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# The Ability of Medical Halophytes to Phytoremediate Soil Contaminated by Salt and Heavy Metals in Lower Volga, Russia

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## ABSTRACT

Most of the soils in the lower Volga region of Russia are polluted by salinity and heavy metals due to the saline water of the Volga River and industrial pollutants. As a result, most of the soils of this region are unsuitable for crop cultivation. In order to assess the potential growth of two medicinal halophytes, *Halocnemum strobilaceum* and *Artemisia absinthium*, in this region, sulphate ($SO_4$)- and chloride (Cl)-containing salts (at 3.0, 5.0, 8.0 and 10.4%, w/v) were added to a lysimeter prior to seeding. In addition, to assess the response of these plants to zinc (Zn) in soil where salt concentration was high (10.4% Cl and $SO_4$ type of salts), zinc sulphate ($ZnSO_4$) was applied at 300, 400 and 500 mg Zn $kg^{-1}$ soil. A three-year trial indicated that both halophytes showed high tolerance to Zn without accumulating this heavy metal in their biomass. The ability of these plants to grow on Zn-polluted and saline soils would allow them to serve the pharmaceutical industry as medicinal raw materials while playing an important role in ecological phytoremediation.

**Keywords:** Salinity, heavy metals, phytoremediation, lowers Volga, Russia

## INTRODUCTION

The United Nations Food and Agriculture Organization and the United Nations Environment Programme estimate that there are currently 4 million $km^2$ of salinized land while approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed, threatening agricultural productivity (Ravindran *et al.* 2007; Rozena and Flowers 2008). Salt-impacted soils show structural problems created by certain physical processes and specific conditions which affect water and air movement, plant-available water holding capacity, and root penetration. Such soils also have devastating effects on plant growth and survival through both ionic and osmotic stresses which act on plants in various ways and at different levels of complexity (White and Broadley 2001; Qadir *et al.* 2003). Since a segment of the world's agricultural fields are exposed to heavy metals, a serious danger of accumulation, translocation and transformation of heavy metals in plants exists which may be passed down the food chain, ultimately negatively impacting human health (Begonia 19988; Franzaring *et al.* 2006; Divan Jr. *et al.* 2008).

The goal of defining metal threshold values serves to minimize the transfer of metals down the food chain to humans. In this case, ideally, plant metal threshold values need to be established first. Once allowable levels for metals in a crop have been set, the next step is to determine threshold levels for metals in particular soil types such that crops growing in those specific soils do not take up metals in excess of the allowable level (Hamon and McLaughlin 2003). Most of the international guidelines enacted to regulate metal levels in agricultural soils have related to the application of sewage sludge ('biosolids') to land (reviewed by McLaughlin *et al.* 2000). **Table 1** shows the guidelines for maximum allowable concentrations of Cd (cadmium), Cu (copper), Pb (lead) and Zn (zinc) in agricultural soils receiving biosolids that have been established in various countries. In plants, the estimated safe and adequate daily intake of Zn, Fe (iron) and Cu is between 10,000-20,000, 1000, 12,000 µg/day (Anonymous 1980; Annan *et al.* 2010), but is 11,000 µg/day for Mn (manganese) (Dey *et al.* 2009). The maximum acceptable concentration of Cd in foodstuff is about 1 µg/g (Linder 1991; Annan *et al.* 2010), 100 µg/day for Ni (nickel) (Das and Dasgupta 2002), and 6, 25, and 75 µg/day for Pb in children, pregnant women and adults, respectively (Anonymous 2012).

**Table 1** Maximum concentrations of metals established by various countries for agricultural soils receiving anthropogenic inputs of metals.*

| Country | Cd ($mg\ kg^{-1}$) | Cu ($mg\ kg^{-1}$) | Pb ($mg\ kg^{-1}$) | Zn ($mg\ kg^{-1}$) |
|---|---|---|---|---|
| Australia | 1 | 100 | 150 | 200 |
| Australia | 3 | 200 | 200 | 250 |
| New Zealand | 3 | 140 | 300 | 300 |
| Europe (limits vary among state) | 1-3 | 50-140 | 50-300 | 150-300 |
| Vietnam, pH 6 (limits vary with pH) | 2 | 120 | 70 | 200 |
| USA | 20 | 750 | 150 | 1400 |

Pb – lead; Zn – zinc; Cd – cadmium; Cu – copper
* Source: McLaughlin *et al.* (2000)

Agricultural production in Russia (about 80%) is concentrated in arid regions of the country. The most severe climatic conditions are inherent to the Pre-Caspian lowland, where the aridity coefficient of desert and semi-desert zones in the Volgograd and Astrakhan regions (European Russia), which occupy more than 2000 ha of natural unproductive stocks, is caused by soil salinity and heavy metal pollutants (Yamnova *et al.* 2010). Salt-affected soils in the area were shaped under the impact of a combination of several factors: (1) salt-bearing deposits of the Caspian Sea, (2) salts transferred by wind with a drop in seawater levels (impulverized salts) and (3) salts from groundwater. Filtering and evaporating river waters may also serve as additional sources of salts in arid climates (Yamnova *et al.* 2010).

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**Table 2** Examples of halophytic plant species used for the purpose of phytoremediation.

| Plant species | Heavy metals | References |
|---|---|---|
| *Sesuvium portulacastrum* | Cd, Pb and As | Zaier *et al.* 2010a, 2010b |
| *Halimione portulacoides*, *Spartina maritima* | Cd, Cu, Pb, and Zn | Reboreda and Caçador 2007, 2008 |
| *Spartina densiflora*, *S. maritima* | As, Cu, Fe, Mn, Pb, and Zn | Cambrolle *et al.* 2008 |
| *Halimione portulacoides* | Zn, Pb, Co, Cd, Ni, and Cu | Sousa *et al.* 2008; Almeida *et al.* 2009 |
| *Juncus maritimus* | Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn | Almeida *et al.* 2006 |
| *Sporobolus virginicus*, *Spartina patens*, *Atriplex nammularia* | Zn, Cu, and Ni | Eid and Eissa 2010 |
| *Tamarix smyrnensis* | Pb and Cd | Kadukova *et al.* 2008; Manousaki *et al.* 2008 |
| *Arthrocnemum macrostachyum*, *Spartina argentinensis* | Cd and Cr | Redondo-Gómez *et al.* 2010a, 2010b |

**Table 3** Heavy metals content of light brown soils in lower Volga of Russia (non active and active forms of heavy metals, mg $kg^{-1}$ dry soil).

| Different districts in lower Volga, Volgograd, Russia | Pb (mg $kg^{-1}$ dry soil) | Zn (mg $kg^{-1}$ dry soil) | Cd (mg $kg^{-1}$ dry soil) | Cu (mg $kg^{-1}$ dry soil) |
|---|---|---|---|---|
| Krasnoarmeisky district | 21.3/4.2 | 186.4/28.5 | 1.6/0.1 | 43.6/2.1 |
| Svetloyarsky district | 13.5/4.1 | 180.3/25.6 | 1.54/0.21 | 38.6/2.2 |
| Kirovsky district | 23.1/4.8 | 185.5/27.1 | 1.13/0.22 | 42.4/1.8 |
| Voroshilovsky district | 22.1/4.6 | 182.6/27.5 | 1.15/0.32 | 38.5/1.4 |
| Threshold concentration of heavy metals for Russia (non active and active form, mg $kg^{-1}$ dry soil) | 32.0/6.0 | 100.0/23.0 | 1.0/0.5 | 55.0/3.0 |

Emissions of polluting substances into the atmosphere of the Volgograd region from stationary sources amounted to about 233,741×$10^3$ tons $year^{-1}$ (including 22,710×$10^3$ tons of solid and 211,031×$10^3$ tons of gas and liquid), with Zn being the main heavy metal pollutant of soils within the territory of the lower Volga region {Administration of Volgograd Region (AVR) 2012}. The main sources of atmospheric pollution in the semi-desertic zone of Volgograd and Astrakhan regions include heavy metals from different power-plant-related enterprises, including the metallurgical industry (Severstal-metiz Ltd.), the oil-processing industry (Lukoil-Volgograd Oil Processing Co. Ltd.), and other industries (AVR 2012).

A halophyte is a plant that grows in waters of high salinity, coming into contact with saline water through its roots or by salt spray, such as in saline semi-deserts, mangrove swamps, marshes and sloughs, and seashores (Glenn *et al.* 1999) and can be used in ecological phytomelioration to improve the quality of saline soils and increase the efficiency of pasturable lands (Ruan *et al.* 2010; Ruan and Teixeira da Silva 2011). These plants are also tolerant to heavy metals. Halophytes are capable of growing in soil polluted by heavy metals without accumulating such metals in their vegetative biomass (Dushenkov *et al.* 1997; Kang 2008). Halophytes are capable of surviving and reproducing in environments where the salt concentration is around 200 mM of NaCl or more and can tolerate salt concentrations that would, under natural conditions, kill 99% of other species. Among these salt-adapted halophytes are annuals and perennials, monocotyledonous and dicotyledonous species, shrubs, and some trees. Examples of some halophytic plant species used for the purpose of phytoremediation are presented in **Table 2**.

Consequently, the advantages of halophytes over non-halophytic plants toward several abiotic factors may simply result from the more efficient performance of several basic biochemical tolerance mechanisms (Bohnert *et al.* 1995). Therefore, halophytes have been suggested to be naturally better adapted to cope with environmental stresses, including heavy metals, compared to salt-sensitive crop plants commonly chosen for phytoremediation purposes for the removal of heavy metals from soils (Jordan *et al.* 2002; Ghnaya *et al.* 2005, 2007). The *in vitro milieu* may serve for the rapid selection of metal-tolerant germplasm (e.g., Aldahhak *et al.* 2010).

Previous research in Pakistan (Khan and Qaiser 2006), India (Vinayak *et al.* 2012) and in the USA (WWS 2012) indicated that *Halocnemum strobilaceum* L. and *Artemisia absinthium* L. have the ability to phytoremediate salt- and heavy metal-contaminated soil. However, in Russia, the prospect of these two halophytes remains unknown. On the basis of this background, a lysimetric greenhouse experiment was conducted over three consecutive years in the lower Volga region of Russia to assess the prospect of both these medical halophytes to phytoremediate salt- and heavy metal-contaminated soil.

## MATERIALS AND METHODS

A three-year (2009-2012) lysimetric greenhouse experiment was conducted in the "Volgograd Administrative Department of Environment Protection" (46° 14' 10" N, 48° 11' 15" E), Volgograd, Russia. For the lysimetric greenhouse research, soils were collected from an open field (fallow land) in the lower Volga region of Russia (**Table 3; Fig. 1**), where soils are light brown, organic matter was 1.84% and pH was 7.45. The greenhouse environment during the experimental period (2009-2012) was 25-30°C (air temperature), 75% relative humidity, 15% soil moisture in the soil arable layer, approx. 0.2% $CO_2$ in the air and 90% light availability.

Seeds were collected from wild halophytes from the southern slopes of Ergeninsky, Volga, then grown in the lysimeter. At the time of the experiment, soil moisture in the lysimeter was always maintained at field capacity through daily watering.

To assess the ability of both species of growing in saline soil from the lower Volga region (**Table 3; Fig. 1**), sulphate ($SO_4$)- and chloride (Cl)-containing salts (at 3.0, 5.0, 8.0 and 10.4%, w/v) were added to the lysimeter prior to seeding. Thus, treatments were: control (soil collected from an open field in the lower Volga region was used); $T_{S1}$ ($SO_4$) = $T_{C1}$ (Cl) = 3.0; $T_{S2}$ = $T_{C2}$ = 5.0; $T_{S3}$ = $T_{C3}$ = 8.0 and $T_{S4}$ = $T_{C4}$ = 10.4% for both $SO_4$ and Cl treatments.

To assess the response to Zn in soil where salt concentration was high (10.4% Cl and $SO_4$ type of salts), zinc sulphate ($ZnSO_4$) was applied at 300, 400 and 500 mg Zn $kg^{-1}$ soil ($T_{zn1}$ (control) = without Zn; $T_{zn2}$ = 300, $T_{zn3}$ = 400, $T_{zn4}$ = 500 mg Zn $kg^{-1}$ soil).

Plants biomass was analyzed by ICP-AES (Inductive Coupled

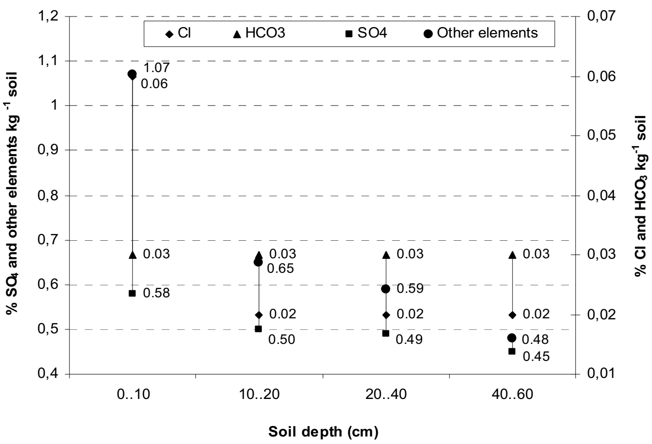


**Fig. 1 Concentration of salts in light brown soils of the lower Volga region of Russia.**

**Table 4** Change of biometric indicators of *Halocnemum strobilaceum* depending on concentration of salts and type of salt structure in the soil. $T_{S1}$ = ($SO_4$) / $T_{C1}$ = (Cl) = 3.0, $T_{S2}/T_{C2}$ = 5.0, $T_{S3}/T_{C3}$ = 8 and $T_{S4}/T_{C4}$ = 10.4 % for both $SO_4$ and Cl content salt treatments.

| Type of salt structure in the soil | Treatments | Concentration (%) | Height, cm | Diameter of a stalk, $cm^2$ | Number of leaves in the main stalk | Dry weight (g) Leaves | Stalk and petioles | Gross weight of elevated parts of plants |
|---|---|---|---|---|---|---|---|---|
| Control (without salt) | | ----- | 77.80 | 8.60 | 9.6 | 12.17 | 16.51 | 35.56 |
| $SO_4$-types salt | $T_{S1}$ | 3.00 | 77.50 | 8.50 | 9.5 | 12.16 | 15.21 | 34.23 |
| | $T_{S2}$ | 5.00 | 77.20 | 8.60 | 9.5 | 12.17 | 16.33 | 35.21 |
| | $T_{S3}$ | 8.00 | 77.40 | 8.60 | 9.5 | 12.15 | 16.43 | 35.52 |
| | $T_{S4}$ | 10.4 | 77.00 | 8.20 | 9.4 | 12.13 | 16.22 | 34.01 |
| Fisher's test | | | ns | ns | ns | ns | ns | ns |
| $Cl^-$ types salt | $T_{C1}$ | 3.00 | 78.80 | 8.60 | 9.5 | 12.17 | 16.23 | 34.23 |
| | $T_{C2}$ | 5.00 | 71.10 | 7.30 | 8.2 | 11.33 | 15.22 | 33.45 |
| | $T_{C3}$ | 8.00 | 67.50 | 6.40 | 7.6 | 11.12 | 14.78 | 32.34 |
| | $T_{C4}$ | 10.4 | 62.10 | 5.90 | 6.9 | 10.10 | 13.55 | 31.78 |
| Fisher's test | | | ns | ns | ns | ns | ns | ns |

ns = not significant

Plasma Atomic Emission Spectrometry) using an Activa–Horiba Jobin Yvon Spectrometer at the Laboratory of the "Volgograd Administrative Department of Environment Protection, Russia".

The availability of heavy metals was evaluated in a two-step sequential extraction procedure, as proposed by Maiz *et al*. (2000). This procedure involved mixing 4 g of soil with 20 ml 0.01M $CaCl_2$, then agitating the solution for 24 h at room temperature. This first suspension was centrifuged at 3000×/min for 15 min, and the supernatant was collected for the analysis of the active fraction. The residue was then rinsed twice with ultra pure water and resuspended in 20 ml of 0.005M diethylene triamine pentaacetic acid (DTPA), 0.01M $CaCl_2$ and 0.1M triethanolamine (TEA), pH 7.3, under continuous agitation, for 24 h. After centrifugation at 3000×/min for 15 min, the supernatant was removed and the mobilizable fraction was analyzed.

The heavy metals of various extracts were measured by ICP-AES. Metal accumulation in plant tissues was evaluated in the two tested species. Plant samples were thoroughly washed in tap water and rinsed three times with distilled water. Samples were then separated into leaves, stems and roots, dried at 40°C until constant weight, ground to a powder, then passed through a 2-mm sieve. Digestion of plant samples was performed using hot nitric concentrated acid (Zarcinas *et al.* 1987; Secu *et al.* 2008).

Data was analyzed using MSTAT-C (Russell 1994). To know the impact of salt and heavy metals on both tested plants, means were separated by analysis of variance and assessed by Fisher's test at $P = 0.05$ (Fisher 1954).

## RESULTS

### Concentration of heavy metals in the light brown soils of the lower Volga region of Russia

The analysis of soil data from different districts in the lower Volga region of Russia (**Table 3**) shows that Zn was the main polluting heavy metal in the light brown soils of the lower Volga region, since there was an excess of maximum available as well as non-active and active forms of Zn. The highest permissible concentration of Zn in non-active and active forms was observed in the Krasnoarmeisky district of the Volgograd region (186.4/28.5 mg $kg^{-1}$ dry soil), and the lowest in the Svetloyarsky district of the Volgograd region (180.3/25.6 mg $kg^{-1}$ dry soil). Others heavy metals (Pb, Cd and Cu) were under the threshold level (**Table 3**).

### Salt concentration in the light brown soils of lower Volga region of Russia

The analysis of soil data for salt concentration from different depths of soil indicated that $SO_4$ and Cl types of salts were higher in the light brown soil of the lower Volga region then other molecules present in the soil. The highest salt concentration was found in the arable level (0-10 cm), but salt concentration decreased with an increase in depth (**Fig. 1**).

### Change of *H. strobilaceum* biometric indicators based on salt concentration in the soil and type of salt structure

A high concentration of salts ($SO_4$ and Cl) did not influence *H. strobilaceum* plant height, stalk diameter and other studied characteristics (**Table 4**). For example, at the maximum concentration of $SO_4$ salt (10.4%), plant height only decreased 1.02% relative to the control (**Table 4**). On the other hand, Cl-type salts negatively impacted the height of plants and other studied characteristics. Cl at 3.0 and 5.0% did not influence plant height, stalk diameter or dry weight, but 10.4% influenced these biometric indicators. For example, 10.4% Cl salt decreased plant height by 20.1% relative to the control (**Table 4**). These findings indicate that *H. strobilaceum* was strongly capable of adapting to saline soil.

### Change of *A. absinthium* biometric indicators based on salt concentration in the soil and type of salt structure

Plant height, stalk diameter and other studied characteristics of *A. absinthium* were not influenced by high concentrations of salts ($SO_4$ and Cl) (**Table 5**). For example, at the maximum concentration of $SO_4$ and Cl salt (10.4%), plant height decreased 2.3 and 4.2% relative to the control (**Table 4**). On the other hand, high concentrations of salts ($SO_4$ and Cl) did not influence the biometric indicators of *A. absinthium* unlike *H. strobilaceum* (**Table 5**). These findings indicated that *A. absinthium* was strongly capable of adapting to saline soil.

### Success of *H. strobilaceum* and *A. absinthium* germination depends on the level of soil Zn

To estimate the tolerance of plants to heavy metals, soil of the highest salt concentration (10.4%) was artificially polluted by zinc sulfate ($ZnSO_4{\cdot}7H_2O$) at three concentrations: 300, 400, 500 mg Zn $kg^{-1}$ soil. Field germination (%) of *H. strobilaceum* and *A. absinthium* was studied when the soil was artificially polluted at different doses (**Fig. 2, 3**). Research findings over three years indicates that the germination level of *H. strobilaceum* and *A. absinthium*on in the control (i.e., without Zn pollution) was 76.4 and 76.3%. Germination of *H. strobilaceum* and *A. absinthium* was not affected by 300 and 400 mg Zn $kg^{-1}$ soil, which was only 1.0% lower than the control (**Fig. 2, 3**). On the other hand, the highest dose of Zn (500 mg Zn $kg^{-1}$) slightly (*F* value insignificant) negatively affected the germination of *H. strobilaceum* (72.8%), which was 4.7% lower than the control (76.4%). Similar results were also observed for the germination of *A. absinthium* (72.9%), which was 4.6% lower (*F* value insignificant) than the control (76.3%) (**Fig. 2, 3**).

**Table 5** Change of biometric indicators *Artemisia absinthium* L. depending on concentration of salts in the soil and type of salt structure of the soil. Treatments detailed in **Table 3**.

| Type of salt structure in the soil | Treatments | Concen-tration (%) | Height, cm | Diameter of a stalk, cm | Number of leaves in the main stalk | Dry weight (g) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Leaves | Stalk and petioles | Gross weight of elevated parts of plants |
| Control (without salt) | | ----- | 68.60 | 7.80 | 8.5 | 11.27 | 15.51 | 34.56 |
| $SO_4$-types salt | $T_{S1}$ | 3.0 | 67.50 | 7.70 | 8.5 | 11.26 | 15.21 | 34.23 |
| | $T_{S2}$ | 5.0 | 67.20 | 7.50 | 8.5 | 11.17 | 15.13 | 34.21 |
| | $T_{S3}$ | 8.0 | 67.40 | 7.10 | 8.4 | 11.15 | 15.03 | 34.12 |
| | $T_{S4}$ | 10.4 | 67.00 | 7.00 | 8.3 | 11.03 | 14.82 | 34.01 |
| Fisher's test | | | ns | ns | ns | ns | ns | ns |
| $Cl^-$ types salt | $T_{C1}$ | 3.0 | 68.10 | 7.60 | 8.5 | 11.27 | 15.13 | 34.33 |
| | $T_{C2}$ | 5.0 | 67.10 | 7.30 | 8.4 | 11.13 | 15.12 | 33.95 |
| | $T_{C3}$ | 8.0 | 66.90 | 6.60 | 8.4 | 11.10 | 14.88 | 33.34 |
| | $T_{C4}$ | 10.4 | 65.70 | 5.90 | 8.4 | 11.11 | 14.55 | 32.78 |
| Fisher's test | | | ns | ns | ns | ns | ns | ns |

ns = not significant

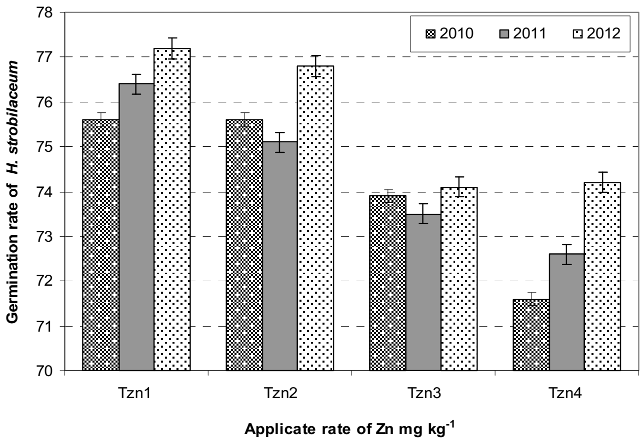


**Fig. 2 Germination (%) of *Halocnemum strobilaceum*, depending on level of soil Zn concentration.** Mean (±SD) was calculated from three replicates for each treatment. $T_{zn1}$ (control) = without Zn; $T_{zn2}$ = 300, $T_{zn3}$ = 400, $T_{zn4}$ = 500 mg Zn kg$^{-1}$ soil.

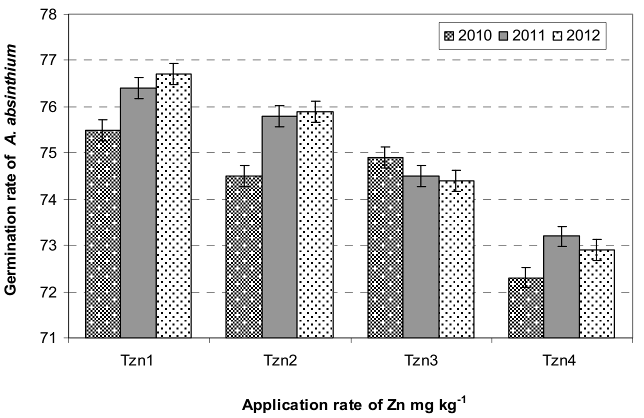


**Fig. 3 Germination (%) of *Artemísia absinthium* L., depending on level of soil Zn concentration.** Mean (±SD) was calculated from three replicates for each treatment. Treatments details as **Fig. 2**.

## Concentration of Zn in *H. strobilaceum* and *A. absinthium* depends on the level of soil Zn

From the three-year field study it was found that high doses of Zn did not increase the Zn concentration in *H. strobilaceum* more than the control. In the control (i.e., without soil pollution caused by Zn), the three-year average concentration of Zn in plants was 40.3 mg Zn kg$^{-1}$ soil, but when soil was polluted by 300, 400, 500 mg Zn kg$^{-1}$ soil, the concentration of Zn in *H. strobilaceum* was 44.2, 49.3, 53.1 mg kg$^{-1}$ biomass, which was statistically similar to the control (**Fig. 4**). Thus, *H. strobilaceum* plants showed tolerance to

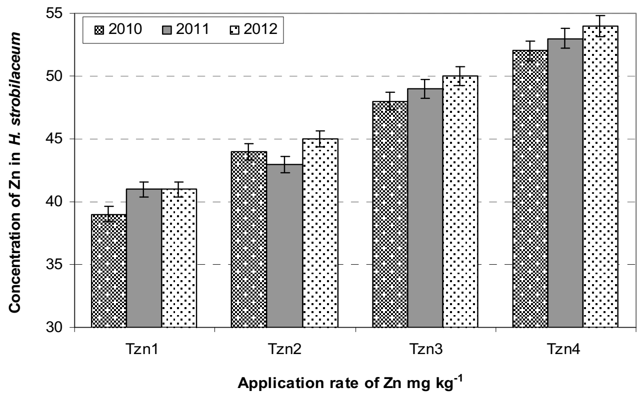


**Fig. 4 Concentration of Zn (mg kg$^{-1}$) in *Halocnemum strobilaceum*, dependence on level of soil Zn concentration.** Mean (±SD) was calculated from three replicates for each treatment. Treatments details as **Fig. 2**.

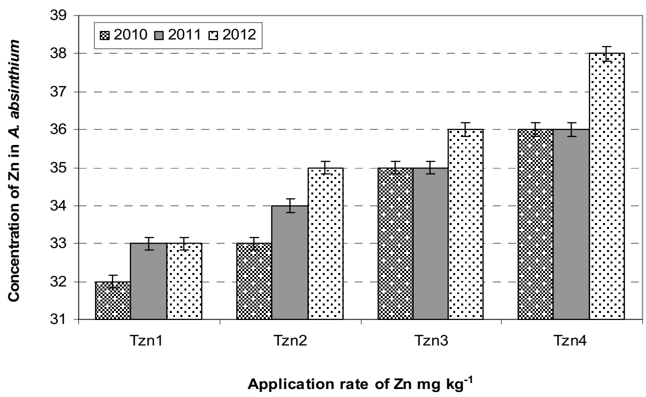


**Fig. 5 Concentration of Zn (mg kg$^{-1}$) in *Artemísia absinthium* plants, depending on level of pollution of the soil zinc.** Mean (±SD) was calculated from three replicates for each treatment. Treatments details as **Fig. 2**.

an increased concentration of Zn in the soil but did not accumulate Zn in the plant biomass.

In the control, the concentration of Zn in *A. absinthium* was 33.2 mg kg$^{-1}$ biomass, which was 21.3% less than the similar treatment of *H. strobilaceum* (three-year average data). When polluted by 300, 400, 500 mg Zn kg$^{-1}$ soil, the concentration of Zn in *A. absinthium* was 43.5, 35.6, 37.4 mg kg$^{-1}$. When soil was polluted by 400 and 500 mg Zn kg$^{-1}$ soil, the concentration of Zn in *A. absinthium* was 38.4% and 41.9% lower than the Zn concentration in *H. strobilaceum* (**Fig. 5**). Thus, *A. absinthium* showed a greater tolerance to a high concentration of Zn in plant biomass than *H. strobilaceum* plants but did not accumulate Zn in the plant biomass.

## DISCUSSION

The present research findings indicate that the two medicinal halophytes (*H. strobilaceum* and *A. absinthium*) growing wild that were studied can potentially be used in ecological phytomelioration to accumulate soil salts in the vegetative biomass of plants. Such halophytes are capable of growing in strongly saline soil and can tolerate a high concentration of Zn. Manousaki *et al.* (2008), Manousaki and Kalogerakis (2011), and Lokhande and Suprasanna (2012) stated that halophytes may be used in phytomelioration due to their biochemical tolerance mechanisms to several environmental factors, including heavy metals and soil salinity. Halophytes have been suggested to be naturally better adapted to environmental stresses, such as heavy metals, salt-sensitivity and thus offer a great potential for the phytoremediation and decontamination of heavy metal-polluted soils (Manousaki *et al.* 2008; Manousaki and Kalogerakis 2011; Lokhande and Suprasanna 2012). Manousaki *et al.* (2008) and Manousaki and Kalogerakis (2011) observed that halophytes are ideal candidates for phytoextraction, phytostabilization, or phytoexcretion of heavy metal-polluted saline and nonsaline soils for soil desalination in arid and semiarid regions.

### Role of halophytes for desalination and stabilization of saline soils

When soil has an electrical conductivity of 4 dS $m^{-1}$ or more (which is equivalent to 40 mM NaCl) and when the osmotic pressure is approximately −0.2 MPa, such soils are termed saline soils (Goudie 1990). Saline soils are problematic around the world, particularly in arid and semi-arid regions, similar to the soil in our study area (**Table 3; Fig. 1**) where insufficient precipitation and inappropriate irrigation systems have been unable to reduce the salt burden in the rhizosphere of plants and where suitable physicochemical methods are too expensive to apply (Shahid 2002; Lokhande and Suprasanna 2012). In this context, research all around the world has developed various physical, chemical, and biological approaches to reclaim such saline soils (Shahid 2002). Among these, a large number of plant species has been utilized for soil desalination (**Table 2**; Lokhande and Suprasanna 2012). Based on this, plants differ greatly in their growth response to saline conditions and are therefore classified as "halophytes", which have the capacity of growing on highly saline environments (Munns and Tester 2008) and can complete their life cycle in a substrate rich in NaCl that is normally toxic to other plant species (Flowers and Colmer 2008). Therefore, studies on halophytes can elucidate the mechanism by which halophytes survive and maintain productivity under abiotic constraints which can be used to define a minimal set of adaptations required in tolerant germplasm (Lokhande and Suprasanna 2012). This knowledge can help to evaluate the overall feasibility of high-salinity agriculture, which depends on more than finding a source of tolerant germplasm (Glenn *et al.* 1997; Lokhande and Suprasanna 2012).

### Role of halophytes in phytoremediation of heavy metal-contaminated soil

Contamination of agricultural soil by heavy metals has become a serious environmental concern due to their mostly negative impact on crop growth (i.e., above physiologically normal conditions) and ecosystems (Almeida *et al.* 2006; Reboreda and Caçador 2007, 2008; Cambrolle *et al.* 2008; Kadukova *et al.* 2008; Manousaki *et al.* 2008; Sousa *et al.* 2008; Almeida *et al.* 2009; Eid and Eisa 2010; Redondo-Gómez *et al.* 2010a, 2010b; Ruan *et al.* 2010; Ruan and Teixeira da Silva 2011; Zaier *et al.* 2010a, 2010b; Lokhande *et al.* 2011; Lokhande and Suprasanna 2012). Such toxic elements are considered to be soil and water pollutants due to their widespread occurrence, and their acute and chronic toxic effect on plants grown in such soils as well as on humans living in their surrounding (Yadav 2010). All heavy metals can be toxic when present above threshold concentrations, although this concentration depends on the metal, the animal/plant species, and also on the environment, which determines the availability (Kennish 1996).

In our present study, soil of the lower Volga regions of Russia contained different types of industrial heavy metals (**Table 3**). Thus, the ability of two medicinal halophytes as soil phytoremediators was tested. From a variety of plant species studied (**Table 2**), it was noted that each species has a species-specific limitation to accumulate toxic metals and detoxify nontoxic compounds through enzymatic reactions. In the course of evolution from marine to freshwater habitats, halophytes are the most successful group of plants which have shown adaptations to a variety of abiotic stresses, tolerance to heavy metal stress (Aken 2008; Lokhande and Suprasanna 2012). Different plant species may have evolved different mechanisms to tolerate excess heavy metals, and even within the one plant species more than one mechanism could be in operation (Hossain *et al.* 2011). Physiological, biochemical, and molecular approaches continue to be employed to identify the underlying mechanisms of heavy metals accumulation, tolerance, and adaptive mechanisms to cope with heavy metals stress (Hossain *et al.* 2011). Some adaptive mechanisms evolved by tolerant plants include immobilization, plasma membrane exclusion, restriction of uptake and transport, synthesis of specific heavy metal transporters, chelation and sequestration of heavy metals, induction of mechanisms contrasting the effects: such as upregulation of antioxidants and the glyoxalase system), induction of stress proteins, the biosynthesis of proline, polyamines, and signaling molecules such as salicylic acid and nitric oxide (Dalcorso *et al.* 2008, 2010; Hossain *et al.* 2009, 2010, 2011; Zhu *et al.* 2011).

Some halophytes (*Aegiceras* and *Avicennia*) have specialized glands or glandular trichomes on abaxial and adaxial leaf surfaces to secrete excessive $Na^{+}$ and $K^{+}$; while such specialized structures are absent in nonsecretors halophytes (*Rhizophora* and *Sonneratia*) (Lokhande *et al.* 2011; Lokhande and Suprasanna 2012). Indeed, mangroves and a number of other estuarine halophytes with glandular tissue are known to excrete heavy metals concomitantly with other solutes (MacFarlane and Burchett 2000). The variation in morphology and function of nutritive root tissue and glandular tissue to deal with the challenges of excess cations in saline environments could become significant for metal accumulation, transport, partitioning, and excretion among halophytic plant species (Zabłudowska *et al.* 2009).

## CONCLUSIONS

This study shows that *H. strobilaceum* and *A. absinthium* have a high tolerance ability to grow in the presence of a high concentration of Zn and salts ($SO_4$ and Cl) without accumulating Zn in plant biomass. Thus, these plants may be used in the pharmaceutical industry as medicinal raw materials as well as in the phytoremediation of salt- and heavy metal-contaminated soil.

## REFERENCES


**Administration of Volgograd Region (AVR)** (2012) Condition of environment of the city of Volgograd, Department of Environment, Ministry of Environment, Volgograd, Russia

**Aken BV** (2008) Transgenic plants for phytoremediation: Helping nature to clean up environmental pollution. *Cell* **26**, 225-227

**Aldahhak O, Salim Z, Teixeira da Silva JA, Abdul-Kader AM** (2010) *In vitro* approach to the multiplication of a halophyte species forage shrub *Atriplex halimus* L. and *in vitro* selection for salt tolerance. *International Journal of Plant Developmental Biology* **4 (1)**, 8-14

**Almeida CMR, Mucha AP, Vascancelos MTSD** (2006) Variability of metal contents in the sea rush *Juncus maritimus* - estuarine sediment system through one year of plant's life. *Marine Environmental Research* **61**, 424-438

**Almeida CMR, Dias AC, Mucha AP, Bordalo AA, Vascancelos MTSD** (2009) Study of the influence of different organic pollutants on Cu accumulation by *Halimione portulacoides*. *Estuarine*, *Coastal and Shelf Science* **85**, 627-632

**Annan K, Kojo AI, Cindy A, Samuel A, Tunkumgnen BM** (2010) Profile of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulations. *Pharmacognosy Research* **2 (1)**, 41-44

**Anonymous** (1980) *Food and Nutrition Board NRC* (9th Edn), Washington, DC: National Academy of Sciences; 1980. NRC Recommended dietary allowances, USA.

**Anonymous** (2012) Lead Content of Soil, Plants, Foods, Air, and Chinese Herb Formulas by Subhuti Dharmananda, Ph.D., Director, Institute for Traditional Medicine, Portland, Oregon. Available online: http://www.itmonline.org/arts/lead.htm

**Begonia GB** (1998) Growth responses of Indian mustard (*Brassica juncea* L.) and its phytoextraction of lead from a contaminated soil. *Bulletin of Environmental Contamination and Toxicology* **61**, 38-43

**Bohnert H J, Nelson DE, Jensenay RG** (1995) Adaptations to environmental stresses. *Plant Cell* **7**, 1099-1111

**Cambrolle J, Redondo-Gomez S, Mateos-Naranjo E, Figueroa ME** (2008) Comparison of the role of two *Spartina* species in terms of phytostabilization and bioaccumulation of metals in the estuarine sediment. *Marine Pollution Bulletin* **56**, 2037-2042

**Das KK, Dasgupta S** (2002) Effect of nickel sulphate on testicular steroidogenesis in rats during protein restriction. *Environmental Health Perspectives* **110**, 923-926

**Dey S, Saxena A, Dan A, Swarup D** (2009) Indian medicinal herb: A source of lead and cadmium for humans and animals. *Archives of Environmental and Occupational Health* **4**, 164-167

**Divan Jr. AM, Oliva MA, Ferreira FA** (2008) Dispersal pattern of airborne emissions from an aluminium smelter in Ouro Preto, Brasil, as expressed by foliar fluoride accumulation in eight plant species. *Ecological Indicators* **2**, 454-461

**Dushenkov S, Kapulnik Y, Blaylock M, Sorochisky B, Raskin I, Ensley B** (1997) Phytoremediation: A novel approach to an old problem. In: Wise DL (Ed) *Global Environmental Biotechnology*, Elsevier Science BV, Amsterdam, pp 563-572

**Eid MA, Eisa SS** (2010) The use of some halophytic plants to reduce Zn, Cu and Ni in soil. *Australian Journal of Basic and Applied Sciences* **4**, 1590-1596

**Fisher RA** (1954) *Statistical Methods for Research Workers* (12th Edn), Oliver and Boyd, pp xv, 356

**Flowers TJ, Colmer TD** (2008) Salinity tolerance in halophytes. *New Phytologist* **179**, 945-963

**Franzaring J, Hrenn C, Schumm A** (2006) Environmental monitoring of fluoride emission using precipitation, dust, plant and soil. *Environmental Pollution* **3 (1)**, 158-165

**Ghnaya T, Nouairi I, Slama I, Messedi D, Grignon C, Adbelly C, Ghorbel MH** (2005) Cadmium effects on growth and mineral nutrition of two halophytes: *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*. *Journal of Plant Physiology* **162**, 1133-1140

**Ghnaya T, Slama I, Messedi D, Grignon C, Ghorbel MH, Adbelly C** (2007) Effects of $Cd^{2+}$ on $K^+$, $Ca^{2+}$ and N uptake in two halophytes *Sesuvium portulacastrum* and *Mesembryanthemum crystallinum*: Consequences on growth. *Chemosphere* **67**, 72-79

**Glenn EP, Brown JJ, Blumwald E** (1999) Salt tolerance and crop potential of halophytes. *Critical Reviews in Plant Sciences* **18 (2)**, 227-255

**Goudie AS** (1990) Soil salinity-causes and controls. In: *Techniques for Desert Reclamation*, John Wiley and Sons Ltd., Wiley, Chichester, UK, pp 110-111

**Hamon R, McLaughlin M** (2003) Food Crop Edibility on the Ok Tedi/Fly River Flood Plain: Report for OK Tedi Mining Ltd. CSIRO Australian Centre for Environmental Contaminants Research A One-CSIRO Centre. 44 pp. Available online: http://www.oktedi.com/attachments/246_030815_Food%20Crop%20Edibility_Hammon%20&%20McLaughlin_CSIRO_FINAL.pdf

**Hossain MA, Hossain MZ, Fujita M** (2009) Stress-induced changes of methylglyoxal level and glyoxalase I activity in pumpkin seedlings and cDNA cloning of glyoxalase I gene. *Australian Journal of Crop Science* **3 (2)**, 53-64

**Hossain MA, Hasanuzzaman M, Fujita M** (2010) Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. *Physiology and Molecular Biology of Plants* **16 (3)**, 259-272

**Hossain MA, Hasanuzzaman M, Fujita M** (2011) Coordinate induction of antioxidant defense and glyoxalase system by exogenous proline and glycinebetaine is correlated with salt tolerance in mung bean. *Frontiers of Agriculture in China* **5 (1)**, 1-14

**Jordan FL, Robin-Abbott M, Maier RM, Glenn EP** (2002) A comparison of chelator-facilitated metal uptake by a halophyte and a glycophyte. *Environmental Toxicology and Chemistry* **21**, 2698-2704

**Kadukova J, Manousaki E, Kalogerakis N** (2008) Pb and Cd accumulation and phytoexcretion by salt cedar (*Tamarix smyrnensis* Bunge). *International Journal of Phytoremediation* **10**, 31-46

**Kang D-H, Tsao F, Wang-Cahill S, Rock AP** (2008) Assessment of landfill leachate volume and concentration of cyanide and fluoride during phytoremediation. *Bioremediation Journal* **3 (1)**, 32-45

**Kennish MJ** (1996) *Practical Handbook of Estuarine and Marine Pollution*, CRC Press, 524 pp

**Linder MC** (1991) *Nutritional Biochemistry and Metabolism*, Appleton & Lange, New York, USA. Available online: http://www.amazon.com/gp/customer-media/product-gallery/0838570844/ref=cm_ciu_pdp_images_all

**Lokhande VH, Suprasanna P** (2012) Prospects of halophytes in understanding and managing abiotic stress tolerance. In: Ahmad P, Prasad MNV (Eds) *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*, Springer Science+Business Media, LLC, Berlin, pp 29-56

**Lokhande VH, Srivastava S, Patade VY, Dwivedi S, Tripathi RD, Nikam TD, Suprasanna P** (2011) Investigation of arsenic accumulation and tolerance in *Sesuvium portulacastrum* (L.). *Chemosphere* **82**, 529-534

**MacFarlane GR, Burchett MD** (2000) Cellular distribution of copper, lead and zinc in the grey mangrove, *Avicennia marina* (Forsk.) Vierh. *Aquatic Botany* **68**, 45-59

**Maiz I, Arambarri I, Garcia R, Millan E** (2000) Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. *Environmental Pollution* **110**, 3-9

**Manousaki E, Kalogerakis N** (2011) Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. *Industrial and Engineering Chemistry Research* **50**, 656-660

**Manousaki E, Kadukova J, Papadonatonakis N, Kalogerakis N** (2008) Phytoextraction and phytoexcretion of Cd by *Tamarix smyrnensis* growing on contaminated non-saline and saline soils. *Environmental Research* **106**, 326-332

**McLaughlin D, Shapley R, Shelley M, Wielaard J** (2000) A neuronal network model of macaque primary visual cortex (v1): Orientation selectivity and dynamics in the input layer 4Cα. *Proceedings of the National Academy of Sciences USA* **97**, 8087-8092

**Munns R, Tester M** (2008) Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**, 651-681

**Qadir M, Steffens D, Yan F, Schubert S** (2003) Sodium removal from a calcareous saline-sodic soil through leaching and plant uptake during phytoremediation. *Land Degradation and Development* **14**, 301-307

**Ravindran KC, Venkatesan K, Balakrishnan V, Chellappan KP, Balasubramanian T** (2007) Restoration of saline land by halophytes for Indian soils. *Soil Biology and Biochemistry* **39**, 2661-2664

**Redondo-Gómez S, Mateos-Naranjo E, Andrades-Moreno L** (2010a) Accumulation and tolerance characteristics of cadmium in a halophytic Cd-hyperaccumulator, *Arthrocnemum macrostachyum*. *Journal of Hazardous Materials* **184**, 299-307

**Redondo-Gómez S, Mateos-Naranjo E, Figueroa ME, Davy AJ** (2010b) Salt stimulation of growth and photosynthesis in extreme halophyte, *Arthrocnemum macrostachyum*. *Plant Biology* **12**, 79-87

**Reboreda R, Caçador I** (2007) Halophyte vegetation influences in salt marsh retention capacity for heavy metals. *Environmental Pollution* **146**, 147-154

**Reboreda R, Caçador I** (2008) Enzymatic activity in the rhizosphere of *Spartina maritima*: potential contribution for phytoremediation of metals. *Marine Environmental Research* **65**, 77-84

**Rozena J, Flowers T** (2008) Crops for a salinized world. *Science* **322**, 1478-1480

**Ruan C-J, Teixeira da Silva JA, Qin P, Lutts S, Gallagher JL** (2010) Halophyte improvement for a salinized world. *Critical Reviews in Plant Sciences* **29 (6)**, 329-359

**Ruan C-J, Teixeira da Silva JA** (2011) Metabolomics: Creating new potentials for unraveling mechanisms in response to salt and drought stress and for biotechnological improvement of xero-halophytes. *Critical Reviews in Biotechnology* **31 (2)**, 152-168

**Russell OF** (1994) MSTAT–C v.2.1 (A computer based data analysis software). Crop and Soil Science Department, Michigan State University, USA

**Secu CV, Iancu OG, Buzgar N** (2008) Lead, zinc and copper in the bioacumulative horizon of soils from Iaşi and the surrounding areas. *Carpathian Journal of Earth and Environmental Sciences* **3 (2)**, 131-144

**Sousa AI, Caçador I, Lillebo AI, Pardal MA** (2008) Heavy metal accumulation in *Halimione portulacoides*: Intra-and extra-cellular metal binding sites. *Chemosphere* **70**, 850-857

**WWS (WorldWideScience)** (2012) Sample records for halophytes. U.S. Department of Energy's Office of Scientific and Technical Information as the Operating Agent for the Worldwide Science Alliance. Available online: http://worldwidescience.org/topicpages/h/halophyte+suaeda+glauca.html

**White PJ, Broadley MR** (2001) Chloride in soils and its uptake and movement within the plant: A review. *Annals of Botany* **88**, 967-988

**Yadav SK** (2010) Heavy metal toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany* **76**, 167-179

**Yamnova IA, Chernousenko GI, Dokuchaev VV** (2010) Soil salinity in the southern Pre-Caspian lowland as related to climate peculiarity and anthropogenic effects. In: Thomas RP (Ed) *Proceedings of the Global Forum on Salinization and Climate Change (GFSC 2010)*, Valencia, 25–29 October 2010, FAO, Rome Italy. pp 9-14. Available online: www.fao.org/uploads/media/BOOK_printing.pdf

**Zabłudowska E, Kowalska J, Jedynak L, Wojas S, Skłodowska A, Anto-**

**siewicz DM** (2009) Search for a plant for phytoremediation – What can we learn from field and hydroponic studies? *Chemosphere* **77**, 301-307

**Zaier H, Ghnaya T, Lakhdar A, Baioui R, Ghabriche R, Mnasri M, Sghair S, Lutts S, Abdelly C** (2010a) Comparative study of Pb-phytoextraction potential in *Sesuvium portulacastrum* and *Brassica juncea*: Tolerance and accumulation. *Journal of Hazardous Materials* **183 (1-3)**, 609-615

**Zaier H, Mudarra A, Kutscher D, Fernandez de la Campa MR, Abdelly C, Sanz-Medel A** (2010b) Induced lead binding phytochelatins in *Brassica juncea* and *Sesuvium portulacastrum* investigated by orthogonal chromatography inductively coupled plasma-mass spectrometry and matrix assisted laser desorption ionization-time of flight-mass spectrometry. *Analytica et Chimica Acta* **671 (1-2)**, 48-54

**Zarcinas BA, Cartwright B, Spouncer LR** (1987) Nitric acid digestion and multielement analysis of plant material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* **18 (1)**, 131-146

**Zhu XF, Zheng C, Hu YT, Jiang T, Liu Y, Dong NY, Yang JL, Zheng SJ** (2011) Cadmium-induced oxalate secretion from root apex is associated with cadmium exclusion and resistance in *Lycopersicon esculentum*. *Plant, Cell and Environment* **34 (7)**, 1055-1064