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Adaptation of the Common Ice Plant to High Copper and Zinc Concentrations and Their Potential Using for Phytoremediation

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Abstract—A facultative halophite *Mesembryanthemum crystallinum* L. (the common ice plant) was shown to grow successively at the high concentrations of Cu and Zn. Although 25 μM $CuSO_4$ or 800 μM $ZnSO_4$ retarded markedly plant growth, they did not interfere with the completion of plant development and the formation of viable seeds. In such plants, leaves accumulated more than 200 μg of Cu and 1700 μg of Zn per 1 g of dry weight. A damaging effect of heavy metals (HMs) was manifested in a reduced content of water in leaves and proline accumulation in them. As copper is a metal with transient valence, copper salts are more toxic than zinc salts, which was manifested in a stronger inhibition of the chlorophyll synthesis. Both HMs induced oxidative stress, as evident from increased activities of guaiacol peroxidase and lipoxygenase. Moderate Cu and Zn concentrations did not damage cell membranes in leaves, as evident from the absence of their action on electrolyte leakage either under optimum conditions or after heat treatment. A capability of a substantial HM accumulation by the common ice plant and their considerable transport to shoots (up to 50 μg of Cu and 560 μg of Zn per plant) make it possible to consider the common ice plant as a promising phytoremediator.

Key words: Mesembryanthemum crystallinum - heavy metals - Zn - Cu - phytoremediation

INTRODUCTION

Heavy metals (HMs) acquire an increasing significance among diverse abiotic stressors. An increasing anthropogenic load on the natural ecosystems is accompanied by a disturbance an equilibrium in biocenoses and accumulation of various toxicants, HMs in particular, in plants [1–4].

One of the basic mechanisms of HM toxicity is their capability of binding to sulfur-containing compounds, SH groups of proteins primarily. This results in the changes in the native conformation of macromolecules and enzyme inactivation [5, 6]. Another damaging effect of HMs can arise from a frequently observed HM-induced disturbance of membrane barrier properties [5, 7] due to HM interaction with protein SH groups and HM-triggered oxidative stress [4].

Zn, Cu, and Ni are of a significant importance because their excessive amounts in soil can arise not only due to human economic activity but also because of natural soil-forming processes. In fact, these HMs occur in some parent material of soils in high concentrations [1]. Thus, Cu content in soil was shown to vary 1000-fold (from 2 to 2000 mg/kg soil); Zn, more than 4000-fold (from 25 to more than 10000 mg/kg) [1]. Taking into account these facts, it is of importance to assess the level of Cu and Zn accumulation in plant shoots when studying the mechanisms of plant adaptation to these HMs. A search for plants capable of accumulating the high concentrations of HMs in their shoots is of interest in respect to phytoremediation technology [8, 9]. Growing of such plants permits natural soil cleaning up from excess HMs, thus helping other, less tolerant plant species to inhabit the region. In such a way, decontaminated soil can be restored for farming [3, 10, 11].

The common ice plant (*Mesembryanthemum crystallinum* L.) is a facultative halophyte dwelling in South Africa deserts. Currently, it inhabits many arid regions of the Earth. This plant is for long used as a convenient model for studying the mechanisms of adaptation to extreme conditions. We have demonstrated [12] that the common ice plant could complete its life cycle at the high concentrations of NaCl in soil (up to 800 mM); it accumulated inorganic ions in the aerial organs. This capability causes to believe *M. crystallinum* a promising species for saline soil phytoremediation.

By now, no systemic investigation of the common ice plant adaptation to HMs was performed. A relatively low tolerance of *M. crystallinum* plants to Cd was shown to be accompanied by HM accumulation in roots, but not

Abbreviations: Chl—chlorophyll; HM—heavy metal; MDA—malondialdehyde; ROS—reactive oxygen species; TBA—thiobarbituric acid.

in shoots [13]. In contrast, Thomas *et al.* [14] demonstrated that the common ice plant could grow at extremely high Cu concentrations (up to 8 mM), which was accompanied by Cu ion accumulation in leaves. This fact permits a supposition that the common ice plant is a promising phytoremediator of areas contaminated with HMs.

At the first stage of this work, we attempted to assess a tolerance of the common ice plant to Cu and Zn salts. To this end, we tried to find out the range of concentrations permitting the common ice plant to complete their life cycle and to accumulate a great amount of HMs in the aerial organs.

MATERIALS AND METHODS

Plants of *Mesembryanthemum crystallinum* L. were grown in the phytotron chamber in water culture at 23–25/18–20°C day/night temperature. Plants were illuminated from HPS Reflux lamps (Reflux, Russia) (350 μmol/(m^2 s), a 12-h photoperiod). Seeds were sown in trays with Perlite. At the age of 4 to 5 weeks, plants were transferred to glass vessels 2 l in volume (three plants per vessel) containing modified Johnson nutrient medium [15] with $FeNO_3$. Cu (10–100 μM) and Zn (50–800 μM) salts were added to nutrient medium when plants developed three or four pairs of primary leaves.

In order to assess the effect of HMs on germination, the seeds of the common ice plant, preliminarily treated with 0.1% $KMnO_4$, were placed in sterilized petri dishes (100 seeds per dish) with Johnson nutrient medium. $CuSO_4$ (up to 200 μM) and $ZnSO_4$ (up to 6000 μM) were added. Seeds germinated under conditions used later for plant growing. The number of germinated seeds was counted after 7 days.

Fresh and dry weights of plant organs (leaves and stems) and the content of water in them were determined with the gravymetric method. Plant material was fixed at 75°C for 30 min and dried at 60°C to constant weight.

The samples for Cu and Zn quantification in plant tissues were prepared as described by Golubkina [16]. The aliquot of air-dry plant material (30–50 mg) was poured over with the mixture of concentrated HNO_3 (1.5 ml) and $HClO_4$ (0.8 ml) and left for a day. Then, using a TDB-40-A thermostat (Biosan, Latvia), the samples were successively heated at 120, 150, and 180°C for 1 h each. After sample cooling to 150°C, 5–6 drops of concentrated H_2O_2 were added. In 10 min, 1 ml of 6 M HCl was added, and the mixture was kept at a temperature of 110°C for 10 min. After decoloration of solutions, the concentration of HM was measured using a Hitachi-207 atom-absorption spectrophotometer (Hitachi, Japan).

Membrane barrier properties (membrane state) were assessed by the amount of electrolyte leakage after high-temperature treatment. Disks (5 mm in diameter) were cut from leaves of the third or fourth pair; every sample comprised 6–10 disks. Samples were rinsed in 10 ml of distilled water for 15 min with stirring to remove damaged cells and the contents of the apoplast. Preliminary experiments showed that such rinsing was sufficient to remove 90% of extracellular electrolytes. Thereafter, samples were blotted, transferred rapidly into pure flasks containing 10 ml of distilled water, and kept at a temperature of 20, 50, or 60°C for 30 min. The content of electrolytes in this solution was the measure of their leakage across the membrane. Electrolytes retained in tissues were extracted with a new portion of water at boiling for 5 min with subsequent stirring longer than for 1 h. The conductivity of solutions was measured with an CM-101 conductometer (Orion Research, United States). Electrolyte leakage was expressed in percents of total intracellular electrolytes, i.e., the sum of electrolytes released after their removal from the apoplast and those extracted by boiling.

Chlorophyll (Chl) content in the leaves of the common ice plant was determined after pigment extraction with ethanol [17]. The sample (200 mg) was ground with 3 ml of 96% ethanol; the homogenate was centrifuged for 3 min at 4°C at 3000 *g* using a K-24 centrifuge (Janezki, Germany). Pigment concentration was estimated at 665 and 649 nm using an SF-46 spectrophotometer (LOMO, Russia) and calculated according to the formulas:

$$C_{\text{Chl } a} = 13.70D_{665} - 5.76D_{649},$$

$$C_{\text{Chl } в} = 25.80D_{649} - 7.60D_{665},$$

where C_{Chl} is a Chl concentration and D is an optical density.

The content of free proline was estimated using an acidic ninhydrin reagent [18]. Proline was extracted by plant sample (300–700 mg fr wt) boiling in 5–15 ml of distilled water for 10 min. After centrifugation, 1 ml of glacial acetic acid and 1 ml of the ninhydrin reagent were added to 1 ml of the supernatant. Samples were kept in a boiling water bath for 1 h, cooled rapidly in ice, and their optical density was measured at 520 nm using an SF-46 spectrophotometer. Proline preparation from Sigma (United States) was used for calibration.

Lipid peroxidation was assessed from the concentration of malondialdehyde (MDA) according to the method of Heath and Parker [19] based on the formation of colored complex between MDA and thiobarbituric acid (TBA). Plant material (250 mg) was ground with 4 ml of 20% TCA; the homogenate was centrifuged at 10000 *g* (K-24 centrifuge) for 15 min. The reaction mixture containing 1 ml of the supernatant and 4 ml of 0.5% TBA in 20% TCA was incubated at 96°C for 30 min, cooled rapidly in ice, and centrifuged at 10000 *g* for 15 min. MDA concentration was measured at 532 nm using a Specol-11 spectrocolorimeter (Carl Zeiss, Germany). The coefficient of MDA molar extinction applied for calculations was 155/(mM cm). Unspecific extinction at 600 nm was subtracted.

Table 1. The effect of Cu and Zn ions on *M. crystallinum* seed germination

Salt concentration, μM	$CuSO_4$	$ZnSO_4$
	% of germinated seeds	
0	53.7	53.7
10	49.3	–
25	61.4	–
50	50.4	60.1
100	44.8	59.3
200	38.6	61.2
400	15.3	64.6
800	–	57.8
1000	–	51.4
6000	–	12.3
$LSD_{0.05}$	5.3	4.9

In order to assay the activities of free and ion-bound peroxidases [20], plant material (500 mg fr wt) was ground with 5 ml of the extraction mixture at 2°C. The extraction mixture contained 50 mM phosphate buffer (pH 7.4), 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 10 mg of insoluble polyvinylpyrrolidone. The homogenate was centrifuged at 4000*g* for 20 min (K-23 centrifuge) at 4°C. The supernatant was used as a source of free peroxidase. The pellet was additionally washed with 3 ml of the extraction mixture and centrifuged repeatedly. Ion-bound peroxidase was extracted with 3 ml of 1 M NaCl in 50 mM phosphate buffer (pH 7.4) with stirring for 30 min and centrifugation at the above regime.

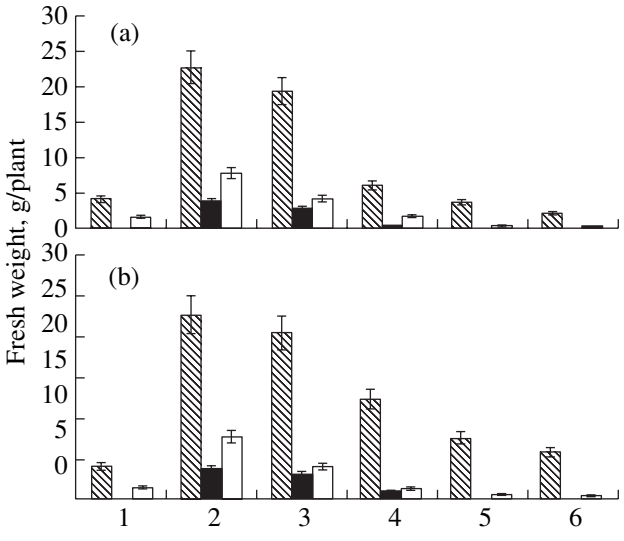

Fig. 1. The effect of 14-day-long plant treatments with Cu and Zn salts on their growth.
(a) (1, 2) Control: (1) Initial plant fr wt; (2) on day 14; (3–6) $CuSO_4$: (3) 10 μM; (4) 25 μM; (5) 50 μM; (6) 100 μM; (b) (1, 2) Control: (1) Initial plant fr wt; (2) on day 14; (3–6) $ZnSO_4$: (3) 100 μM; (4) 250 μM; (5) 500 μM; (6) 800 μM.
▨ Leaves and stems; ■ lateral shoots; □ roots.

In the assay of peroxidase activity, H_2O_2 was used as a substrate, and guaiacol (Fluka, Switzerland), as a hydrogen donor. The reaction mixture contained 0.05 ml (for free peroxidase) or 0.25 ml (for ion-bound peroxidase) of the enzyme preparation, which corresponded to 50–200 μg of protein, 0.25 ml of 7 mM guaiacol and 0.25 ml of 6 mM H_2O_2 (added immediately before measuring into the cuvette). The volume was adjusted to 2 ml with 66 mM phosphate buffer (pH 7.4). The change in the optical density was recorded after 1 min at 470 nm using a Specol 11 spectrocolorimeter. Peroxidase activity was expressed in mmol guaiacol/(mg protein min) using a constant of 5.6 for calculations.

Protein content was determined by the method of Bradford [21] using BSA (Sigma) for calibration.

Every experiment was repeated at least three or four times with three replications each. Tables present the mean values and LSD at a 95% level of significance. Bars in figures indicate standard deviations [22].

RESULTS

Germination of *M. crystallinum* seeds turned out to be relatively resistant to Cu and Zn salts (Table 1). Within the concentration range from 10 to 50 μM, $CuSO_4$ did not inhibit seed germination; moreover, some slight stimulatory effects were observed, at 25 mM in particular. Only 100 μM $CuSO_4$ and its higher concentrations reduced significantly seed germinability. Seeds were much more resistant to $ZnSO_4$: its adverse effect was evident only at the concentration of 6000 μM.

Another pattern was observed when the common ice plant was grown in the presence of HMs for a long time. It turned out that the common ice plant could not acclimate to Cu concentrations above 100 μM and Zn concentrations, above 1000 μM; they perished in three or four days after treatment. In our further experiments, we used Cu salt concentrations from 10 to 100 μM and Zn concentrations, from 150 to 800 μM. However, even at these concentrations, HMs suppressed substantially plant growth.

Figure 1 presents data about the effects of Cu and Zn salts on the growth of the common ice plant. For 14 days, control plants increased the fresh weight of their primary leaves from 4.1 to 23 g and started to produce lateral shoots. In the presence of the lowest $CuSO_4$ concentration (10 μM), plant growth and development were close to control plant growth. At 25 μM $CuSO_4$, plant growth was greatly retarded; at higher concentrations, plant fresh weight was even lower that the initial one. The common ice plant manifested a better capability of adaptation to higher $ZnSO_4$ concentrations. 100 μM $ZnSO_4$ only slightly reduced the growth rate,

whereas 800 μM $ZnSO_4$ retarded considerably fresh weight accumulation; however, it did not block the development of lateral shoots.

In next experiments, we evaluated Cu and Zn ion accumulation in plant aerial organs. The results of a typical experiment are presented in Table 2. In control plants, Cu content was 15.3 μg/g leaf dry wt and Zn content was 30.7 μg/g leaf dry wt. In the presence of HMs in medium, their concentrations in leaves rose from the beginning of experiment. At a higher used $CuSO_4$ concentration (200 μM), Cu content increased to 174 μg/g leaf dry wt as soon as after three days and to 215 μg/g leaf dry wt after seven days. Zn accumulation was still higher: its concentration exceeded 1700 μg/g leaf dry wt on the 7th day (at 800 μM $ZnSO_4$ in medium). Similar ion accumulation was observed when chlorides of HMs were used (Table 2).

A longer growing of the common ice plant on the medium containing HMs did not result in further HM accumulation in leaves (Figs. 2a, 2b). Thus, after 7-day-long growth on medium with 100 μM $CuSO_4$, Cu content in leaves attained 198 μg/g leaf dry wt; this value exceeded the level in control plants 11-fold. However, after 14-day-long growth, there was no further substantial Cu accumulation, especially at it high concentrations. The highest Cu content was 212 μg/g leaf dry wt. The highest Zn content in leaves was about 1.9 mg/g leaf dry wt, which was 40 times higher than in control leaves.

In one experiment, we calculated a total content of HMs studied per entire plant, i.e., HM absorption and translocation to shoots (Fig. 3). It turned out that, when the common ice plant were grown at 25 μM $CuSO_4$, the total Cu content per plant was about 50 μg, which exceeded 4.4-fold its content in control plants. At a higher Cu concentration in medium, Cu content in plants was considerably lower (to 35 μg) because of strong growth retardation. The much better plant growth in the presence of even high Zn concentrations resulted in almost 30-fold higher Zn content per plant, as compared to control plants. The highest value of Zn absorption by the common ice plant and its translocation to shoots exceeded 560 μg/plant.

In order to elucidate the causes of HM-induced plant growth suppression, we studied the effects of HMs on some physiological indices. Taking into account the fact that, after HM, especially Cu, addition to nutrient medium, plants demonstrated some signs of wilting (a decrease in turgor pressure) and leaf bleaching, we assessed some indices of the water status and pigment content.

It turned out that, when plants grew in the presence of HMs, even of the lowest concentrations of $CuSO_4$, the water content in leaves reduced substantially (Table 3). This reduction in water content was manifested already at early stages of Cu action; its damaging effect strengthened with time. The calculation of water content per dry matter, presented in Table 3, indicates clearly a Cu-induced disturbance in water status. As distinct from Cu, Zn effect on water content was less expressed.

Table 2. Accumulation of copper and zinc by the leaves of the common ice plant

Treatment	Salt concentration, μM	3 days	7 days
		μg/g dry wt	
Control	medium*	–	15.3
$CuSO_4$	50	84	125
	100	130	185
	200	174	215
$CuCl_2$	50	85	107
	100	119	147
	200	199	191
Control	medium*		30.7
$ZnSO_4$	250	181	304
	500	410	1248
	800	913	1710
$ZnCl_2$	250	210	317
	500	395	1312
	800	1023	1405
$LSD_{0.05}$		23	31

* Nutrient medium contained 0.25 μM $CuSO_4$ and 1 μM $ZnSO_4$.

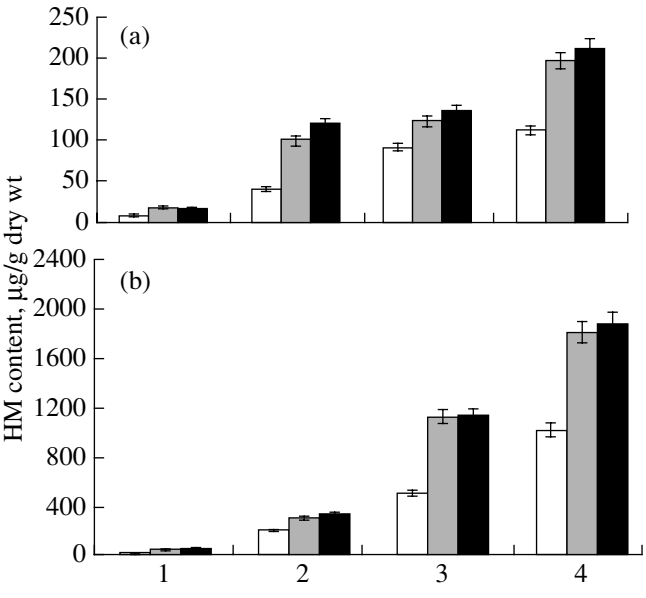

Fig. 2. Accumulation of (a) Cu and (b) Zn in the leaves of the common ice plant for □ 3, ▨ 7, and ■ 14 days. (a) (1) Control; (2–4) $CuSO_4$: (2) 25 μM; (3) 50 μM; (4) 100 μM; (b) (1) Control; (2–4) $ZnSO_4$: (2) 250 μM; (3) 500 μM; (4) 800 μM.

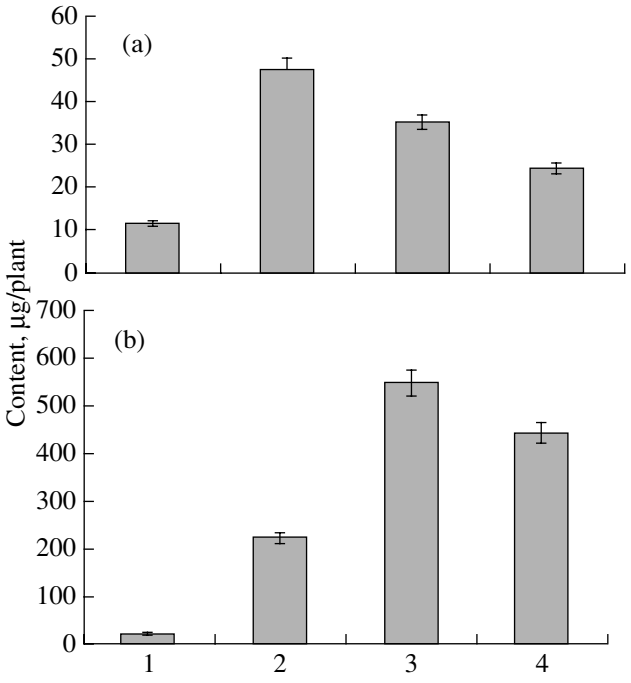

Fig. 3. Total content of (a) Cu and (Zn) in the aerial organs of the common ice plant for 14 days.
(a) (1) Control; (2–4) $CuSO_4$: (2) 25 μM; (3) 50 μM; (4) 100 μM; (b) (1) Control; (2–4) $ZnSO_4$: (2) 250 μM; (3) 500 μM; (4) 800 μM.

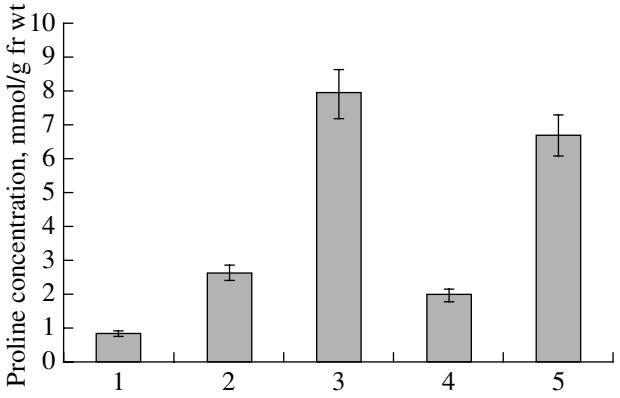

Fig. 4. Proline accumulation by the common ice plant in the presence of Cu and Zn for 14 days.
(1) Control; (2) 25 μM $CuSO_4$; (3) 50 μM $CuSO_4$; (4) 250 μM $ZnSO_4$; (5) 500 μM $ZnSO_4$.

After a drop in water content, proline, one of the basic osmoprotectants in the common ice plant [12, 23], was accumulated in HM-treated plants. On day 14, proline concentration increased threefold in the presence of 25 μM and eightfold at 50 μM $CuSO_4$. In Zn-treated plants, proline concentration could be increased by 7 times (Fig. 4).

High HM concentrations reduced Chl content rather rapidly. The responses of mature and young developing leaves to HMs differed. In mature leaves, bleached necrotic lesions appeared up to 1 cm in diameter (Fig. 5), whereas young leaves developed in the presence of HMs demonstrated the signs of chlorosis. Quantification of Chl in plants grown on media containing HMs showed that $ZnSO_4$ was efficient only at its highest concentration (Table 4): 500 μM $ZnSO_4$ did not affect significantly Chl content but changed the Chl *a/b* ratio. Low Cu concentrations exerted only weak action; however, 50 μM $CuSO_4$ not only changed the Chl *a/b* ratio but reduced markedly Chl content.

One of the chief causes for detected Chl degradation and a disturbance in its synthesis in the leaves of the common ice plant might be HM-induced oxidative burst [19, 24]. In the system of plant defense against reactive oxygen species (ROS), peroxidase plays a key role, preventing excess accumulation of hydrogen peroxide. Changes in the guaiacol peroxidase activity indicate the induction of oxidative stress by HMs (Fig. 6). Especially strong peroxidase activation (2.5-fold) was induced by 50 μM $CuSO_4$. A comparable effect of $ZnSO_4$ was observed at its tenfold higher concentration

Table 3. Water content in the leaves of the common ice plant

Treatment	Salt concentration, μM	3 days	10 days	3 days	10 days
		% of fr wt		g H_2O/g dry wt	
Control	medium*	97.11	96.83	33.60	30.64
$CuSO_4$	15	96.43	94.97	27.01	18.95
	25	96.12	92.11	24.77	11.72
	50	94.28	89.49	16.48	8.81
$ZnSO_4$	150	96.81	95.18	30.35	19.76
	250	96.36	95.12	26.47	19.62
	500	96.12	94.35	24.77	16.70
$LSD_{0.05}$		0.45	0.53	1.5	1.2

* Nutrient medium contained 0.25 μM $CuSO_4$ and 1 μM $ZnSO_4$.

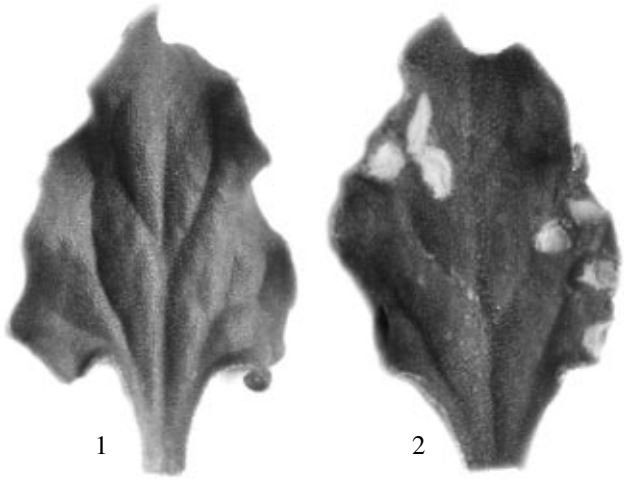

Fig. 5. Necrotic lesions on the leaves of the common ice plant growing on the medium contained Cu for 14 days.
(1) Control; (2) 50 μM $CuSO_4$.

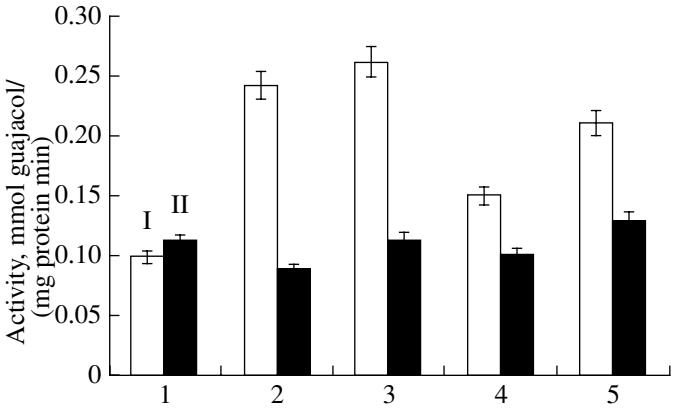

Fig. 6. The effects of 14-day-long treatment of the common ice plant with Cu and Zn on activities of (I) free and (II) bound peroxidases
(1) Control; (2) 25 μM $CuSO_4$; (3) 50 μM $CuSO_4$; (4) 250 μM $ZnSO_4$; (5) 500 μM $ZnSO_4$.

(500 μM). It is worth mentioning that Cu and Zn did not affect the activity of bound peroxidase in plant leaves.

Another index characterizing the strength of oxidative stress is an activity of lipoxygenase. As evident from data presented in Fig. 7, high Cu and Zn concentrations significantly and similarly (by 70%) activated this enzyme, whereas low concentrations did not affect its activity.

Frequently, basic cell membranes, plasma membrane and tonoplast, are the main targets for ROS [7, 25]. In order to assess membrane state and HM effects on membrane integrity, we used such an integral index as electrolyte leakage from leaf cells. In all treatments (control plants and those grown in the presence of Cu and Zn salts), the conductivity of solutions containing the apoplast (extramembrane fraction) contents was within a narrow range (0.22–0.29 μS), which comprised 10–15% of total amount of electrolytes in the samples. Membrane leakage was assessed as a percent of electrolyte leaned of their total content (see Materials and Methods section). Electrolyte leakage was measured not only at room (20°C) but also at greatly elevated (test) temperatures of 50 or 60°C (Table 5).

In control plants, electrolyte leakage through the plasma membrane at room temperature comprised only 12% of their total content in cell protoplasts. However, it increased sharply at elevated temperatures: to 40% at 50°C and to 77% at 60°C. It turned out that HM accumulation did not exert any substantial damaging effect on electrolyte retaining in protoplasts either at 20°C or at elevated temperatures, which weaken considerably membrane barrier properties.

DISCUSSION

Both Cu and Zn are elements vitally important for plants. For example, Cu is known to be a key component providing for functioning of some enzymes, such as cytochrome oxidase, ascorbate oxidase, and galactose oxidase, and nonenzymic proteins (for example, plastocyanin, the element of the photosynthetic electron-transport chain, and others) [26]. Among Zn functions, its involvement in transcription factor functioning attracts now an especial attention [27]. As these elements are essential ones, Cu and Zn salts are always added to nutrient media for plant growing. Thus, in experiments with the common ice plant, we used the medium containing 0.25 μM $CuSO_4$ and 1 μM $ZnSO_4$ [15].

As evident from our first experiments, the seeds and young seedlings (until 7 days) of the common ice plant demonstrated a high tolerance to HM salts. The highest Cu and Zn concentrations, which did not suppress seed germinability, were 50 μM and 1 mM, respectively. These values exceeded the concentrations of these metals in standard medium 200- and 1000-fold. Similar tol-

Table 4. The effect of 10-day-long treatment of the common ice plant with Cu and Zn ions on chlorophyll content

Treatment	Salt concentration, μM	Chl, mg/dm^2	Chl *a*/*b*
Control	medium*	5.1	2.3
$CuSO_4$	15	5.0	2.0
	25	5.3	1.8
	50	4.1	1.5
$ZnSO_4$	150	5.3	2.2
	250	5.6	2.3
	500	5.0	3.3
$LSD_{0.05}$		0.2	0.1

* Nutrient medium contained 0.25 μM $CuSO_4$ and 1 μM $ZnSO_4$.

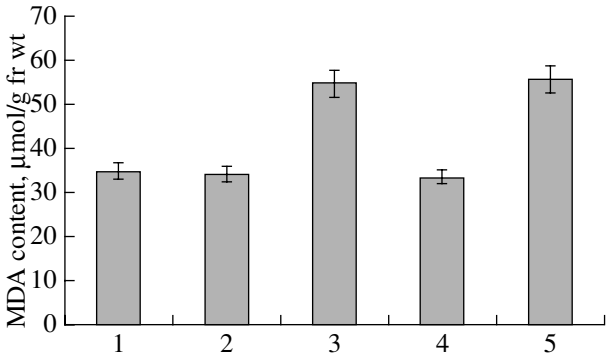

Fig. 7. The effects of 14-day-long treatment of the common ice plant with Cu or Zn on the level of lipid peroxidation. (1) Control; (2) 25 μM $CuSO_4$; (3) 50 μM $CuSO_4$; (4) 250 μM $ZnSO_4$; (5) 500 μM $ZnSO_4$.

erance of the common ice plant to copper was reported earlier [14].

Basing on the results of Thomas *et al.* [14], who used high concentrations of $CuSO_4$ (up to 8 mM) in experiments with the common ice plant, we firstly attempted to grow the common ice plant on high concentrations of HM in medium for a long time. However, it turned out that a long-term growth of the common ice plant was possible only at considerably lower concentrations: not higher than 50 μM for Cu salts and 800 μM for Zn salts. These concentrations permitted a completion of plant life cycle, although plant fresh weight was reduced and necrotic lesions appeared in the presence of Cu salts. Thus, the common ice plant could grow at $CuSO_4$ concentrations 200-fold and $ZnSO_4$ concentrations 800-fold higher than those in standard nutrient medium providing for normal plant development. Nevertheless, the concentrations of studied HM, which did not suppressed fresh weight accumulation, were much lower: 10 μM for Cu and 100 μM for Zn, i.e., they were 40- and 100-fold higher than in nutrient medium, respectively.

Thus, copper salts turned out to be much more toxic than zinc salts for the common ice plant as for most other plant species [6, 24]. This conclusion is in agreement with substantially lower Cu concentrations in plant tissues, as compared with Zn concentrations (1–10 μg/g dry wt for Cu and 10–100 μg/g dry wt for Zn) and the minimum toxic concentrations of these HM (20–30 μg/g dry wt for Cu and 300–500 μg/g dry wt for Zn) [2, 28, 29].

The difference between salts of these HM on the common ice plant were manifested also in the following: Zn (at the concentration up to 1 mM) substantially retarded growth but did not prevent the accumulation of shoot fresh weight; in contrast, low $CuSO_4$ concentrations (only 100 μM) stopped plant development and induced even a decrease in the fresh weight due to the death of some leaves (Fig. 1).

At present, much attention is given to phytoremediation, and plants-hyperaccumulators of HM play a key role in this process [8, 10, 11, 28, 30]. Hyperaccumulators are the plants that can grow at high HM concentrations in soil, maintaining the capability of normal

Table 5. The effect of Cu and Zn on the value of electrolyte leakage, μS

Treatment	*t*, °C	Leakage from tissues	Extraction from killed tissues	Total content of intracellular electrolytes	Leakage, % of total content
Control	20	0.101	0.715	0.816	12
	50	0.304	0.464	0.768	40
	60	0.524	0.155	0.679	77
$CuSO_4$, 25 μM	20	0.136	0.800	0.936	14
	50	0.305	0.602	0.907	34
	60	0.600	0.308	0.908	66
$CuSO_4$, 50 μM	20	0.150	0.904	1.054	14
	50	0.352	0.600	0.952	37
	60	0.634	0.377	1.011	62
$ZnSO_4$, 250 μM	20	0.068	0.593	0.661	10
	50	0.219	0.325	0.544	40
	60	0.346	0.115	0.459	75
$ZnSO_4$, 500 μM	20	0.074	0.423	0.597	12
	50	0.192	0.264	0.456	42
	60	0.334	0.124	0.458	72
$HCP_{0.05}$		0.511	0.632	0.401	

development and accumulating in shoots HM in concentrations exceeding some definite concentration for each HM [8]. Such a critical concentration for Cu is 1 mg/g dry wt; for Zn, 10 mg/g dry wt; these values exceed 100-fold and more the average concentration of these HM in the aerial organs of most plant species [2, 8, 30]. The first place among Zn hyperaccumulators belongs undoubtedly to *Thlaspi caerulescens* (51.6 g/kg dry wt) [8, 31]; *Anthoxanthum odoratum* and *Medicago truncatula* [8, 31] also accumulate high Zn concentrations. *Aeollanthus biformifolius* and *Ipomea alpina* (12.3 g/kg) [8, 11, 33] are most efficient in Cu accumulation.

When to follow these criteria, we cannot ascribe the common ice plant to hyperaccumulators of HM tested. Really, in spite of the fact that plant growing on medium containing 800 μM $ZnSO_4$ resulted in the accumulation of almost 2 mg Zn/g dry wt in leaves, this value was substantially lower than concentrations characteristic of Zn hyperaccumulators. In addition, strong growth retardation was observed under these conditions, which maybe did not occur in soil culture.

In respect to Cu, its toxicity for the common ice plant has primarily engaged our attention. It was observed earlier by Thomas *et al.* [14], although was not adequately discussed by the authors. A strong damaging effect of Cu was manifested early, during the stage of seedling growth, and was expressed in a rapid decrease in the Chl content in the presence of 50 μM [14]. However, a critical state of plants on media with extremely high Cu content is still more evident from a drop of water content in the common ice plant tissues. Such data were obtained by Thomas *et al.* [14]. Thus, on medium with 8 mM $CuSO_4$, the water content in leaves was only 5 g H_2O/g dry wt after 48 h *vs* 38 g H_2O/g dry wt in control plants. Even at the lower concentration of 800 μM, water content decreased to 7.4 g H_2O/g dry wt on day 7, which indicates a rapid accumulation of injurious effects. For *M. crystallinum* plants with its high water content, a 5–7-fold decrease in this content undoubtedly indicated an irreversible damage. In our experiments, growth was completely ceased after 14 days already at 100 μM $CuSO_4$, when water content in leaves decreased only 3.5-fold.

Along with disturbance in plant water status, which evidently occurred not only at the level of water uptake by the root system, the obvious sign of HM injurious action was oxidative stress. In our experiments, it manifested in a marked Chl degradation, the suppression of its synthesis, and activation of peroxidase and lipoxygenase. It is characteristic that all these signs of oxidative stress were more pronounced in the presence of Cu salts, which corresponds to its chemical nature as an element with transient valence [26, 34, 35].

It is of importance that, in spite of the accumulation of high Cu and Zn amounts in the common ice plant leaves, HM-induced oxidative stress was not accompanied by the disturbance in membrane barrier properties. Even at elevated temperatures, which sharply weakened the intercellular substance compartmentation, HM did not induce an additional membrane destabilization and did not enhance electrolyte leakage through the plasma membrane. The results obtained support the conclusion drawn from the study of Cu action on *Silene cucubalus* plants [5] about the key role of the maintenance of membrane barrier properties for plant tolerance to this HM. The type of defense responses of the common ice plant (activation of free peroxidase and proline accumulation) also implies that ROS-induced intracellular, possibly chloroplastic, damages occurred, but they did not interfere much with the functions of the tonoplast and plasma membrane.

In general, the data obtained permit a conclusion that the common ice plant is relatively tolerant to Cu and especially Zn salts. Although $CuSO_4$ substantially retarded growth at the concentration of 25 μM, plants could complete their development and produce well-quality seeds. Under these conditions, plant leaves accumulated more than 100 μg Cu per 1 g dry wt, which also indicates a high tolerance of the common ice plant because exceeds toxic Cu level in most other plant species [2, 29].

In its capability of Zn accumulation in the aerial organs (up to 2 μg/g dry wt), the common ice plant not only exceeds most other plants but approach to the level characteristic of plant-hyperaccumulators [8, 11]. Plant growth on medium with Zn salts, a high level of Zn carrying away (more than 500 μg/plant), and tolerance to Zn during seed germination—all these facts permit a consideration of the common ice plant as a promising phytoremediator of areas contaminated with Zn. Although so far we cannot recommend the common ice plant for practical use in region decontamination, it is reasonable to continue investigations in this direction.

In conclusion, we have to answer the question why the common ice plant can accumulate rather high concentrations of HM in their leaves without strong disturbance of normal metabolic processes. A necessary condition for a tolerance of plants accumulating HM at high concentrations is the isolation of excessive HM from the sites of active metabolism, which is achieved either by HM detoxification or by their compartmentation to the vacuole [36]. For some plant species, it was shown that Zn transport with the involvement of membrane transporters play an important role in Zn accumulation [27, 28, 36]. The mechanisms of Cu detoxification by plants are less studied. The involvement in this process of metallothioneins, the cysteine-enriched small proteins, seems quite probable [37, 38], which will be the objective of our further investigations.

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