high compared with other studies, 0.49-1.3 μg g^{-1} in *U. lactuca* from Hong Kong (Ho 1990) and 1.0 μg g^{-1} in *U. rigida* from Greece (Haritonidis and Malea 1999). For the Gulf of California, Páez-Osuna et al. (2000) found concentrations of Cd of 1.3 μg g^{-1} in *U. lactuca* collected in Manzanillo (*ca.* 1000 Km south to the area in the present study) and 0.9 μg g^{-1} for populations of this alga in Yavaros, Sonora (about 200 Km north to the area in the present study). Other studies carried out in Sinaloa coastal lagoons have shown Cd concentrations of 5.6 to 18.2 μg g^{-1} for oysters from Navachiste Bay. Such high concentration of Cd could be explained as result of enrichment of the coastal seawater due to upwelling waters from the Gulf of California (Delgadillo-Hinojosa, et al. 2001), as well as to the intense use of phosphate fertilizers, which can contain great amount of Cd (Förster and Wittmann, 1979).
In the same way, previous works have shown high concentrations of Zn in clams, mussels and oysters (*ca.* 342-806; 900-1300; 400-1500 μg g^{-1}, respectively) from different coastal lagoons in Sinaloa. High concentrations of this metal have been related with agricultural effluents and associated with an intensively fertilized agriculture drainage basin where organophosphorous pesticides, carabamates, and metallic fungicides enriched in heavy metals as Zn, are extensively used (Szefer, et al. 1998). Thus, high levels of Zn observed in *U. lactuca* could be the result of the enrichment, which is produced by metallic fungicides. In this study, concentrations of Zn were three to four times higher than values proposed by Say et al. (1990) for high contaminated environments (> 150 μg g^{-1}). Although Zn concentrations found in this study are not the highest ever reported for *U. lactuca* (798 μg g^{-1}, Hornung et al. 1992), it can be considered that Navachiste Bay presents contamination for this metal, according to the criteria of Say et al. (1990).

Similar to other metals, Pb concentrations in algae were higher from October to February. It has been pointed out that small boats are probably the source of Pb in Sinaloa coastal lagoons, especially in sites where fishing activities are carried out intensively (Páez-Osuna et al. 2002). Navachiste Bay plays an important rolle in artesanal catch of shrimp and scale fishes, which are carried out by *ca.* 950 small boats (Navachiste Bay Fishermen Society, personal com.). These activities increase from September to April, when prohibition of shrimp catch ends (out season), and more boats increase the fish effort in the bay.

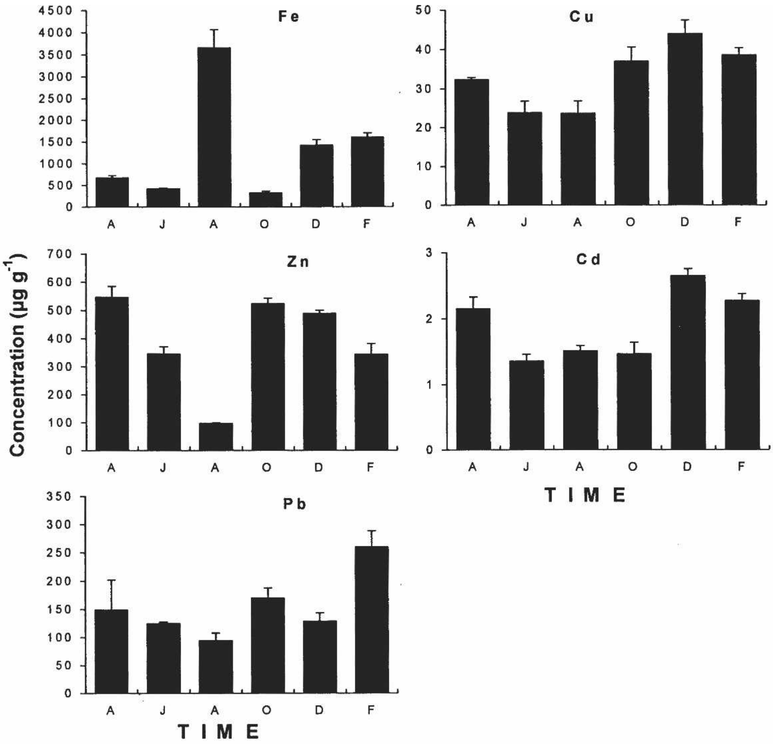

Figure 1. Seasonal variations of mean (± SD) metal concentrations in *Ulva lactuca* (µg g^{-1} dry weight).

Excepting Fe, all metals showed their maximum concentrations in winter and their minimum in summer. Seasonal changes in metal content in different algae species have been related to both biological and physical aspects.

Seasonal pattern of concentrations of several metals in *Ulva rigida* and *Enteromorpha linza* have been attributed to a growth effect (Haritonidis and Malea, 1995, 1999). According to these studies, increase in the biomass (high growth rate) resulted in the decrease of metals per mass unit due to a dilution effect. Similar observations were reported for *U. lactuca* and *Gracilaria verrucosa* (Sawidis and Voulgaropoulos, 1986), *U. rigida* and *Enteromorpha intestinalis* (Villares, et al. 2002). Another possible source of seasonal variation in metal concentration in algae might be due to an increase in the mean levels of metals in seawater due to increasing inputs to the coastal lagoon during winter. According to Comision Nacional del Agua (Water National Commission), irrigation of inland crops is carried out more intensively during winter than in summer time. Thus, it is possible that this increase in the runoff raised the level of suspended particles and metal contents in the discharge areas. This effect has been reported previously by Bryan and Hummerstone (1973) who concluded that factors controlling the seasonal concentrations of metals in seaweeds included the concentrations of metals in seawater. Also, it has been reported that the highest concentration of Cu and Zn in five seaweed species were detected during the rainy season (de Lacerda et al., 1985). Similarly, in the present study it was found that concentrations of Cu, Zn, Cd, and Pb in *U. lactuca* were highest in fall-winter, coincidently with storm season in the subtropical west coast of Mexico from September to November, which increases the fluvial inputs with a consequent augment in suspended particles and dissolved metals in seawater.

Lack of metal related industries in the area indicates that all metals have mainly an anthropogenic origin derived from agriculture practices. Furthermore, high Zn concentrations found in *U. lactuca* could indicate and excessive use of agrochemical products that finally are discharged in this coastal lagoon through watering channels. Mean concentrations of metals analyzed in *U. lactuca* were higher than those found in other studies with the same species, particularly Zn, Cu and Pb. Thus, it can be considered that populations of these algae from Navachiste Bay are polluted with these metals.

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