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Cu and Zn Uptake by *Halimione portulacoides* (L.) Aellen. A Long-Term Accumulation Experiment

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Salt-marsh vegetation has for long attracted attention, especially by its adaptation to waterlogged saline soils. Coastal areas and plant species colonizing salt-marshes are often exposed to a large diversity of pollutants, with emphasis on heavy metals.

The important role of marsh vegetation in the accumulation of metals has been shown by several authors (Beeftink *et al.*, 1982; Menon and Ghuman, 1985; Reboredo, 1985). Some of these studies were carried out *in loco*, but others were performed under experimental controlled conditions (Ribeyre and Boudou, 1982; Taylor and Crowder, 1983; Huiskes and Nieuwenhuize, 1985), in order to know in advance the behaviour of the selected species - e.g.,unicellular algae, halophytes, or even crop plants, in the presence of increased concentrations of heavy metals.

In a previous work (Reboredo, 1988) some aspects of the ecophysiology of *Halimione portulacoides* collected in its natural habitat were studied. This species is one of the most representative halophytes of the salt-marshes of the river Sado estuary. The Sado basin receives an important input of residual elements from chemical industry, metallurgy, shipbuilding, extractive industry (Fe and Cu sulphides), paper pulp, fertilizers and canned food production, and also the untreated domestic sewage from Setúbal, a populous city located in the mouth of the estuary.

Agricultural wastes from extensive rice fields located upstream were also responsible for several damages in the fauna (especially in fishes) as refered by Lima and Vale (1978).

In order to study the uptake and pattern of accumulation of Cu and Zn by the roots, stems and leaves of *Halimione*, plants were cultivated *in vitro* and treated daily with solutions containing 5, 25 and 50ppm Cu (as $CuCl_2$) and 50, 100 and 150ppm Zn (as $ZnCl_2$).

MATERIALS AND METHODS

Plants used *in vitro* experiments were obtained from cuttings and cultivated under daylight in pots with ca. 1700 cm^3 volume, in a growth-room, in a soil mixture (pH 6.5; Eh 411.3mv) whose characterization was previously described (Reboredo, 1988).

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Plants were treated daily with 100ml of sea water, containing 5, 25 and 50ppm Cu and 50, 100 and 150ppm Zn, while control plants were treated daily with an equal volume of solution without any of the tested elements.

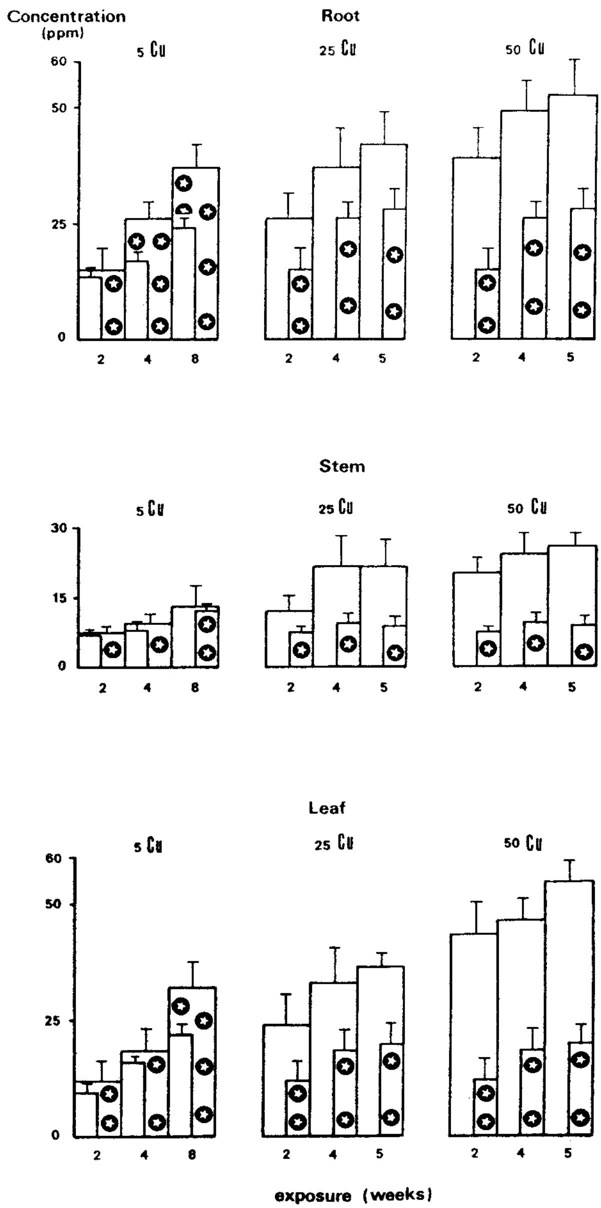

Figure 1. Copper levels in the roots, stems and leaves of *Halimione* control-plants (■) and in plants treated with 5, 25 and 50 ppm Cu (□) during different exposure times.Thin vertical bars (I) indicate the standard deviation.

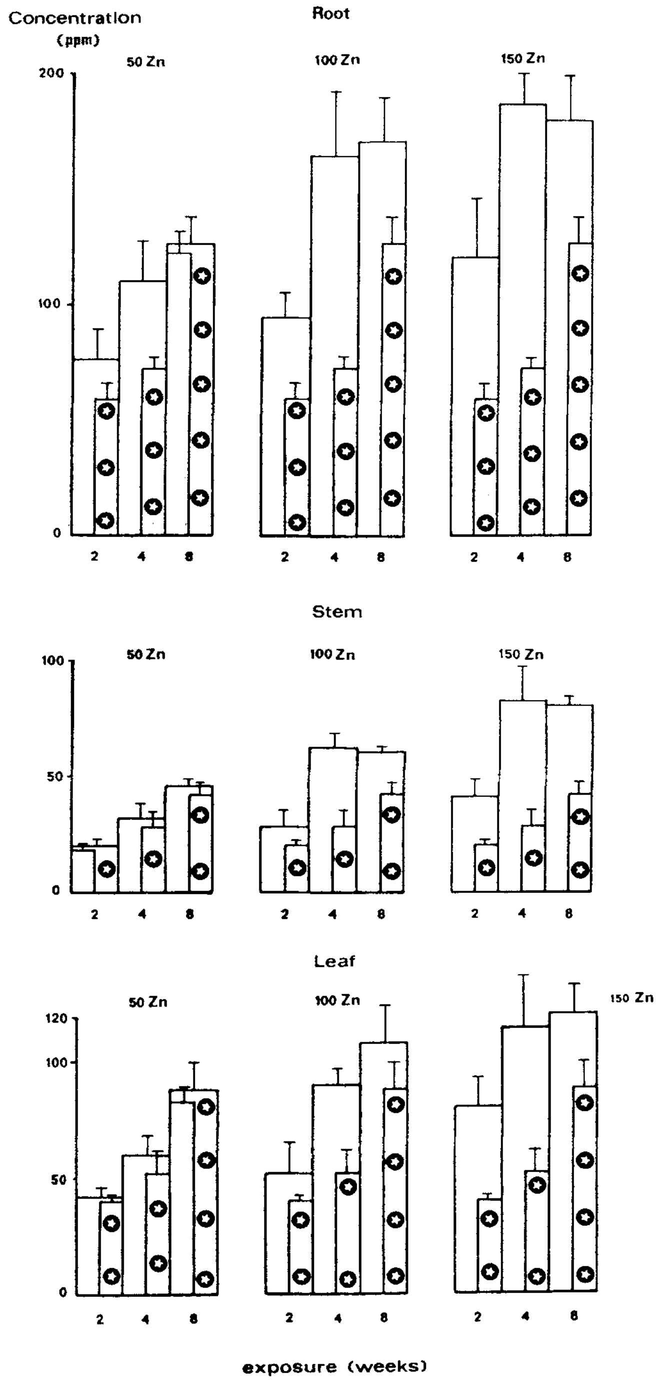

Figure 2. Zinc levels in the roots, stems and leaves of *Halimione* control-plants (■) and in plants treated with 50, 100 and 150 ppm Zn (□) during different exposure times.Thin vertical bars (I) indicate the standard deviation.

Roots, stems and leaves were collected in triplicate after 2, 4 and 8 weeks of treatment with both metals, as well as immediately after toxicity symptoms were detected. In this particular case the treatment was suspended.

Lignified stems (without phothosynthetic activity), yellow-green leaves and young leaflets were not considered for Cu and Zn analysis. Samples (roots, stems and leaves) were carefully rinsed with distilled water and dryed at 70°C to constant weight.

Each sample of dry plant and soil (1 gram) was placed in a 100ml borosilicate beaker (previously cleaned with 10% HNO3 aqueous solution) and digested according to the method described by Agemian and Chau (1976). The metals were determined by atomic absorption spectrometry using a Perkin-Elmer model 5000 spectrometer fitted with a deuterium background corrector. The operating conditions were those recommended by the manufacturer.

Pro-analysis grade reagents (E.Merck) were used in every case. Standards were prepared by serial dilution of commercially available stock solutions (Fisher Sci. Company). Each analysis (treated or non treated plants) was carried out in triplicate, taking as the final result the arithmetic mean of the three.

RESULTS AND DISCUSSION

Halimione plants were treated with 5ppm Cu during 8 weeks without apparent damage, while plants treated with 25 and 50ppm of the element exhibited several injuries during the 5th week of treatment. The treatment was suspended.

These injuries, such as foliar necrosis of mature leaves and the withering of a great majority of the young leaves of the shoots, were probably caused by high levels of Cu in the leaves (36.5 and 53.8ppm for plants treated with 25 and 50ppm Cu, respectively), compared with the leaves of control-plants (20.0ppm) - Fig. 1.

Higher Cu levels were generally found in the roots, although levels in the leaves were similar (Fig. 1). Plants treated with the higher levels of Cu had more Cu than the control-plants, in contrast to plants treated with 5ppm Cu which had generally less Cu than controls.

From the analysis in Fig. 1, and taking into account the differences between the levels of Cu in treated and control plants, Cu accumulated in the first two weeks. After that time the accumulation was insignificant.

The Cu root conc./Cu leaf conc. ratio varied between 0.9-1.4 for Cu treated plants and 1.2-1.4 for control plants (Fig.3A). In natural conditions (three different sampling points), this ratio was a little bit higher for samples collected in 1982 with a variation between 1.5-1.9, while for the next year the variation was between 0.8-1.2 (Reboredo, 1988).

Soils from pots enriched with Cu during the experimental work reached levels (min. 135.7; max. 329.5ppm) above the soils of the salt-marshes of the Sado river estuary, where plant species live in natural conditions.

The tested Zn levels (50, 100 and 150ppm) and exposure times (2, 4 and 8 weeks) did not induce changes in the external morphology of *Halimione portulacoides*. The average Zn levels of the roots, stems and leaves, in the first two weeks of treatment with Zn, was generally small, especially for the lower levels (50 and 100ppm Zn).

The enrichment during the 2nd to 4th week was 2-3 times higher in a great majority of the cases (Fig. 2). The uptake of Zn was stimulated between that period, independent of the Zn applied. From that time onwards the Zn treated-plants accumulate very low levels of the element in their organs in contrast to control-plants which accumulate more Zn.

Zinc levels associated with the roots of *Halimione* were much higher than those observed in the stems and leaves, in contrast to previous *in vivo* observations in which the leaves presented the highest levels.

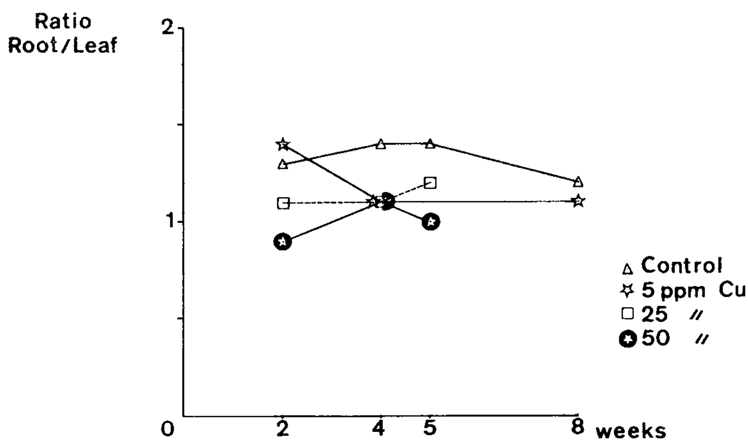

Figure 3A. Variation of the ratio values (Cu root conc./ Cu leaf conc.) with the exposure time and the different Cu concentrations applied to the plant.

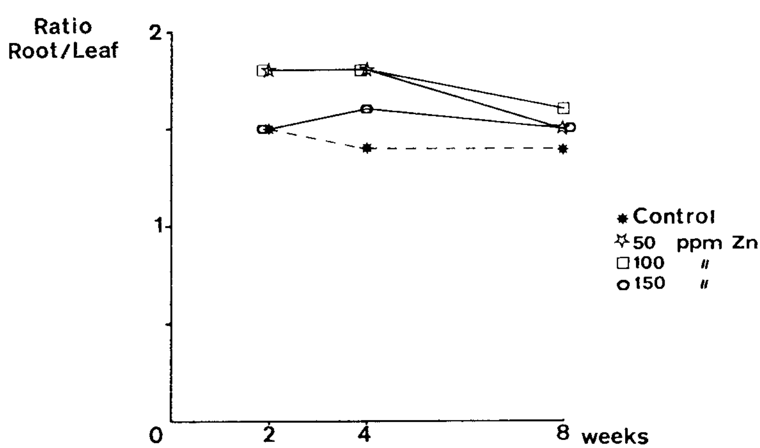

Figure 3B Variation of the ratio values (Zn root conc./ Zn leaf conc.) with the exposure time and the different Zn concentrations applied to the plant.

A very interesting feature related to the root conc./leaf conc. ratio was observed. Small variations (Fig. 3B) contrasted with the variations observed under natural conditions - 0.6-1.4 and 0.8-1.0, for samples collected in 1982 and 1983, respectively (Reboredo, 1988).

By the end of the experiment, soils enriched with Zn showed levels ranging from 370.6 to 884.0ppm Zn. These values were the differences between the levels observed in both enriched and control-soils.

The appearance of injuries in plants treated with 25 and 50ppm Cu was probably related to the increase of this element in an available form in the soil, although its solubility was considerably reduced by high pH and by the presence of sulphides and carbonates. In the present work, soil pH ranges from 6.5 in the beginning to 8.1-8.4 at the end of the experiment, constituting a strong impediment to uptake by the roots, especially at alkaline pH values.

Although the uptake of Cu by plants varies considerably, the concentration in most agricultural crops seldom exceeds 30ppm Cu, even at high rates of Cu fertilization (Mackay *et al.* 1966).

According to Howeler (1983), the toxicity for plant leaves ranges between 15 and 50ppm Cu. Leaves of *Halimione* plants treated with 25 and 50ppm Cu exhibited toxicity symptoms, respectively when leaves reached 36.5 and 53.8ppm Cu. Control leaves with 20.0ppm Cu showed no morphological damage or decrease in Chl. a and b content (Reboredo, 1988).

Roots of Cu-treated and control plants are the main accumulator organs, with a single exception in plants treated with 50ppm Cu, the leaves showing a Cu content (53.8ppm) similar to the roots (52.5ppm). Similar observations were done for the water hyacinths - *Eichhornia crassipes* (Kay *et al.* 1984), *Atriplex griffithii* and *Juncus maritimus* (Joshi *et al.* 1987) but in the latter case the studied specimens were collected in their natural habitat.

Concerning the treatments with Zn it was observed that all the Zn levels applied did not induce changes in the external morphology of *Halimione* plants. Stiborová *et al.* (1986), reported a total lack or only a tenuous influence of different Zn concentrations on ribulose-1,5-biphosphate carboxylase activity in *Hordeum vulgare* L., as well on Chl. a and b content.

From the results here presented and taking into account the maximum Zn levels observed in the roots (185.8ppm) and leaves (120.4ppm) it would be expected that a Zn-stress (due to the lowering of pH to acid values) in the natural habitat does not occur since these levels are far above those measured in populations *in situ* (Reboredo, 1988).

Halimione plants in hydroponic cultures containing 150ppm Zn exhibited after 15 days exposure pronounced changes in the starch grains (in respect to size and number) of the chloroplasts of the chlorophylline parenchyma cells. Zinc content of these leaves was 194.1ppm, clearly higher than the above mentioned level (Reboredo, *op. cit.*). These observations demonstrated how inconsistent are the extrapolations exclusively based on laboratory especially if short-term experiments were used.

Roots of Zn-treated and control plants accumulate more Zn than the leaves (1.4-1.8 times more). Thus, *in vitro* conditions roots function as a barrier to the translocation of Zn to the above-ground organs.

From the analysis of the root conc./leaf conc. ratios it is apparent that roots control at every moment the translocation of Cu and Zn to the leaves, both in treated and control plants. However, in the natural habitat it was observed that the variation of these ratios was much larger than that observed under *in vitro* conditions (Reboredo, 1988).

The Cu and Zn accumulation rate by *Halimione* growing in contaminated pots is relatively low. After 8 weeks exposure to 5ppm Cu and 150ppm Zn leaves presented 21.6ppm Cu and 120.4ppm Zn, respectively. Plant leaves grown in hydroponic culture, after 15 days exposure to the same levels had 43.2ppm Cu and 194.1ppm Zn (Reboredo, 1988).

It may be concluded that absorption from contaminated soils is not negligible, although it will be slow if compared with absorption from water, which is relatively rapid. This assumption is based on the Cu and Zn levels translocated to the leaves.

The accumulation of Cu and Zn in the different organs increased with both time and increasing concentrations of the elements in the solution, according to the pattern:

roots conc. > leaves conc. > stems conc.

A similar finding was reported by Stiborová *et al.* (1986), who observed that Cu, Zn, Cd and Pb were much more accumulated in barley (*Hordeum vulgare* L.) roots than shoots and the levels in these organs also increased, in a great majority of the cases, with increasing concentrations of the elements in the solution.

Our findings are of particular interest since other results from studies *in vivo* indicated that the leaves generally contained the highest Zn levels (Reboredo, 1988).

Typha latifolia plants grown in laboratory also accumulate considerably more Cu and Ni than plants growing in the field under contaminated conditions (Taylor and Crowder, 1983). The same authors conclude that it is not possible to make direct comparisons between the availability of metals in natural soils and in nutrient solutions. We share the same opinion, emphasising that all the extrapolations derived from plant behaviour *in vitro* must be avoided or done with great care.

Although long-term experiments are not commonly used, the effects and accumulation of contaminants in natural ecosystems remained, through decades or more, without a true perception of the multivariate interactions between compartments. Short-term experiments can give us a signal, long-term experiments perhaps a tendency. Only by resorting to both and using hydroponic and soil cultures could one get an idea of the reality.

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