Chapter 8
Plants Under Heavy Metal Stress in Saline Environments

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8.1 Introduction

The adaptation of plants to heavy metals (HMs) under conditions of salinity is an increasingly important problem due to the increasing pollution of salinized lands with HMs. At present, about 25% of all land is saline to some degree. The greatest degree of salinity occurs in arid and semiarid regions. In this context, recent years have seen much interest in river estuaries and salt marshes, in regions with ecological catastrophes – for example, the death of the Aral Sea and the extreme salinity of vast areas of land in countries surrounding it – and also in urban areas in northern latitudes, where salt is used in deicing technologies.

On the other hand, more than 200 years of human industrial development have resulted in much pollution of the environment – including salinized lands – with HMs. Such areas include those where fossil fuels are extracted and treated, those that have undergone active industrial development, urban territories, lands alongside major motorways, and agricultural lands polluted with HM due to the use of fertilizers and other methods of protecting plants. The total HM contents of polluted soils can exceed those of unpolluted soils 10–1,000-fold. Some HMs, such as Zn, Ni, Cu, Fe, and some others, are required for plant growth and development at low concentrations, whereas others, such as Cd and Hg, are not needed by plants and are very toxic to all living organisms.

Plants have developed various mechanisms that allow them to tolerate soils that are highly polluted with HMs. One of their main protective mechanisms is excessive HM chelation by SH-containing amino acids, proteins (metallothioneins), or peptides (phytochelatins) in the cytoplasm, with the subsequent transport of the resulting complexes into the vacuole. The systems of HM transmembrane movement,

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intracellular traffic, long-distance translocation throughout the plant, and accumulation in metabolically less active organs and tissues all play important roles. However, the functioning of plant defensive systems under saline conditions remains poorly studied, despite previous attempts to study plant responses to HMs under saline conditions (Helal et al. 1998).

In this work, we attempted to fill this gap by analyzing our own data and that found in the literature from the last decade. Our attention focused on analyzing the characteristics of the responses of plants from various groups (glycophytes, halophytes, and macrophytes) to the stress resulting from the combined effects of two damaging factors – HMs and salinity – at the level of integral physiological processes such as linear growth and biomass accumulation, as well as the abilities of plants to absorb HMs, transport them to aboveground organs, and accumulate them, which is a characteristic feature of many metallophytic species. The interactions between the effects of HMs and salt are described in terms of antagonism, additivity, and synergism. In addition, we attempted to understand the cellular and molecular mechanisms that determine plant life under heavy metal stress in saline environments.

8.2 Combined Effects of HMs and Salinity on Plants from Various Ecological Groups

The main task of this chapter is to consider the effects of salinity on plant tolerance to HMs and on HM accumulation in plants. Special attention is paid to the partitioning of HMs between underground and aboveground plant organs.

8.2.1 Halophytes of Salt Marshes

Many of the investigations of halophytes have been performed with plants from natural habitats that differ in terms of the ratios between important factors such as the degree of salinity and HM concentrations. Such differences are well manifested in river estuaries and coastal lagoons. Investigations in this direction were started some time ago and performed in several estuaries, such as the York River (USA) (Drifmeyer 1981), Ems Estuary salt marshes (Holland) (Otte et al. 1991), Suir Estuary salt marshes (Ireland) (Fitzgerald et al. 2003), and some others.

Relatively independent changes in the two parameters, HM concentration and salinity, are characteristic of such habitats, and this hampers any analysis of the action of each factor. Problems also arise due to the incomplete characterization of each of the locations where plant material was collected, and differences in plant species composition. Therefore, the most valuable reports are those where several plant species that inhabit the same region are compared.

Among the early studies, the investigation performed in salt marshes along the salinity gradient of the York River estuary (Drifmeyer 1981) seems characteristic. While a 3.5–4.5-fold salinity gradient was attained across the six locations tested, the levels of Mn, Cu, Zn, and Ni in *Spartina alterniflora* plants varied 2.5–3.5-fold, and the level of Fe by as much as ninefold. However, the authors concluded that, in plant tissues, "the levels of these metals … did not correlate strictly with the salinity of either river or sediment pore water." In the absence of direct data on the metal content of the water, and due to the high probability that there were several sources of contamination across the rather large territory studied (more than 40 km along the coast), which had a dense human population, the author's conclusion that "… trace element uptake … is not greatly affected by salinity" can only be considered a preliminary one.

The work of Otte et al. (1991) presented information on the accumulation of Zn, Cu, and Cd in the roots and shoots of four plant species in salt marshes at two locations in the Ems Estuary, upstream (Dyksterhusen) and downstream (Petkum) of Ems (a difference of 2′ N in the northerly direction). Rather representative are data for *Triglochin maritima* (Juncaginaceae) and two dicotyledonous species (*Aster tripolium* and *Spergularia maritima*, Cariophyllaceae), which grew under moderate salinity and strong HM pollution (Dyksterhusen) or heavy salinity and moderate HM pollution (Petkum). It turned out that, in the region of severe pollution (Dyksterhusen), all three HMs (Cd, Cu and Zn) accumulated to the greatest degree, whereas in the area with high salinity (Petkum), they reduced their accumulation in the roots but favored HM – especially Zn – translocation to shoots. As a result, the accumulation factor increased from 0.1 to 3.5 for Cu and from 0.4 to 6.3 for Zn (data for Cd were not significant). In contrast, the Zn and Cd contents in the roots of *T. maritima* and *A. tripolium* doubled in the region of high salinity (the effect of salinity on Cu was less pronounced or absent), but their translocation to shoots was suppressed.

More complete data were presented by Fitzgerald et al. (2003) for the Suir Estuary (Ireland). It turned out that, at four locations along the inner Suir Estuary that were analyzed in the study, Cu and Pb salt concentrations in sediment samples declined approximately twofold from the upper to lower point along the stream. However, there was no significant difference between the Cu concentrations in the roots of six tested plant species. Another pattern was observed for Pb. In two species, the highest Pb accumulation in the roots was detected mostly downstream (Checkpoint: the area with the least metal but with the highest salt concentration). In this area, the Pb content was 239.0 μmol kg^{-1} dry weight in *Aster tripolium* and 517.4 μmol kg^{-1} dry weight in *Spartina* spp., which was more than twice as high as in plants growing in other habitats. Correspondingly, the highest Pb concentrations in shoots were 360.5 and 290.5 μmol kg^{-1} dry weight, which also differed from those in plants growing in other habitats. Despite the fact that there were no significant differences between the Cu concentrations in the roots of all of the studied species at different locations in the estuary, the highest concentration in the shoots of *Schoenoplectus tabernaemontani* was observed immediately downstream of the site of pollution, significantly (threefold) exceeding the value for the unpolluted region; in *Spartina spp*., the highest Cu concentration was detected in the next region downstream (fourfold higher than in the region located upstream). In general, the authors concluded that there was a common trend in the Cu and Pb contents of the two dicotyledonous species studied: the shoot/root ratio displayed a tendency to increase as the salinity increased. This trend was particularly evident for Pb in *A. tripolium*.

Only indirect data are available on the effects of salinity on HM accumulation by plant tissues in Tagus Estuary salt marshes (Portugal) (Reboreda and Cacador 2007; see references therein concerning the Tagus Estuary). Cu, Pb and Cd accumulation in the roots, stems and leaves of two plant species was investigated. *Spartina maritima* (Poales, Poaceae) plants inhabited the low marsh, and so were subjected to more severe salinity than *Halimione portulacoides* (Caryophyllales, Chenopodiaceae) plants, which inhabited the middle marsh. It was established that *H. portulacoides* accumulated twice as much Cd and Pb as *S. maritima* (taking into account the difference between the HM contents of the habitat sediments). Relative Cu absorption by the roots of *S. maritima* was significantly lower than that of the roots of *H. portulacoides*. However, under severe salinity, the Cu concentration in the leaves of this plant species was slightly higher than in *H. portulacoides*.

Among the studies of HM pollution in the Sheldt (Belgium) Estuary, Du Laing et al. (2008; see also references on the Sheldt Estuary therein) applied a special approach. Soil samples and sediments from four locations were flooded with waters containing different levels of salinity (0.5, 2.5, and 5 g l^{-1} NaCl for 250 days), and duckweed (*Lemna minor*) was grown (for 4 weeks) on the surface water. The salinity was found to primarily enhance the mobility of Cd, and its uptake by duckweed increased by as much as fivefold compared with the control. Moreover, the effect was also observed at a lower salinity. When the salinity of the flood water was increased from 0.5 to 5 g l^{-1} NaCl, the Cd concentrations in duckweed increased by a factor of 4. However, Zn concentrations in duckweed were only slightly enhanced by the salinity, while Ni uptake was not affected at all (excluding one treatment, where a large increase was observed). Although an effect of salinity on the total Cu concentration in surface water samples was not detected, the copper concentrations in the plants slightly (but significantly) increased.

8.2.2 *Other Halophytes*

In some studies, terrestrial halophytes have been used to study tolerance to HMs and as putative candidates for phytoremediation. Such works are based on the idea (Ghnaya et al. 2005) that "salt-tolerant (halophytic) plants would be better adapted to coping with environmental stresses, including HM than salt-sensitive (glycophytic) crop plants." To confirm such a possibility, the authors point to the rather high tolerance of two halophytes, *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*, to a high concentration (100 μM) of Cd, one of the most toxic HMs. After 15 days of treatment, the authors did not observe any visible damages, although the whole-plant biomass of *S. portulacastrum* decreased by 40%, and that of *M. crystallinum* dropped by 70% (Ghnaya et al. 2005, 2007a).

Most investigations of the effects of salinity on HM accumulation in plants have been performed with Cd. In work focusing on one of the aforementioned halophytes, *S. portulacastrum* (sea purslane), experiments were performed with rooted plants (Ghnaya et al. 2007b). Plant growth on a medium containing 50 or 100 μM Cd for 30 days resulted in a 50% inhibition of biomass accumulation and in the appearance of necrotic lesions on the leaves. Upon combined treatment with 100 or 400 mM NaCl and cadmium, the necrotic lesions were absent. Plant dry weight approximated and in some cases significantly exceeded control plant biomass. Salinity strongly reduced the concentration of Cd in roots and shoots; however, due to the enhanced growth of NaCl-treated plants, the Cd content per plant increased significantly.

In the work performed with rooted cuttings of a typical Mediterranean halophyte *Tamarix smyrnensis* Bunge (salt cedar) (Manousaki et al. 2008), $Cd(NO_3)_2$ was added to the soil at a concentration of 16 ppm per 1 g of soil dry weight. During ten weeks of plant growth in the presence of Cd or 0.5% NaCl, the plants did not display any signs of toxicity due to these stressors. In contrast, 3.0% NaCl strongly suppressed salt cedar growth in terms of height and biomass accumulation. The concentration of Cd in the absence of salt increased to 2.45 ppm in roots and 3.3 ppm in shoots. As a result, total Cd removal from the soil by the whole plant increased from 9.4 μg in the absence of salt to 19.7 μg at 0.5% NaCl and to 38.3 μg at 3.0% NaCl. In other words, total Cd removal increased by a factor of four when the salinity was increased from 0 to 3%.

Lopez-Chuken and Young (2005) presented data on the effect of NaCl on Cd absorption by seven species of halophytes (and also by four crops, see below). Plants were grown in soil collected from a sewage disposal farm run and containing 28 μg of Cd per 1 l of soil pore water**.** Salinity was created through the addition of 100 mM NaCl (or $NaSO_4$) to the calculated water-holding capacity (WHC) of soil. For five of the seven tested species – *Cynodon dactylon*, *Sorghum* × *drummondii*, *Paspalum vaginatum*, *Atriplex hortensis*, and *Kochia scoparia* – a significant increase in the Cd concentration in shoots was observed (not more than double the average; the highest increase, 2.8 fold, was observed for *A. hortensis*). No significant effect was detected for *Asparagus* sp., *Parthenium argentatum.* When calculating per vessel (per plant), the salinity-induced increase in the Cd content was also especially strong in *A. hortensis* (threefold), just like the absolute value (taking into account the large biomass of this plant, which exceeds those of other halophytes 15-fold).

An investigation of the effect of salinity on Cd and Zn uptake was performed with *Leucaena leucocephala* (a leguminous tree) seedlings in Egypt, where the usage of saline waters for irrigation is a common practice (Helal et al. 1999). It turned out that, after thirty years of attempting to improve poor soils where this work was performed, the soil had become almost tenfold more polluted with Cd and fourfold more polluted with Zn. It was established that, after six months of supplying 10 mM NaCl, the Cd concentration in leaves increased 2.3-fold and the Zn concentration 1.5-fold. The salinity affected neither HM concentrations in the roots and stems nor biomass production. It significantly increased the total HM content per plant (1.23–1.25-fold for each HM) and also the transfer factor of each HM (metal concentration in plant/metal concentration in soil) by 2.36-fold for Cd and 1.46-fold for Zn.

Another work with four salt-tolerant plant species was performed in Beirut, Lebanon (Zurayk et al. 2001). These halophytes were *Hordeum vulgare*, recommended by the authors as a salt-tolerant crop, and also *Plantago coronopus* L., *Portulaca oleraceae* L. and *Inula crithmoides* L.; the two latter species “are edible and have been recommended for usage in saline agriculture“ (we consider the data obtained for barley below, together with other works performed with this crop). These authors studied the effects of two levels of NaCl salinity (9 and 18 dSm^{-1} approx. 100 and 200 mM, respectively) (control plants were treated with tap water containing 0.5 dSm^{-1} NaCl) on HM accumulation in shoots. Experiments were performed with Cd, Cr, and Ni at concentrations of 2, 4, and 10 ppm, respectively. By the twenty-first day of saline treatment, Cd and Ni had caused a significant decrease in the dry biomass accumulation of *P. oleracea* but had exerted no effect on the other plant species. Metal accumulation in these four plant species was generally enhanced by the 9 dSm^{-1} but not the 18 dSm^{-1} treatment. Significant shoot metal accumulation was demonstrated for Cd and Ni in *P. oleracea.* In contrast, in *P. coronopus*, a significant decrease in the concentrations of these metals in shoots was observed at both salinity levels, and it was proportional to the degree of salinity, whereas the Cr concentration was increased. A very high degree of scatter in the data did not allow significant differences to be established for other species.

Kadukova and Kalogerakis (2007) studied the effect of salinity on Pb accumulation in roots and leaves of salt cedar (*Tamarix smyrnensis* Bunge). $Pb(NO_3)_2$ was added to the soil at the concentration of 800 ppm per dry weight of soil organic substance; salinity was created by watering the soil with tap water containing commercial edible sea salt. For ten months, the salinity did not exert any influence on dry weight accumulation, but biomass decreased significantly with Pb in the absence of salt or at a high salt concentration (3%). The highest biomass was produced by plants treated with Pb and watered with 0.5% salt solution; it exceeded the control treatment by 34%. Although Pb concentrations in roots that underwent this treatment did not differ from those in other Pb treatments, the concentrations in leaves were more than twofold lower than in those from other Pb-treated plants. However, because of a high degree of scatter in the data, no significant differences from the control treatment were recorded.

In our laboratory, the mechanisms of adaptation to salinity and HM action of another facultative halophyte, the common ice plant (*Mesembryanthemum crystallinum* L.), were investigated over many years (Kholodova et al. 2002). These plants can develop under conditions of rather severe salinity (up to 400 mM), and manifest substantial tolerance to Cu and especially Zn; they completed their life cycles at $CuSO_4$ or $CuCl_2$ concentrations of up to 50 μM and $ZnCl_2$ concentrations of up to 800 μM (Kholodova et al. 2005). Nevertheless, damage from the Cu manifested itself as a decrease in or loss of turgor, the appearance of necrotic lesions, and a sharp suppression of biomass accumulation. In the presence of excess Zn, growth suppression was less substantial.

In these studies, it was shown that 400 mM NaCl applied together with HMs partially and in some experiments almost completely neutralized the damaging actions of copper and zinc on biomass accumulation; the leaves retained their turgor; other signs of metal toxicity were much less pronounced. At the same time, this severe salinity did not reduce HM concentrations in the leaves of the common ice plant (Fig. 8.1), which exceeded the control values by factors of 7–8 for Cu and 20–40 for Zn. These concentrations were maintained for both separate HM addition and HM addition in combination with NaCl. Taking into account the great biomass accumulation that occurred upon the combined action of the two factors, the total Cu and Zn accumulation per plant (vessel) increased by 30–80% (Fig. 8.2).

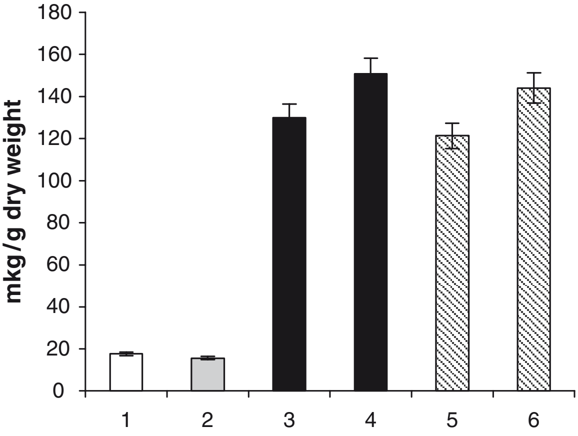

Fig. 8.1 Copper accumulation in common ice plant leaves on the seventh day of the experiment. *1*, Control; *2*, 400 mM NaCl; *3*, 25 µM $CuSO_4$; 4, 50 µM $CuSO_4$; *5*, 400 mM NaCl + 25 µM $CuSO_4$; *6*, 400 mM NaCl + 50 µM $CuSO_4$

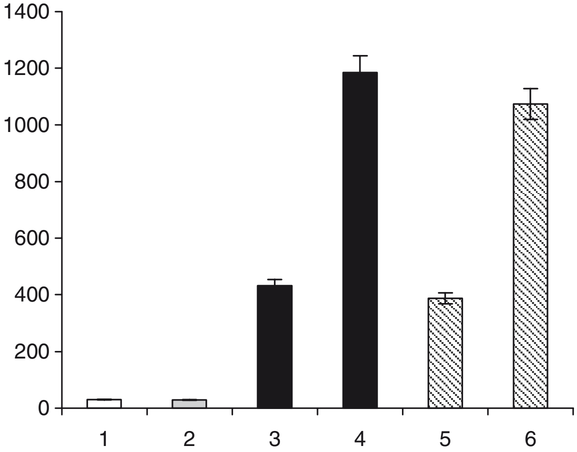

Fig. 8.2 Zinc accumulation in common ice plant leaves on the seventh day of the experiment. *1*, Control; *2*,400 mM NaCl; *3*, 250 µM $ZnSO_4$; 4, 500 µM $ZnSO_4$; *5*, 400 mM NaCl + 250 µM $ZnSO_4$; *6*, 400 mM NaCl + 500 µM $ZnSO_4$

8.2.3 Crop Plants

Most crops are glycophytes, and although it is quite evident that their initial salt tolerance is lower than that of halophytes, relatively salt-tolerant cultivars have been created by breeding. At the same time, the large aboveground green masses of particular plants from this group, or large stand densities (in the case of cereals), make crops promising candidates for use in HM phytoremediation.

8.2.3.1 Monocotyledonous Crops

Investigations of the effects of salinity on plant tolerance to HMs (Cd primarily) and the accumulation of HMs in the roots and aboveground organs of some species and cultivars of wheat (*Triticum* spp.) have been performed in various regions.

Greenhouse experiments with several wheat (*Triticum aestivum* L. and *T. durum* L.) genotypes grown on Cd-polluted soil from a field in Qom province, central Iran, have been performed (Khoshgoftar et al. 2004; Khoshgoftarmanesh et al. 2007). Different salinity levels (0, 60, 120, and 180 mM NaCl) were created by adding NaCl to irrigation water. Increasing the salinity significantly reduced the shoot weight (*T. aestivum*, cv. Rushan) (the strongest inhibition was approximately twofold); however, the concentration of Cd in shoots significantly increased (by a factor of 3.5 in comparison with the controls at the highest NaCl concentration). The highest ability to accumulate Cd was observed for *T. aestivum*, cv. Kavir and *T. durum*, cv. Durum; these accumulated about 0.1 mg Cd per kilogram dry weight at 180 mM NaCl.

Salinity exerted a different effect on Zn accumulation. Increasing the NaCl concentration actually slightly decreased the Zn concentrations in shoots (Khoshgoftar et al. 2004). However, none of the cultivars displayed a significant effect of salinity on HM accumulation in wheat shoots (Khoshgoftarmanesh et al. 2006). For the cultivars tested, a negative correlation was observed between Cu and Zn accumulation, in particular upon additional Zn fertilization (Khoshgoftar et al. 2004).

Some researchers showed that the presence of chlorine ions in the soil solution reduced the cadmium absorption of the soil, which ultimately resulted in enhanced HM accumulation in plants (Smolders et al. 1997; Weggler-Beaton et al. 2000).

Weggler (2004) performed a pot experiment with sludge application rates of 0, 20, 40, and 80 g sludge per kilogram soil and chloride concentrations in the soil solution ranging from 1 to 160 mM. The Cd concentrations in shoots and soil solution increased with sludge application rate up to 40 $\mathrm{g\,kg^{-1}}$, but slightly decreased with the 80 $\mathrm{g\,kg^{-1}}$ sludge treatment. The soil and plant shoot concentrations of Cd were positively correlated with the soil chloride concentration.

Muehling and Lauchli (2003) studied two wheat genotypes (*T. aestivum* and a salt-tolerant amphiploid, *T. aestivum* x *Agropyron elongatum* Host.); the plants were grown hydroponically. NaCl (75 mM) stress and Cd (10 μM) stress led to significant decreases in shoot yield in both wheat genotypes; however, combined treatment with the two stresses did not lead to further decreases in shoot and root biomass. Upon the combined action of HM and NaCl, a significant increase in the Cd concentration was only detected in shoots of the salt-sensitive genotype.

The high toxicity of Cd makes it important to check for its possible presence in the edible parts of crops. Norvell et al. (2000) (North Dakota, USA) compared 124 samples of grain collected from a field of durum wheat (*T. turgidum* L. subsp. *durum* (Desf.) Husn.), cv. Munich, along with appropriate soil samples differing in their levels of salinity. The amount of Cd in the durum grain varied widely, from 0.025 to 0.359 mg kg^{-1}; its accumulation in the grain was strongly and positively associated with soil salinity (as represented by soluble chloride, soluble sulfate, or extractable Na). A strong association of the Cd in durum wheat grain with the soluble Cl^- in soil from the region of the root zone 0–15 cm deep was observed.

Some studies on the effects of salinity on plant tolerance to HM have been performed with barley (*Hordeum vulgare* L.) plants. Thus, Huang et al. (2006a, b) showed that adding NaCl to a water culture reduced Cd accumulation in barley plants.

Smykalova and Zamecnikova (2003) showed that biomass accumulation in barley plants grown hydroponically for seven days was equally suppressed in the presence of 10 μM Cd and 100 mM NaCl (down to 76.7% and 74.4%, respectively); the combined action of these factors only slightly enhanced the negative effect (suppressed to 61.6%). A similar inhibitory effect on Cd accumulation in the roots (61.0% of the accumulation of the control) was exerted by a combined treatment; a slightly lower effect was observed on shoots (69.3% of the accumulation of the control). This indicated that Cd translocation to shoots was slightly less inhibited than its accumulation in the roots.

The purpose of the experiment performed by Wahla and Kircham (2008) was to determine the effect of NaCl irrigation on the displacement of HMs applied to soil columns containing barley plants. The concentrations of Cd, Fe, Mn, Ni, Pb, and Zn that leached out of the columns upon irrigation with NaCl (10 g l^{-1}) were higher than those obtained with tap-water irrigation. NaCl significantly increased the concentrations of Cd and especially Mn (up to 200% of control) and Ni (up to 150% of control) in the shoots of barley plants. The presence of NaCl in the irrigation water does not affect the concentrations of Cu, Pb, or Zn in shoots or roots, whereas the Ni concentration in roots strongly (but insignificantly) decreased under saline conditions (Wahla and Kircham 2008).

Zurayk et al. (2001) demonstrated a dependence of HM accumulation in barley plants grown on perlite on the level of salinity. None of the HMs applied at the concentrations used (Cd at 2 ppm, Cr at 4 ppm and Ni at 10 ppm) in combination with NaCl (9 or 18 dSm^{-1}, i.e., ~100 or ~200 mM) inhibited biomass accumulation. It was established that 100 mM NaCl enhanced HM accumulation in shoots of barley plants; however, doubling the NaCl concentration slightly reduced HM accumulation.

The effect of salinity on the tolerance of maize (*Zea mays* L.) to HM was evaluated in pot experiments utilizing polluted desert soil (Helal et al. 1996). NaCl increased both the concentrations of the HMs studied (Zn, Cu and Cd) in soil saturation extracts and their accumulation in roots, but it accelerated root mortality.

Plants irrigated with saline water accumulated more HM than those watered with tap water. In another study with maize plants (Lopez-Chuken and Young 2005), after three weeks of plant growth on a sewage disposal farm run, root dry weight was significantly reduced for the plants undergoing 200 mM NaCl treatment, and this was accompanied by a threefold enhancement of the Cd concentration compared to that of the control.

8.2.3.2 Dicotyledonous Crops

In experiments performed with potato (McLauchlin et al. 1994; McLaughlin et al. 1997), the Cd concentrations in 89 samples of tubers from various regions of Australia were compared with some indices characterizing the soils from the exact same locations at each site. The total Cd concentrations in soils varied from 0.01 to 0.17 mg kg^{-1}, with some soil solutions having Cd concentrations of up to 222.4 nM. A wide variation in tuber Cd concentration was observed across sites, from 0.005 to 0.232 mg kg^{-1} fr wt. Potato tuber Cd concentrations were positively related to soil water-extractable Cl ($R^2=0.62$, $P<0.001$) in the topsoil.

A team of researchers performed a series of studies on Swiss chard (*Beta vulgaris* L., cv. Fordhooe Giant), which is known to be a Cd-accumulating plant species (Smolders and Laughlin 1996; Smolders et al. 1998; Weggler-Beaton et al. 2000). In soil culture, NaCl (or $NaNO_3$) addition to the nutrient solution decreased plant growth significantly, starting from a concentration of 60 mM. The addition of NaCl (but not $NaNO_3$) increased the Cd concentrations in shoots: in 120 mM NaCl treatment, the Cd concentration was almost twice as high as in the zero-salt treatment (Smolders et al. 1998).

In another series of experiments, plants were grown in biosolid-amended soil. Treating the plants with moderate NaCl concentrations (not exceeding 1.6 $g\,l^{-1}$) for 30 days did not affect plant growth. Cd concentrations in shoots of Swiss chard increased linearly with increasing Cl concentration in soil solution (Weggler-Beaton et al. 2000).

Lopez-Chuken and Young (2005), along with data on several halophytes (see “Other Halophytes”) and *Zea mays*, presented information on two crops, *Brassica juncea* and *Medicago sativa*, that are putative candidates for Cd phytoremediation. Salinity was created with 100 mM NaCl (and also Na_2SO_4) treatments for 3–6 weeks. These treatments did not significantly affect plant biomass but they did markedly influence Cd accumulation in them. Under saline conditions, the Cd concentrations in *B. juncea* shoots increased by factors of 3–4 compared with those of the controls, whereas the Cd concentrations in the roots increased by factors of 2.3 or less. Therefore, the Cd_{shoot}/Cd_{root} ratio strongly increased (from 1.10–1.63 in the absence of NaCl to 2.02–2.65 under saline conditions). In one of the cultivars, the transfer factor ($Cd_{shoot}/Cd_{solution}$) increased under salinity from 339 to 439. When calculated for plants in a single pot, the Cd content in shoots was 15-fold higher than in roots. NaCl treatment increased the Cd content in *M. sativa* as well, although this effect was less pronounced, and much of the (additionally) absorbed NaCl remained in the roots.

These authors also showed that NaCl treatment of *B. juncea* significantly increased Zn uptake (data not shown), but that Cu concentrations exhibited an irregular pattern among the treatments.

In an investigation of the adaptive mechanisms of another member of the *Brassica* genus, *B. napus*, which was started in our laboratory, it was demonstrated that a combined plant treatment with 100 μM Cu and 200 mM NaCl weakened the symptoms of Cu toxicity, whereas Cu concentrations in different leaves exceeded control values by factors of 3–4.

The effect of salt water irrigation (0.8 $g\,l^{-1}$ for 9 weeks) on the uptake of Cd and Ni by *Spinacea oleracea* L. (Helal et al. 1998) was investigated. Salt water irrigation, which is a commonly accepted practice in northwest Egypt, stimulated root development and enhanced the extractability of Cd and Ni from the soil as well as their concentrations in plants.

Experiments were also performed with *Sesamum indicum* L., an important oil seed crop predominantly grown on dry and salt-affected soils in India (Bharti and Singh 1994). To this end, seedlings were grown for five days on 1 mM HM (Pb^{2+}, Cu^{2+} and Cd^{2+}) solutions, which were used in pairs in the ratio 1:1. Pb + Cd and Cu + Cd induced significant and especially strong growth inhibition. The presence of NaCl largely eliminated the negative effects of the metal combinations on the fresh weight of roots, and it caused an increase in the tissue dry mass in most cases. However, a recovery in the leaf fresh weight was only observed when NaCl (2 EC or 10 EC) was added together with Cu^{2+}+ Cd^{2+}.

It was established that sunflower (*Helianthus annuus* L.) plants grown on soils with increased levels of chlorides resulted in enhanced Cd accumulation in the seeds (Li et al. 1994). Thus, in one of two regions that contained very similar Cd concentrations (0.43 and 0.40 μg) but differed twofold in their chloride contents, the content of Cd in seeds was 3.5-fold higher in plants grown in an elevated chloride concentration.

Singh et al. (2003) established that NaCl enhanced the damaging effects of lead acetate on five-day-old *Vigna radiata* L. seedlings. Although these stressors (separately or together) barely affected germination, significant inhibition of root and shoot growth was observed. One millimolar Pb only slightly reduced seedling dry weight (that of shoots by 8% and of roots by 20%). Combined action of the two stressors exerted a synergistic effect that enhanced with increasing NaCl concentration. The addition of NaCl markedly reduced lead accumulation in the seedling roots, from 5 to 1.3–1.0 mg g^{-1} dry weight upon the addition of 6 EC and 12 EC NaCl, respectively. Lead concentrations in the roots exceeded those in the shoots by a factor of 20, which was evidently due to its low capacity to translocate to the aboveground organs.

8.2.4 Water Macrophytes

The results of Du Laing et al. (2008), who demonstrated a significant increase in Cd accumulation with a moderate increase in the NaCl concentration in water for a species of duckweed (*Lemna minor* L.), have already been described (see the end of "Halophytes of Salt Marshes"). At the same time, salinity did not have any significant influence on Cu or Ni uptake.

An interesting study was performed with the same species of aquatic macrophyte (*Lemna minor* L.): it was established that the technetium (Tc) radionuclide ^{99}Tc is usually present as pertechnetate (TcO_4^-), the only form of Tc that is known to be taken up by plants (Hattink et al. 2001, The Netherlands). Earlier, these authors showed that there was no competition between anions during their uptake. In the case of Tc, absorption evidently occurs through the leaves because, under anoxic conditions, this element is reduced in the sediment to forms that are unavailable to plants.

In this study, it was established that salinity was positively correlated with accumulation because Tc mainly accumulates in the cell walls and water free spaces of plant tissues. But that the intracellular uptake of TcO_4^- by duckweed was independent of chloride concentration. This report also indicated that, in marine microalgae, increasing the salinity enhanced the rate of Tc uptake.

Demirezen (2007) studied the effects of Ni and salinity on another *Lemna* species (*Lemna gibba*, fat duckweed). The Ni concentration was 20 mg l^{-1} (~340 μM). It turned out that applying moderate salinity (125 mM NaCl, 25% of the salinity of seawater) for 10 days resulted in a substantial increase in biomass accumulation for this typical freshwater plant. However, this was accompanied by an almost twofold decrease in the Ni concentration in the plant. Greater levels of salinity (250–375 mM NaCl) suppressed Ni accumulation further and inhibited plant growth (at 500 mM NaCl, the net growth rate became negative). It appeared that there was a negative relationship between water salinity and tissue Ni concentration ($R = -0.72$, $P < 0.05$).

More comprehensive work was performed by a team of Swedish researchers with a typical water macrophyte, pondweed (Greger et al. 1995). For 2 weeks, plants of the submerged macrophyte *Potamogeton pectinatus* L. were grown either in Cd-contaminated sediment and water of varying salinity or in water with Cd and varying salinity but no sediment (Greger et al. 1995). It was shown that Cd uptake by *P. pectinatus* from water decreased with increasing salinity, but that Cd uptake increased with increasing salinity in the presence of sediment.

In one of the recent works from this team (Fritioff et al. 2005), along with *Potamogeton natans* Michx. (which has thick leaves that float on the surface of the water), *Elodea canadensis* L. (which has very thin submerged leaves) was used in experiments. These plants were grown in the presence of Cd (1 μM), Cu (1.5 μM), Zn (20 μM), and Pb (4 μM) in combination with salinities of 0, 0.5 (slightly increased salinity), and 5% (one of the highest salinity levels found in stormwater). This work permitted a comparison of the effect of salinity on the accumulation of four HMs in two plant species.

For 48 h, the salinity and HMs did not suppress plant growth significantly; no signs of their toxicity were observed either. The effect of salinity on the concentrations of the HMs tested was rather specific. Pb accumulation was generally unaffected or barely affected by (temperature and/or) salinity. At the same time, its concentration in *Elodea* was more than tenfold higher than in pondweed. This fact, and also the absence of a temperature dependence of Pb accumulation, permitted the hypothesis that the main mechanism of its uptake was its absorption on the leaf surface, which is much more substantial in *Elodea* than in pondweed. Concentrations of Cd and Cu also were much higher in *Elodea*, by twofold or more. In both *Elodea* and pondweed, salinity resulted in a decrease in the concentrations of the HMs in the plants. Finally, the two plant species barely differed in their Zn accumulation from a 10 μM solution, in spite of the fact that Zn concentrations were 3–6 times higher in control *Elodea* plants than in pondweed. Unlike other HMs, moderate salinity induced only a slight inhibition of Zn uptake, and severe salinity was also not very efficient at producing inhibition.

Due to pondweed having a much greater biomass than *Elodea*, the total Zn accumulation (mg/container) in pondweed greatly exceeded the corresponding value for *Elodea*. However, the total accumulation of Cd and Cu at optimum temperature was roughly the same for the two plant species. In the case of Pb, this value in *Elodea* was six- to eightfold higher than in pondweed in all treatments. It seems probable that such great values are due to passive uptake via absorption on the leaf surface and in the leaf apoplast. It does not appear to be very probable that such effects could be maintained under long-term HM action. (This problem could be clarified by studying the dynamics of HM uptake by plants).

8.3 Mechanisms of the NaCl–HM Interaction

Let us now consider the basic principles that determine the responses of various plant species and ecological groups to the combined action of salinity and HMs (or how salinity modifies plant responses to HM), and what the causes of typical effects may be.

Comparative data on the effect of each factor and combinations of them on the basic integral biological indices, linear growth and biomass accumulation, have been presented in only a few works. Nevertheless, it seems evident that all three possible responses to the combined action of a HM and salinity (in comparison to the action of the HM without salinity) were observed: (1) enhanced biomass accumulation; (2) suppressed biomass accumulation, and; (3) the absence of any effect.

Significant data indicating the weakening or neutralization of HM toxicity and an improvement in the plant state are presented in only a few reports, and were obtained mainly for halophytes, such as sea purslane, salt cedar, the common ice plant, and the common duckweed (Ghnaya et al. 2007b; Kadukova and Kalogerakis 2007b; Volkov et al. 2006; Demirezen 2007).

A neutral effect, where salinity did not substantially affect the plant state, did not enhance biomass accumulation, and also did not lead to its reduction, in addition to HM-induced growth suppression, was observed more frequently (Helal et al. 1998, 1999; Weggler-Beaton et al. 2000; Weggler 2004). Such responses have not only been observed for halophytes.

However, for glycophytes, a salt-enhanced negative effect of HMs on biomass accumulation is more characteristic. Such a situation even arises at moderate salinities (75–100 mM); in some cases, a synergistic inhibitory effect was observed (wheat, barley)

(Muehling and Lauchli 2003; Khoshgoftar et al. 2004; Khoshgoftarmanesh et al. 2006; Smykalova and Zamechnikova 2003).

In some works, when the effects of varying degrees of salinity were examined, different responses were obtained for different salt concentrations: positive, neutral, or negative responses (Zurayk et al. 2001; Demirezen 2007; Lopez-Chuken and Young 2005; Kadukova and Kalogerakis 2007; Khoshgoftarmanesh et al. 2006).

Bearing in mind such observations, it is not entirely unexpected that salinity exerted a positive effect that manifested in an increased root length (up to 50%) in *Spinacea oleracea* (Helal et al. 1998), because the concentration of NaCl used was not high (~14 mM). It is more difficult to explain the results obtained by Bharti and Singh (1994) on *Sesamum indicum* seedlings. Combined treatment with two HMs at extremely high concentrations (1 mM each) strongly inhibited growth, as evaluated by the accumulation of root or leaf biomass. However, the addition of a relatively low NaCl concentration (2 or 10 EC) abolished this toxic effect or reduced it markedly.

The effect of salinity on HM accumulation in various organs of plants of various species and various ecological groups has been studied more comprehensively. Most works present data on the HM concentration per dry weight of plant organs; also, relatively frequently, data are calculated per total organ biomass or per pot (container) (when the same number of plants are used in each treatment), or (for crops) per unit of stand area.

Many researchers have reported an effect of NaCl on HM accumulation in shoots alone; only two reports have focused on this effect in underground organs. An increase in the Cd concentration under salinity was reported for potato tubers (McLauchlin et al. 1994, 1997) and the shoots of some halophytes, barley, and Swiss chard (Lopez-Chuken and Young 2005, Zurayk et al. 2001; Smolders and McLaughlin 1996; Smolders et al. 1998). In some cases, an observed increase in the HM concentration occurred with biomass reduction, and the authors consider this to be a positive effect of salinity (Lopez-Chuken and Young 2005).

In other research, there was no substantial change in the HM concentration in shoots (Cd in duckweed, Cu and Zn in the common ice plant, Cu in rapeseed; Volkov et al. 2006, and presented here). However, it was repeatedly stated that there was a significant decline in the HM concentrations in the shoots of some plant species (Cd and Cu in barley) (Smykalova and Zamechnikova 2003; Demirezen 2007; Huang et al. 2006a, b; Wahla and Kirkham 2008). The effect depended on NaCl concentration (Weggler 2004).

In a few works permitting a comparison of the effect of salinity on HM accumulation in roots and in shoots, similar, very strong increases in the Cd concentration in salt cedar were found (Manousaki et al. 2008). A most interesting result was obtained by Helal et al. (1999), when the HM concentration in *Leucaena leucocephala* shoots was found to be markedly and significantly higher than in its roots; this was considered as increase in the transfer factor (i.e., enhanced translocation from the root into aboveground organs).

Although under the combined action of salinity and HM increased biomass accumulation has never coincided with an increase in HM concentration, the HM content per plant (shoot) or area (volume of nutrient medium, container) has increased, and this parameter is important when plants are used for HM phytoextraction (Salt et al. 1998; Lopez-Chuken and Young 2005; Manousaki et al. 2008).

In general, the information available at present reliably indicates that salinity could favor the extraction of HMs from the rooting medium (substrate, soil, sediment) and their accumulation in aboveground plant organs. Such a response to salinity has reliably been shown for Cd in several plant species at various Cd and NaCl concentrations, and at various ratios of them. It was established that this effect was (1) species and cultivar specific; (2) not reproduced in some cases, even for the same plant species, evidently due to differences in the conditions used in the experiments or some specific features of the natural populations; (3) regularly manifested for Cd but less frequently for other HMs (Zn, Cu, Ni); (4) was not usually reproduced when NaCl was replaced with Na_2SO_4; and (5) was regularly manifested only when the plants were grown on solid substrate (soil, bottom sediment, etc.). This last point makes it rather probable that the positive action of salinity (including sea water) was due to increased HM mobility, evidently due to ion exchange, with the transition of the HM from an oxidized to a reduced form (Du Laing et al. 2008). The formation of organic complexes was considered to be another possible cause, because it was demonstrated that "… NaCl treatment raised the concentration of organic carbon" (Helal et al. 1999; Kirkham 2006).

A deeper study permitted an elucidation of deeper interrelations between HMs and salinity.

Earlier, in works performed mainly on aquatic organisms, a so-called free ion activity model (FIAM) was formulated that postulates that the uptake of metal by an organism is proportional to the free ion concentration of the metal in the surrounding solution (Allen et al. 1980, according to Degryse et al. 2006). However, some contradictions with this model were soon found, even in early studies of the effect of salinity on HM accumulation (see, for example, McLaughlin et al. 1994, 1997, and references therein). A large body of information is now available which indicates that HM chlorocomplexes but not free ions play a major role at the stage of HM penetration into the plant root system. This conclusion is based on numerous works that demonstrate a tight correlation between the amount of HM accumulated by the plant (Cd mainly) and the amount of chlorocomplexes in the solution close to root surfaces. Appropriate programs have been applied for the assessment of chlorocomplex speciation and content (GEOCHEM PC, McLauchlin et al. 1997, MINTEQA2, Khoshgoftar et al. 2004; WHAM–VI, Lopez-Chuken and Young 2005); these permit the concentrations of different species of a metal to be calculated on the basis of the total concentration of the metal and some additional parameters of the system [pH and the concentrations of NaCl, some inorganic ions, dissolved organic compounds (DOC), and some others].

On this basis, it was reliably stated that the positive effect of NaCl on Cd accumulation in plant tissues of various species depends markedly on the concentrations of specific chlorocomplex species that are designated $CdCl_n^{2-n}$.

Thus, Lopez-Chuken and Young (2005) observed a strong salinity-induced activation of Cd accumulation in shoots; some data regarding the activities of Cd species in soil pore water were presented. These authors showed that, under the influence of 100 mM NaCl treatment, the total Cd concentration increased threefold (the Zn concentration increased by a factor of 1.34); however, the concentration of Cd^{2+} increased by only by 20%, while the concentrations of $CdCl^+$ and $CdCl_2^0$ increased 12-fold (at 200 mM NaCl, the concentration of $CdCl^+$ increased 14-fold and the concentration of $CdCl_2^0$ 24-fold).

It was established that the concentrations of Cd in the shoots of *B. juncea* and *Z. mays* showed a higher correlation with Cl^--dependent factors (Cl^-, $R^2=0.93$ and 0.86; $CdCl^+$, $R^2=0.91$ and 0.87; $CdCl_2^0$, $R^2=0.93$ and 0.89) than with Cd^{2+} ($R^2=0.32$ and 0.04) or even the total Cd concentration in the soil pore water ($R^2=0.84$ and 0.75). Correlations with SO_4^{2-}-dependent factors were negative.

Khoshgoftarmanesh et al. (2006) presented similar data regarding the effect of salinity on Cd in wheat. In 120 and 180 mM NaCl treatments, which induced the highest Cd accumulation, more than half of the total Cd was found to be in the form of chloride complexes. In contrast, free Zn^{2+} was the dominant species at all NaCl levels in soil. Increasing the soil salinity decreased the free Zn^{2+} concentration, which decreased the shoot Zn concentrations.

Similar correlations between salinity and Cd accumulation have been found for most plant species (although the opposite effect has also been obtained). This effect is usually explained by the salt-induced desorption of Cd that is tightly bound to the soil and its conversion into a bioavailable form. Therefore, this effect only manifests itself in plants growing in soil or on bottom sediment; it disappears in experiments with soil-free solutions and with free-floating plants, like *Elodea* (as distinct from pondweed), where Cd penetrates through the leaf surface and predominantly into the intercellular space.

However, it is not clear whether increased Cd bioavailability alone determines the positive effect of salinity. Some researchers propose that plants could take up Cd directly in the form of intact Cd–chloride complexes. However, this supposition is yet to find experimental support (Smolders and McLaughlin 1996; Smolders et al. 1998). Another (also hypothetical) possibility is that chlorocomplexes help to overcome diffusive resistance and, when they break down on the root surface, they increase the local Cd concentration in the vicinity of the root, in the zone of availability for membrane transporters.

Information concerning other HMs is extremely scarce. A significant effect of salinity on Zn mobility (availability) has not been strongly established. Khoshgoftarmanesh et al. (2006) and Du Laing et al. (2008) concluded that free Zn^{2+} was the dominant species at all NaCl levels in soil. Increasing the soil salinity decreased the free Zn^{2+} concentration, which caused a decrease in shoot Zn concentrations. It is also not clear how salinity influences Cu accumulation. It is supposed that one of the causes of the contradictory results could be low Cu–chloride complex stability. On the other hand, in marine water, sulfides (which exhibit low solubility) could play a decisive role in Cu and Ni accumulation in plants (Du Laing et al. 2008). In general, the results available at present do permit some conclusions.

It has repeatedly been established that, for natural habitats (estuaries, salt marshes), increased salinity is correlated with enhanced accumulation of HMs, mainly in halophytes. A stimulatory effect of salinity on the accumulation of HMs in plants and their transport to aboveground organs has also been demonstrated in laboratory and field experiments. However, systematic investigations have not been performed so far, and only rather fragmentary data are available on the combined action of these two stressors on plant physiological processes.

It is not surprising that, in the cases where combined crop treatments resulted in a clear suppression of biomass accumulation, some important physiological functions were also disturbed. Thus, in wheat and several barley genotypes, the combined action of these two stressors enhanced the damaging effects of each, additionally reducing the chlorophyll content, suppressing photosynthesis, destroying transpiration, inducing membrane injuries and ionic imbalances, and suppressing the activities of the enzymes involved in nitrogen metabolism, among others (Muehling and Lauchli 2003; Smykalova and Zamecnikova 2003; Huang et al. 2006a,b; Kadukova and Kalogerakis 2007). A deeper analysis of the causes of this effect of two stressors showed that "… a significant interaction exists between Na and Cd in their influence on antioxidant enzyme activity and the accumulation of each element in the plant" (Muehling and Lauchli 2003).

In contrast, in two works performed with halophytes, an optimization of physiological parameters was demonstrated upon combined NaCl and HM treatment (both halophytes belonged to the same family, Aizoaceae). In *Sesuvium portulacastrum*, 50–100 μM Cd halved the values of the basic physiological parameters, while salinity (100 or 400 mM NaCl) completely restored biomass accumulation and even increased the relative growth rate to above control values. In spite of a salinity-induced shift of the K/Na ratio towards Na, the total optimization of the physiological state of the plant upon the combined action of both stressors was evident. This manifested itself, in particular, in the maintenance of chlorophyll content at almost control levels, even though plant treatment with Cd alone severely reduced its concentration (to 44% of the control level) (Ghnaya et al. 2007).

In the studies conducted in our laboratory with another member of the same family, the facultative halophyte *Mesembryanthemum crystallinum*, strong disturbances in plant water status by Zn and Cu attracted special attention. It was shown that supplementation of a Cu-containing medium with NaCl (400 mM) markedly increased the total water content (Fig. 8.3) and stabilized transpiration, improving the principal indices of plant water status. The combined action of the two factors (NaCl and $CuSO_4$) also resulted in sharp drops in the leaf osmotic potential (Fig. 8.4). These drops, to values (–3.0 to –4.5 MPa) that were significantly lower than the osmotic potential of the rooting medium (–1.8 MPa), resulted in an influx of water to the aboveground organs of the plants. Finally, NaCl stimulated the rapid accumulation of proline, one of the most important osmolytes found in plants (Fig. 8.5). As a result, the highest proline concentration (15–16 μmol g^{-1}) was found in plants treated with both stressors together, and this level was twice as high as those observed after treatment with each of them separately.

It was thus evident that salinity favored the adaptation of the common ice plant to the toxic action of the HM, triggering mechanisms that are specific to halophytes, such as a strong reduction in the osmotic potential and enhanced proline accumulation, a universal low molecular chaperone that protects macromolecules and cell structures against toxic HM action (Kholodova et al. 2000).

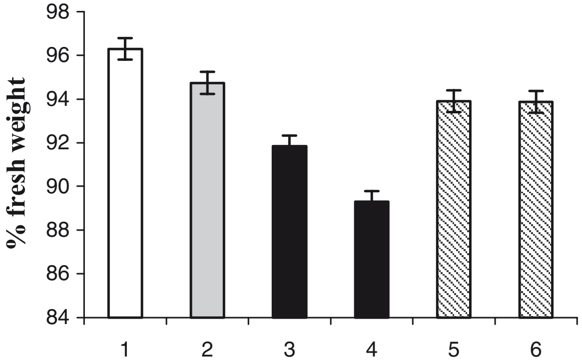

Fig. 8.3 Water content in common ice plant leaves on the seventh day of the experiment. *1*, Control; *2*, 400 mM NaCl; *3*, 25 μM $CuSO_4$; 4, 50 μM $CuSO_4$; *5*, 400 mM NaCl+25 μM $CuSO_4$; *6*, 400 mM NaCl + 50 μM $CuSO_4$

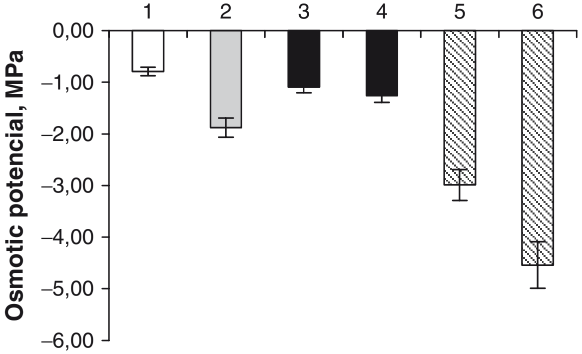

Fig. 8.4 Osmotic potential of common ice plant leaves on the seventh day of the experiment. *1*, Control; *2*, 400 mM NaCl; *3*, 25 μM $CuSO_4$; 4, 50 μM $CuSO_4$; *5*, 400 mM NaCl+25 μM $CuSO_4$; *6*, 400 mM NaCl + 50 μM $CuSO_4$

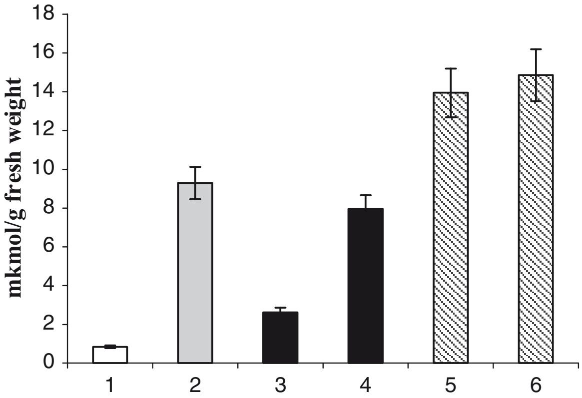

Fig. 8.5 Proline accumulation in common ice plant leaves on the seventh day of the experiment. *1*, Control; *2*, 400 mM NaCl; *3*, 25 μM $CuSO_4$; 4, 50 μM $CuSO_4$; *5*, 400 mM NaCl+25 μM $CuSO_4$; *6*, 400 mM NaCl + 50 μM $CuSO_4$

8.4 Conclusion

It has become clear that, in some cases, moderate salinity can rather efficiently improve the HM tolerance of plants, and this should be taken into account when developing innovative phytoremediation technologies. Halophytes and some crops are especially promising for these tasks. However, so far, researchers have directed their attention to studying the effects of salinity on processes that occur in the soil before HM penetration into the plant. These studies have helped us to understand the causes of the positive influence of salinity on Cd bioavailability, whereas the interactions of NaCl with other HMs (Zn, Cu, Ni and others) still require further investigation. The joint efforts of biologists, soil scientists, and chemists are needed to this end.

Regretfully, the basic principles of the adaptation of plants to the joint action of salinity and HMs have still not been elaborated, and so we cannot make use of the additional possibilities of the adaptive potential of plants. Thus, the available information indicates that HM hyperaccumulation is not always the most efficient approach of phytoremediation. In contrast, lowering the HM concentrations in shoots – often observed under saline conditions – could lead to the optimum result. In this case, an overall improvement in the state of the plant and enhanced biomass accumulation result in increased HM extraction from the polluted substrate, despite a relatively low HM content in shoots, thus increasing the efficiency of the phytoremediation technology. Inadequate knowledge of the physiological basics of plant adaptation to the joint action of salinity and HMs also impose other limitations. In fact, when developing phytoremediation technologies, universal solutions are not expected: particular plant forms should be selected to decontaminate particular polluted territories.

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