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Cadmium Uptake by *Halimione portulacoides:* An Ecophysiological Study

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The accumulation of Cd by *Halimione portulacoides* collected in the salt marshes of River Sado estuary was studied (Reboredo, 1992). The results showed that the element is easily absorbed and translocated to the leaves – the main accumulation organ.

The great mobility of Cd was also detected in *Azolla filiculoides* - the relative concentration of the roots was similar to that found in the floating leaves (Sela et al. 1988) although in the aquatic plant *Eichhornia crassipes* (Jamil and Hussain, 1992), a different pattern accumulation was detected (root concentration>petiole>leaf concentration).

Cadmium is a non-essential element with reported toxic effects on plants specially at the ionic balance (Sela et al. 1988), enzymatic activity (Tu and Brouillette, 1987), ultrastructural level (Baszynski et al. 1980) and pollen germination and germ tube elongation (Chaney and Strickland, 1984).

H. portulacoides is well adapted to waterlogged saline soils, and sodium chloride absorbed by the roots is translocated and excreted throught salt glands. Taking into account the levels of Cd in the leaves of *H.portulacoides* collected *in vivo* (Reboredo, 1992), experimental studies with *Halimione* plants in hydroponic cultures containing Cd were initiated, to verify if cadmium can be excreted to the leaf surface through salt glands.

MATERIALS AND METHODS

Plants used in hydroponic cultures were cultivated according the methods described (Reboredo, 1988, 1997). Plants obtained from cuttings that had been in pots for 2 months and transported *a posteriori* to water cultures, presented a similar degree of development; average values - height (21 cm), stem biomass (0.80g) and leaf biomass (1.13g), on a dry weight basis.

Nutrient solution (Baumeister and Schmidt,1962) contained 1.0 and 5.0 μg ml^{-1} Cd as $CdCl_2$, or was free of Cd (controls) at a final pH of 5.5. Although in the natural habitat (Sado river estuary) soil pH varies between 6.5-7.6 (Reboredo, 1992), acid discharges from pyrite mines have already occurred in upstream areas, with acute consequences to the fauna and flora.

Five batteries of 20 individuals were submitted to each of the concentrations used (three of them were used for analytical purposes and the others for the determination of the mortality rate), the pH and volume being kept constant during the experiment. Plants were observed daily for chlorosis, necrosis, browning of tissues and other toxicity symptoms.

Leaves were collected 15 days and 1 month after the beginning of treatment; the Cd and Mg content was determined by atomic spectrometry using a Perkin-Elmer model 403, according to Reboredo (1988) and the chlorophylls and total carotenes contents according to Arnon (1949) using a spectrophotometer Shimadzu UV-160. For the determination of the biomass, leaves and stems were dried at 100ºC to constant weight.

Each analysis was carried out in triplicate in each battery. An analysis of variance (ANOVA) was performed and mean values were compared by the Tukeys test (Sokal and Rohlf, 1994).

The absorption and emission spectra were performed using a spectrophotometer Shimadzu UV-160 and SPEX Fluourolog FIII (λ_{exc} = 450 or 470 nm), respectively. Scanning electron microscopy studies (SEM) were performed on fresh vegetal material using a Jeol 330A coupled with a X-ray microanalyser Tracor Northern Series II. All analyses were performed at 20 Kv, using a static beam spot under standardized conditions for 60s live time. Peak to background ratios (K ratios) were calculated for Cd using the routine supplied by Tracor Northern.

RESULTS AND DISCUSSION

The morphological changes as a result of the Cd stress, were expressed at the roots – lack of lignification, absence of root hairs and wrinkling. At the day 30, the mortality of the initial population was 30% and 55% for plants treated with 1.0 and 5.0 μg ml^{-1} Cd, respectively.Conversely, the mortality of control plants was 0%. Fargasova(1994) observed very low toxic effects of different metals, including Cd, on seed germination of *Sinapis alba,* while the deleterious effects on root growth were more pronounced.

In control plants the levels of Chl.*a*, and to a lesser extent, Chl.*b* and Cd, remained constant during the experiment while in plants treated with Cd these levels have decreased from the 15^{th} day of treatment (Table 1).

Table 1. Concentrations of chlorophylls, carotenes, Cd and Mg in *Halimione* leaves and stem and leaf biomass of plants treated with 1.0 and 5.0 μg ml^{-1} Cd, under different exposures times - 15 and 30 days.

	Control		1.0μg ml^{-1} Cd		5.0μg ml^{-1} Cd	
	15th day	30th day	15th day	30th day	15th day	30th day
Chl. *a**	264±7.8a	267±16a	265±30a	213±8.9bc	267±9.8a	236±14b
Chl. *b**	106±6.6b	116±16a	107±6.0b	89,1±17c	117±26a	105±12ab
Carot.*	26±4.4a	37±5.0a	27±7.5a	22±3.2b	23±4.7a	26±4.8b
Cd**	0,8±0.2c	0,6±0.1c	4,5±0.8b	2,2±0.3b	6,0±1.6a	3,7±0.5a
Mg***	0,93±0.12a	0,95±0.08a	0,91±0.09a	0.84±0.09b	0.92±0.06a	0,90±0.07a
L.b.#	1.12±0.08a	1.11±0.07a	1.07±0.15a	1.00±0.13a	1.09±0.08a	1.07±0.15a
S.b.#	0.81±0.07a	0.83±0.08a	0.80±0.09a	0.80±0.09a	0.82±0.03a	0.83±0.05a

*Mean values expressed as μg g^{-1} of fresh weight or as μg g^{-1} of dry weight** or as % g^{-1} of dry weight***; # Mean values expressed as g of dry weight
L.b.=leaf biomass; S.b.=stem biomass. Comparable means (at the same day) not followed by a common letter are significantly different (P≤0.05) ± S.deviation

Nutrient solution was free of Cd, although plants used and previously collected in contaminated salt-marshes, presented different Cd concentrations, according to sampling points, seasons and plant organs (Reboredo,1992).

Comparatively with controls, the Chl. *a* levels of *H.portulacoides* plants treated with 1.0 e 5.0 μg ml^{-1} Cd decreased (at day 30), 20% and 12%, respectively, while total carotenes decrease 39.7% and 30.3% for the same concentrations (Table 1). Baszynski et al. (1980), observed approximately a 50% reduction of the Chl. *a* and *b* contents for plants treated with 20μM Cd, while carotenoids decrease 38.9%.

The Mg content of the leaves at day 15th was not statistically different at the 0.05 significance level, the concentrations being approximately 5 times higher than the adequate concentration in dry tissues – 2000 μg/g (Stout, 1961). At day 30th and comparatively with the controls, the reduction of the Mg content was 11.6% and 5.3% for plants treated with 1.0 e 5.0 μg ml^{-1} Cd, respectively (Table 1), these percentages being approximately half of the reduction of the chlorophyll levels.

Surprisingly, in Cd-treated plants the Cd levels of the leaves decreased from the day 15 to the day 30 (Table 1), which is probably related with either an active process of excretion through the salt glands (Figs. 4 and 5), or with the use of younger leaves (since mature succulent leaves usually fall), or even due to a defficient uptake decreasing translocation, or the coexistence of all the referred phenomena.

At day 15, only two specimens were slightly withered (1 specimen per each Cd treatment) while subsequent 15 days, were lethal for the majority

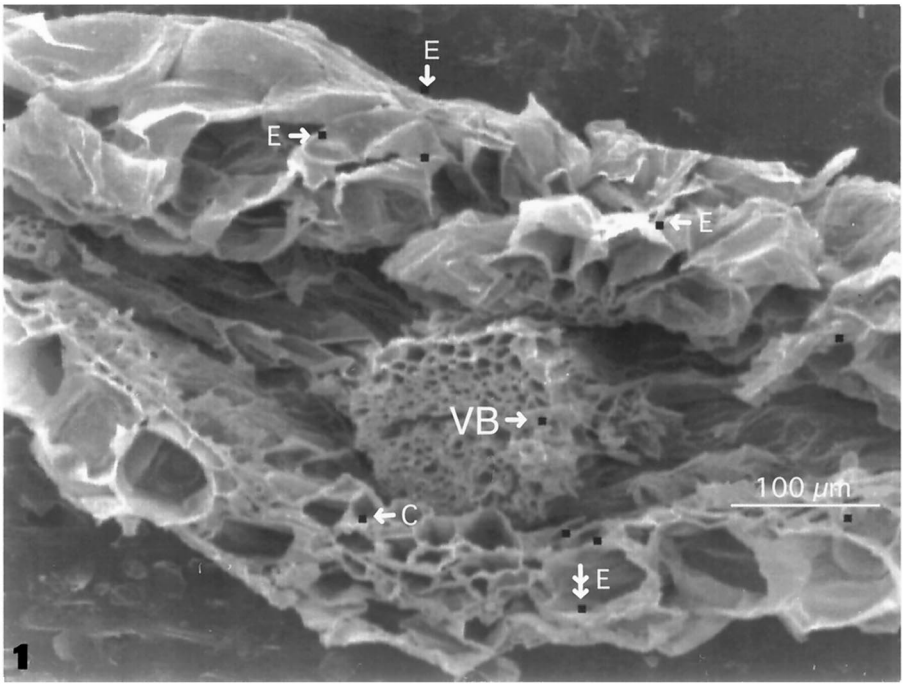


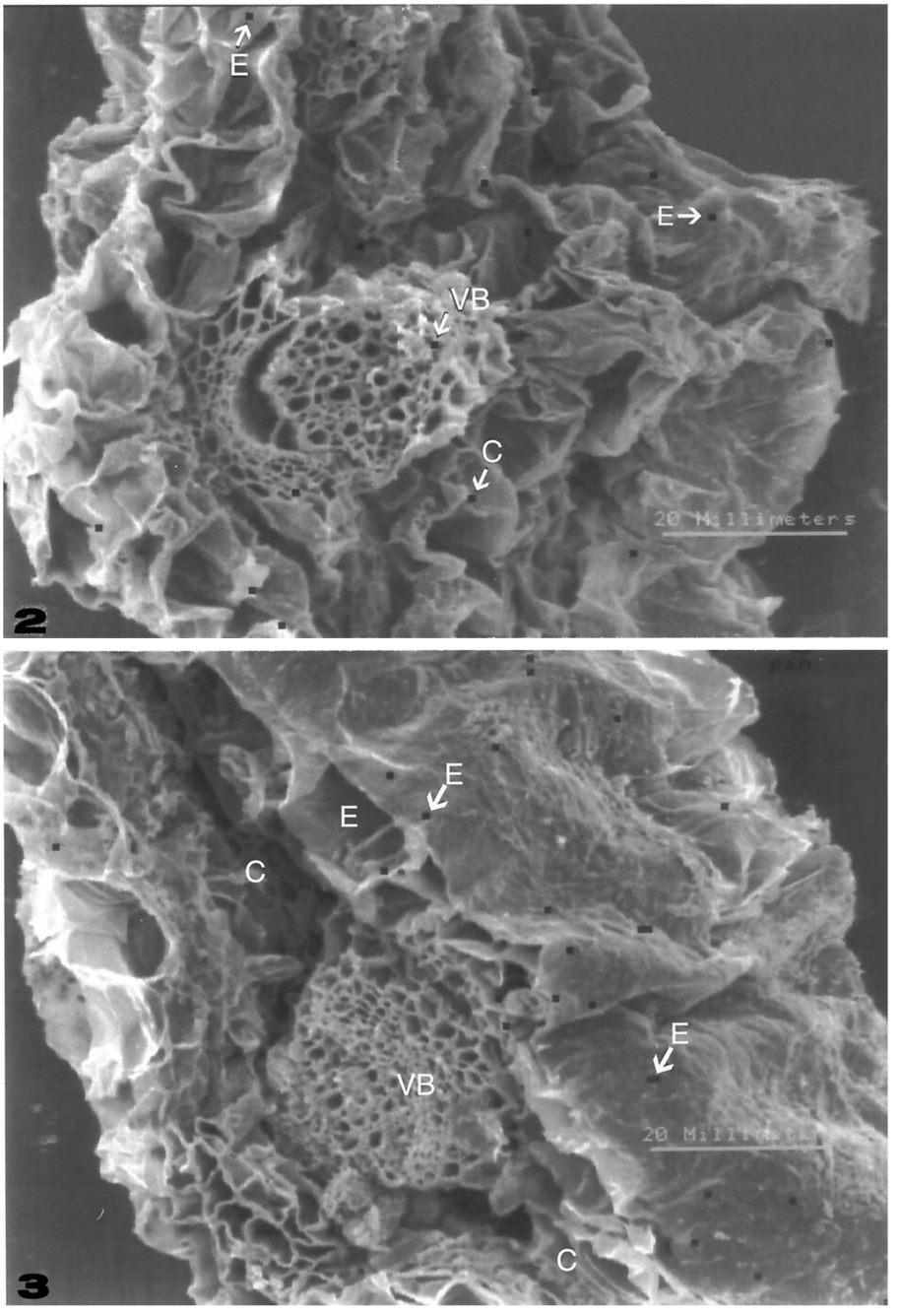
Figure 1. Cadmium localization (arrow) over the epidermis (E), in the epidermis (double arrow), chlorenchyma (C) and vascular bundle (VB) of the leaf of *Halimione portulacoides*. Control plants - day 30

of the initial population in spite of the decline of the Cd levels - from 4.5 to 2.2 $\mu g\ g^{-1}$ and from 6.0 to 3.7 $\mu g\ g^{-1}$ for plants treated with 1.0 $\mu g\ ml^{-1}$ Cd and 5.0 $\mu g\ ml^{-1}$ Cd, respectively (Table 1).

If we subtract the background Cd value of control plants (0.6 $\mu g\ g^{-1}$ Cd) at the 30th day, from the values measured in Cd-treated plants, we observed a recovery of 160% and 62% for plants treated with 1.0 $\mu g\ ml^{-1}$ Cd and 5.0 $\mu g\ ml^{-1}$ Cd, respectively, emphasising the great capacity of accumulating Cd in the leaves – as also seen *in vivo* (Reboredo, 1992) and the regulation of intracellular levels through salt excretion, although no data concerning adsorption on test vessel walls had been presented.

The biomass of leaves and stems of control and treatments did not seem to be affected by Cd since mean values were not statistically different at the 0.05 significance level (Table 1).

The studies by X-ray microanalysis allowed to detect Cd at the chlorenchima, vascular bundles (Figs 1 and 2) and in the epidermis and particularly on the epidermis of the leaf (Figs 1, 2 and 3), confirming an active process of Cd excretion through salt glands especially for plants



Figures 2 and 3 - Cadmium localization (arrow) over the epidermis (E), chlorenchyma (C) and vascular bundle (VB) of the leaf of *Halimione portulacoides*. Fig. 2 - Plants treated with 1µg.ml^{-1} Cd - day 30; Fig. 3 – In plants treated with 5 µg.ml^{-1} Cd (day 30), chlorenchyma (C) and vascular bundle (VB) are free of cadmium deposits.

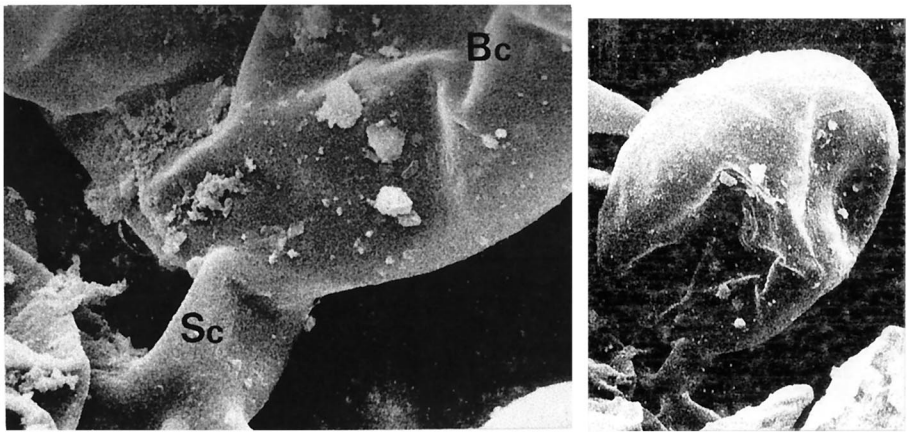


Figure 4. Salt gland of *H. portulacoides* leaf with the stalk cell (Sc) and bladder cell (Bc). Partial aspect (3000**x**) and general aspect (1000**x**)

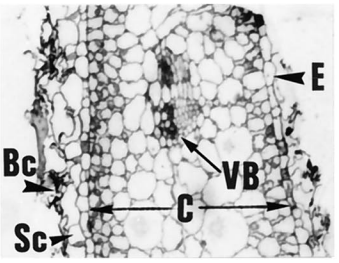


Figure 5. Transverse section of the leaf of *Hportulacoides* showing the epidermis (E) chlorenchyma (C) and vascular bundle (VB). See the salt glands on the epidermis with the stalk cell (Sc) and bladder cells (Bc) collapsed (170**x**)

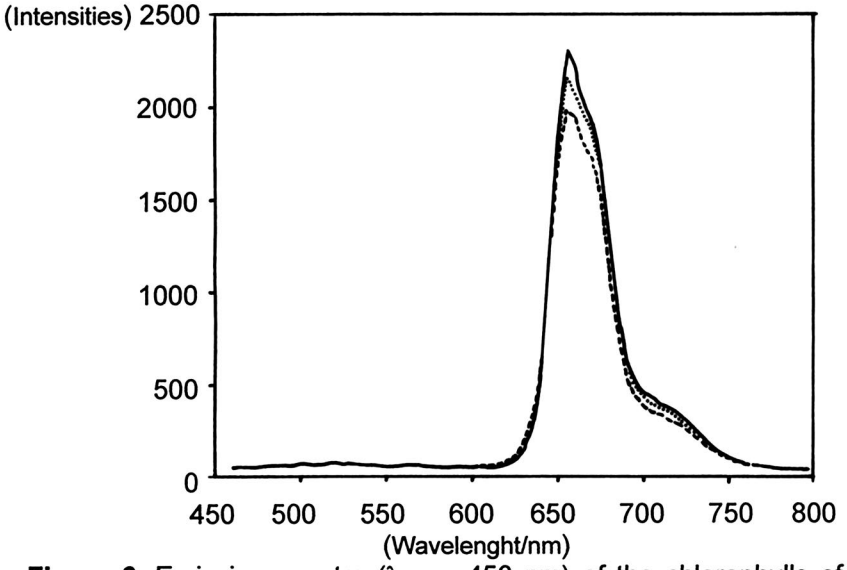


Figure 6. Emission spectra (λ_{exc} = 450 nm) of the chlorophylls of the leaves of *H.portulacoides* treated with 1.0 μg ml^{-1} Cd (---) 5.0 μg ml^{-1} Cd (····) and controls (—).

treated with 5.0 μg ml^{-1} Cd (Fig. 3) - an abundant and almost exclusive deposition of Cd on the epidermis of the leaf was observed (Fig. 3), comparatively with the scarce deposits noted for plants treated with 1.0 μg ml^{-1} Cd (Fig. 2) or controls (Fig. 1).

This means that the excretion of Cd must occur when the internal concentration of the mesophyll reach a certain critical concentration, emphasising that some heavy metals could be removed and recycled within the ecosystem (on a temporal basis), which is a benefit mainly for the plant - intracellular Cd would be harmful.

Van de Geijn and Petit (1978), observed a major accumulation of ^{115}Cd in the epidermal and cortical layers of the stems of *Lycopersicon esculentum*, after two days of Cd supply indicating a lateral migration, which can also occur in *H.portulacoides*. Rauser and Ackerley (1987) detected Cd inside the root parenchyma cells of *Agrostis gigantea* and *Zea mays* exposed to 3mmol m^{-3} $CdSO_4$ but failed to detect it in epidermal cells and cell walls in both differentiating and mature root segments.

The analysis of the emission spectra show us an intriguing characteristic, traduced by an emission peak for plants treated with 5.0 μg ml^{-1} Cd, higher than that observed for plants treated with 1.0 μg ml^{-1} Cd. Nevertheless, control peak emission is higher than the treatments (Fig. 6)

The shape of the chlorophyll fluorescence spectra depends upon the chlorophyll content of the leaves (Lichtenthaler et al. 1986) and the decrease of the fluorescence intensity with the increasing chlorophyll content of the leaves of *Phaseolus vulgaris* L. is due to reabsorption of the shorter wavelength fluorescence around 690 nm by the chlorophylls (Lichtenthaler and Buschmann, 1987).

Measurements using diluted concentrations of chlorophyll with absorbances lower than 0.2, show that at lower Chl.*a* concentrations, correspond lower emission peaks, so there is a linear correlation between intensity of emission and concentration. If a complexation phenomenum of Cd by the chlorophyll would occurred, the emission spectra of treated plants would be affected by comparison with the spectrum from control plants.

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