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# Amount of Mn and Zn in herbaceous plants growing on industrial area of steel production companies in southeast of Ahvaz, Iran

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## ABSTRACT

In the present study, a field study was performed on some herbaceous plants growing in the southeast of Ahvaz, where some metal producing industries are active. The aim of this study was to investigate and compare manganese (Mn) and zinc (Zn) accumulation in seven dominant herbaceous plants in this area. Plant samples were collected randomly. Associated soils were sampled from the same sites next to the root of individual plants. The metals concentration in the soil and the plant samples were determined by flame atomic absorption spectrometry. Highest Mn and Zn concentrations were observed in the shoots of *Halocnemum strobilaceum*, *Taraxacum kotschyi*, *Malva parviflora,* and *Solanum nigrum*. Moreover, elevated accumulation of Mn was found in the roots of *Lolium temulentum*, and *Convolvulus arvensis*. Regarding to defined standards for phytoremediation purposes, studied plants could not be classified as hyperaccumulators, at least under field conditions. Nevertheless, based on accounted bioconcentration and translocation factors, it seems that the majority of investigated plants have the metals accumulation capacity in shoot parts.

**Keywords:** bioconcentration, metal accumulation, soil concentration, translocation factor.

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## Introduction

Manganese and zinc are the component of heavy metals which act as micronutrient essential elements for normal plant growth, development and functioning. Routine concentration of Mn and Zn is 10-100 and 15-50 μg $g^{-1}$ dry weight of plant tissue, respectively (1). Manganese, as a cofactor of Mn-superoxidase dismutase, caused to protect the cells from the oxidative stress. Moreover, Mn includes in activation of some enzymes which are involved in transcription, gibberellic acid and fatty acid biosynthesis and nitrogen metabolism (2). Zinc is also required to maintain integrity of biomembranes. It is supposed that Zn has an important role in DNA and RNA synthesis, seed maturity, signal transduction pathway and some Zn-dependent hydrolytic routes in plant cells (1). Despite significant functions of mentioned metals, their high concentrations are very toxic and lead to metabolic and developmental disorders at cellular-molecular level in plants (3). Mining and industrial activities produce heavy metal particles such as, Mn, Zn, Cd, Ni, Fe, Hg and so forth. in the environment. Heavy metals do not destroy and remain in the biosphere for a long time, and exert extreme harmful effects ecologically and environmentally. High heavy metals concentrations of soil result in reducing growth, reproduction and development of various organisms (4). Due to unfavorable consequences of chemical and physical techniques on environment, there have been attempts to find more safety and efficient manners to eliminate many toxic materials such as heavy metals from the environment. Phytoremediation, the idea to clean-up contaminated environments using plants and the eco-friendly technology, is a relatively valuable and effective manner and a low-cost solution to decontaminate metal-rich environments (5). The plants exploit three strategies to grow and survive in metal-rich soils (6): excluder species, which concentration of element in the shoots is kept in low amounts in spite of its high concentration in the roots. They detoxify most of heavy metals in the roots, through reduced translocation to the shoots. In indicator species, metal concentration in the plant is equivalent to its content in the soil. Accumulator species that have the ability to concentrate on metal in their shoot parts more than its amount in the soil. Hyperaccumulators are subgroup of accumulator plants which have very high potential to accumulate significant extents of metals particularly in their aboveground parts without any symptoms indication of toxicity at levels 100-1000 fold which are higher than those observed in non-accumulators (7, 8). Hyperaccumulators may be advantageous for remediation of polluted soils by means of phytoextraction. Regarding plant species which grow on the same soil area, comprise different potential to uptake the metals in their organs (9). Some surveys have been conducted to determine heavy metals concentration and accumulation capacity of the plants growing on mining and industrial regions (for example, 10, 11, 12, 13, 14, 15, 16). Due to more adjustment with climatic and environmental conditions of each zone, native plant species are suggested for phytoremediation objectives (17). Thus, screening of accumulation and uptaking capacity of the plants growth on metalliferous soils and industrial regions can be useful to present a consistent management plan for the removing heavy metals from the environment.

In addition to oil and gas resources, many industries such as steel production industries have developed in Khouzestan Province located in south of Iran. The activity of these industries may be led to produce and release various metals dust such as Mn and Zn in the environment. This work was carried out as a field screen to assess and compare content, accumulation and distribution of Mn and Zn, as micronutrient heavy metals, in dominant herbaceous plants growth on areas surrounding steel production industries in southeast of Ahvaz, Khouzestan Province, Iran.

## Materials and Methods

### Corpus of the study

Several steel producing industries are located in Ahvaz, Khouzestan Province of Iran. In this study, areas surrounding some of these industries in southeast of Ahvaz (sited in Ahvaz-Bandare Imam road) were screened. Ahvaz has a tropical climate with annual average temperature more than 25ºC, wind speed 9 m $s^{-1,}$ and annual rainfall 213 mm. Investigated area has been located geographically the northen latitude 31º20´ and the eastern longitude 48º 40´.

### Soil and plant sampling

The soil and plant sampling were performed during winter (February and March) due to some reasons as follows: 1) Production activity of steel industries is elevated throughout winter since energy costs are very high in summer. 2) The region herbs often complete their life cycle during winter. Therefore, previous study during summer season in this area was mainly directed on tree and shrub types to assay the metals content such as Mn and Zn (18). Soil samples were also collected from the rhizosphere of each plant (0-20 cm depth). After sieving through 2 mm mesh, soil samples air-dried in room temperature for one week. Dominant herbaceous plants include; *Convolvulus arvensis* L. (Convolvulaceae), *Halocnemum strobilaceum* (Pall.) Bieb. (Amaranthaceae), *Lolium temulentum* L. (Poaceae), *Malva parviflora* L. (Malvaceae), *Salsola soda* L. (Amaranthaceae), *Solanum nigrum* L. (Solanaceae) and *Taraxacum kotschyi* Soest (Asteraceae) were sampled. The plant samples immediately transported to laboratory and washed thoroughly with tap water and deionized water to eliminate dust and soil particles adhere to plant surfaces. Shoot and root parts were separated and oven-dried at 70ºC for 72 h, subsequently, dried matters were grounded to get plant extraction ready. In this study, the collection of soils and plants was randomly performed in six replicates for each plant species. Soil or plant samples were then mixed to obtain a whole soil or plant sample according to Yanqun *et al*. (2004). Three replicates were used to estimate metals concentration of soil and plant samples.

## Assay of heavy metals concentration

Soil samples were analyzed the total and bioavailable forms of heavy metals. To evaluate Mn and Zn total concentration, 1 gr of sieved soils were mixed with 15 ml 65% $HNO_3$ and 10 ml 37% HCl. After heating and desiccating, 30 ml of 1 N HCl was added. The digests were then filtered and made up to volume 50 ml with deionized water (19). For determing bioavailable metal concentration which was consistent with Lindsay and Norvell (1978), 10 gr of air-dried and sieved soil samples were put in polythene bottles and mixed with 20 ml of DTPA solution (0.005 M, pH= 7.3). The suspensions were shaken for 2h and then filtered. Grounded shoot and root samples were extracted using 65% $HNO_3$ and 30% $H_2O_2$ to assay concentrations of Mn and Zn (21). 10 ml of 65% $HNO_3$ was added to 1 gr of powdered samples. The digests were kept at room temperature overnight and then heated at 85 ºC. After cooling, the digests were mixed with 1 ml of 30% $H_2O_2$ then filtered. Plant extracts were diluted with deionized water to 50 ml. The amount of mentioned metals was determined by using a Flame Atomic Absorption Spectrometer instrument (GBC, Avanta model, Australia). Standard samples were prepared from $MnSO_4$ and $ZnSO_4$ solutions.

Translocation factor (TF) and bioconsentration factor (BF) are used as one of the description aspects of a hyperaccumulator plant that reveal metal concentrating and translocating capacity in the shoot parts. TF was calculated as the ratio of metal concentration in the shoot to the root (22). Bioconsentration factor (BF), as an enrichment coefficient, was evaluated as the ratio of metal concentration either in the shoot or in the root to the soil. (23).

## Data analysis

The average metals concentration was estimated from the sum of three replicates. Obtained data were compared by Duncan's Multiple Range Test at $P < 0.05$ confidence level. Statistical analysis was carried out using SPSS 20 software (version 20, Novin Pendar Engineering Co.).

## Results

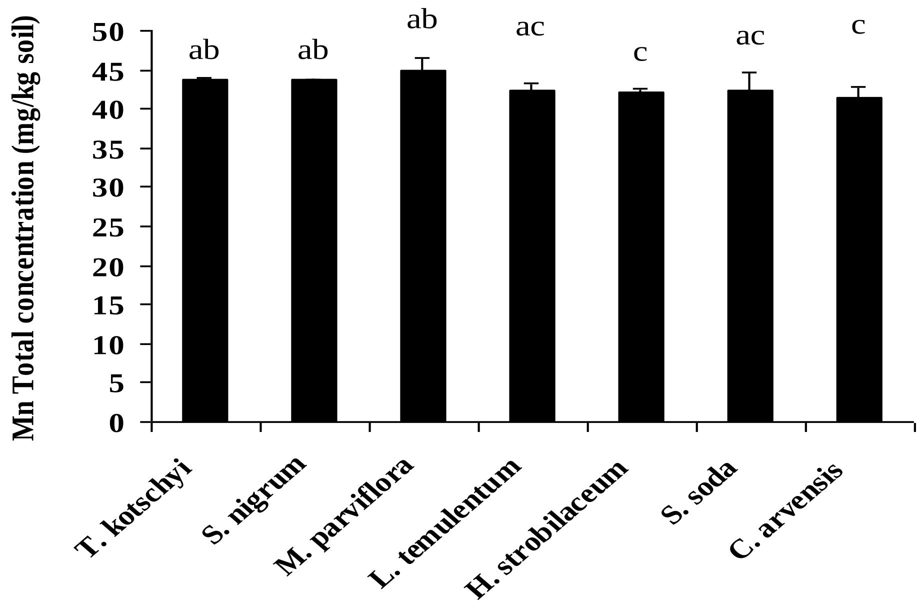
### Metal concentration in soils

The total and bioavailable heavy metals concentrations in the soils surrounding steel production industries in Ahvaz were analyzed. As represented in Table 1, average concentration of Mn illustrated a more elevated level compared to Zn concentration in screened soils, as either total or bioavailable.

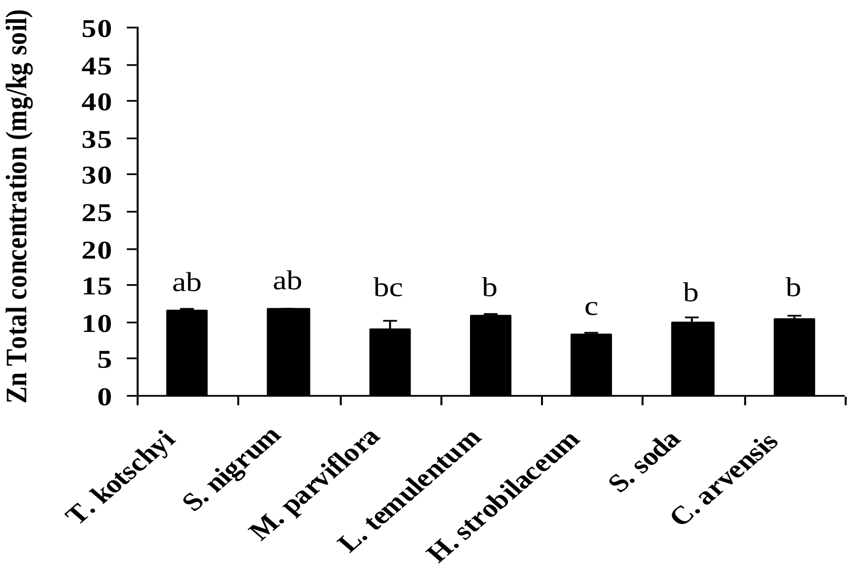
**Table 1. Avarage concentration of Mn and Zn in the area (mg kg$^{-1}$) soils and (mg kg$^{-1}$ DW) plants**

| | Soil total concetration | Soil bioavailable concentration | Plant concentartion |
|---|---|---|---|
| **Mn** | 42.93 ± 0.18 | 5.79 ± 0.05 | 77.97 ± 0.55 |
| **Zn** | 10.32 ± 0.15 | 1.27 ± 0.15 | 41.49 ± 0.15 |

In the adjacent soils of screened plants, there was approximately no notable difference between the other soil samples in the magnitude of Mn (Fig. 1A). The lowest Zn content was assayed in *H. strobilaceum* soil 8.38 ± 0.2 mg kg$^{-1}$ (Fig. 1B). Figure 2 shows the bioavailable concentrations of Mn and Zn in the soil samples. Mn concentration exhibited a significant decrease ($P<0.05$) in the soils of *S. soda* (1.47±0.1 mg kg$^{-1}$) and *C. arvensis* (1.22±0.2 mg kg$^{-1}$) compared to the other soil samples. It was not almost observed any considerable difference in Mn bioavaiability between soil samples of the other investigated species (Fig. 2A). Zn concentration displayed a remarkable increase (2.9±0.62 mg kg$^{-1}$, $P<0.05$) in the soil of *S. soda* in comparison to the other samples (Fig. 2B). Minimum extent of Zn was nearly assayed zero in the soil around *H. strobilaceum* (0.019±0.01 mg kg$^{-1}$).

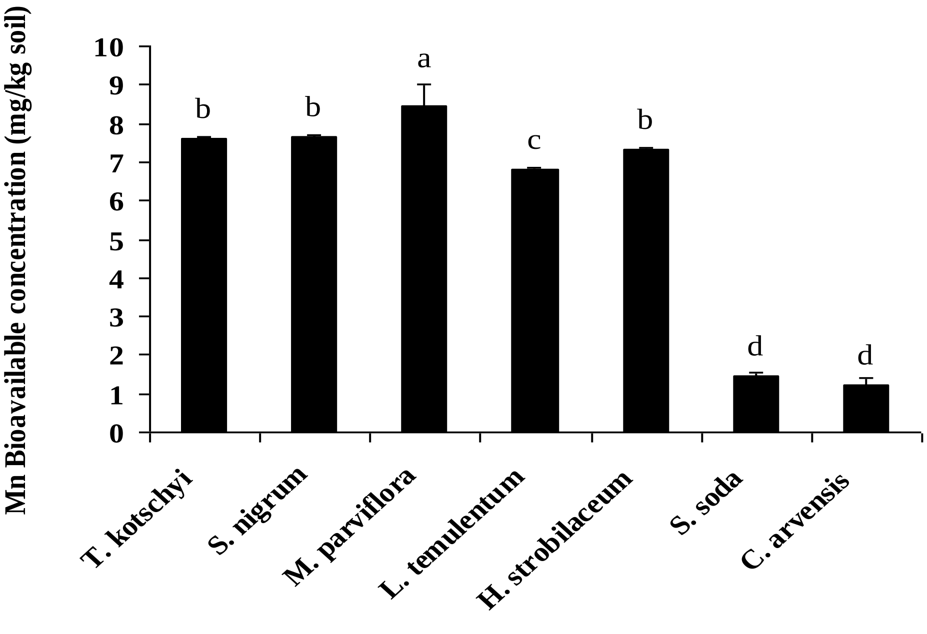



(a)

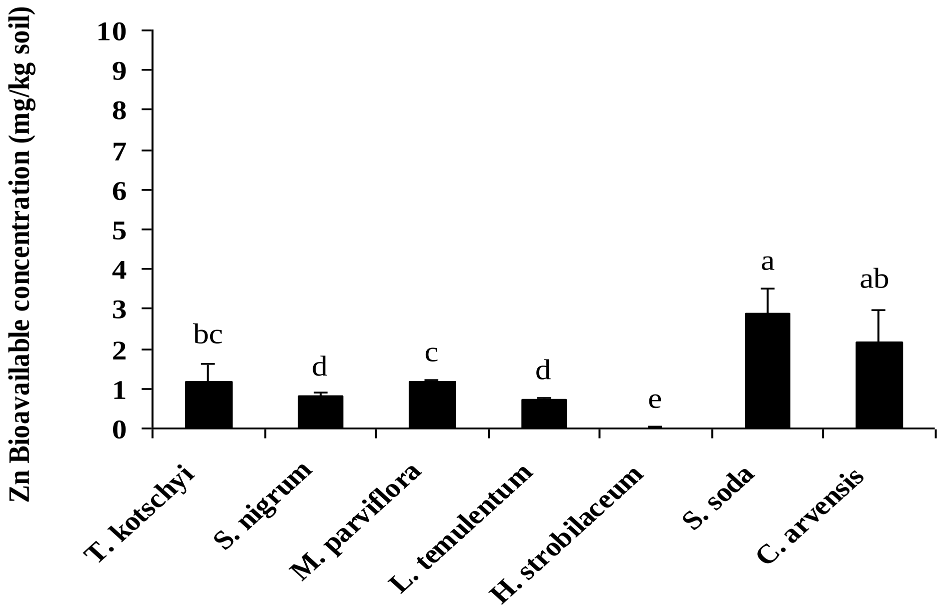



(b)

**Figure 1. Total Mn (A) and Zn (B) concentation (mg kg$^{-1}$) in the soils. The values are mean of three replicates ± SD. The same letters are not significantly different at confidence level P < 0.05.**




(a)

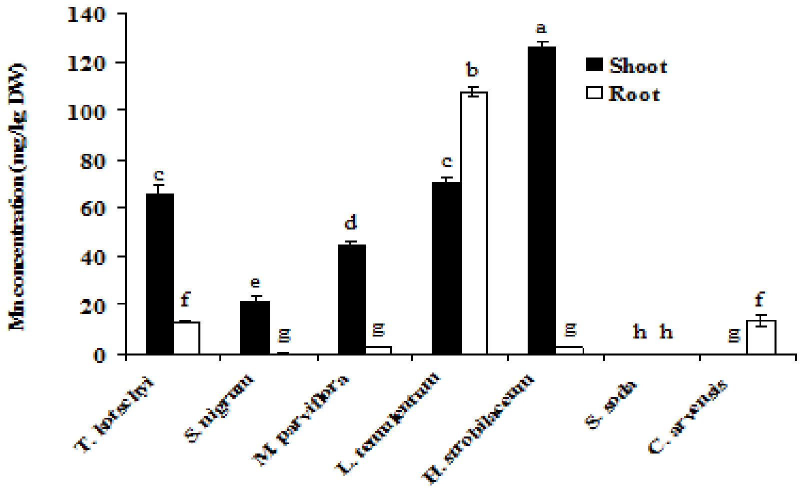



(b)

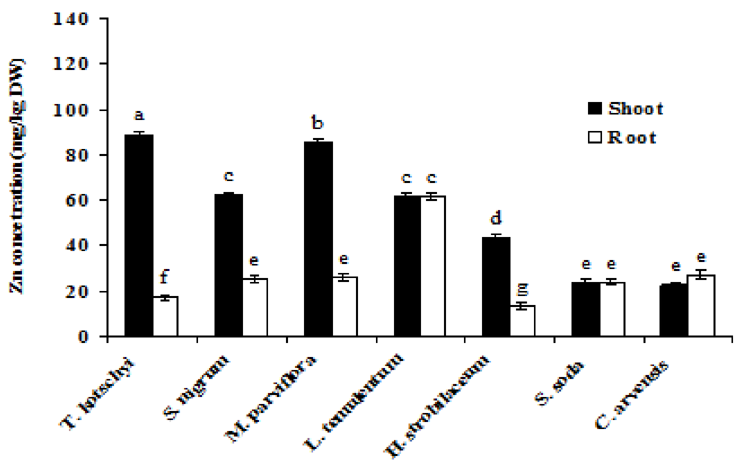
**Figure 2. Bioavailable Mn (A) and Zn (B) concentation (mg kg$^{-1}$) in the soils. The values are mean of three replicates ± SD. The same letters are not significantly different at confidence level P < 0.05.**

## Metal concentration in plants

The average concentration of metal in area plants showed the same trend as in the soils, *e. g*. the order of Mn > Zn (Table 1). Distribution of Mn and Zn quantities in the shoot and the root of plants was widely varied (Fig. 3). In spite of Mn higher accumulation in aboveground parts of *H. strobilaceum*, *T. kotschyi*, *M. parviflora* and *S. nigrum* compared to the roots, a significant increase (P < 0.05) was assayed in the roots of *L. temulentum* and *C. arvensis* in comparision with the shoots (Fig. 3A). The highest Mn concentration was found in the shoot of *H. strobilaceum* and the root of *L. temulentum*, 125.71±2.5 and 108±1.6 mg $kg^{-1}$, respectively. No acceptable content of Mn was determined in the shoot and the root tissues of *S. soda*. According to Figure 3B, accumulation of Zn in the shoots was significantly (P<0.05) greater than that in the roots of *T. kotschyi*, *M. parviflora*, *S. nigrum* and *H. strobilaceum*, 89.00±1.0, 86.00±1.0, 62.00±1.4 and 43.5±1.0 mg $kg^{-1}$, respectively.




(a)




(b)

**Figure 3. Concentation of Mn (A) and Zn (B) in the plants (mg $kg^{-1}$ DW). The values are mean of three replicates ± SD. The same letters are not significantly different at confidence level P < 0.05.**

There was no significant difference between shoots and roots of *L. temulentum*, *C. arvensis* and *S. soda* with respect to Zn concentration. The results of Mn and Zn translocation and bioconcentration factors have been represented in Table 2. Due to lacking Mn accumulation in the shoots and the roots of *S. soda*, translocation and bioconcentration factors were calculated zero. TF for Mn was higher than 1 in *S. nigrum*, *H. strobilaceum*, *M. parviflora* and *T. kotschyi*. For TF of Zn, all investigated species with the exception of *L. temulentum*, *S. soda* and *C. arvensis*displayed the values of more than 1. In the other plant species, excepting *C. arvensis*, shoot BF was greater than 1 for Mn. *L. temulentum, C. arvensis,* and *T. kotschyi* showed root BF more than 1 for Mn. All the plant species exhibited BF more than 1 for Zn, either in the shoots or in the roots.

## Discussion

Different forms of a metal exist in the soil, from the simply available to the highly unavailable. All these metal fractions constitute total pool of the metal in the soil which is known as total metal concentration. However, metal fractions present in the soil solution are only forms which are available for plant uptake. Thus, the metal concentrations in the plants are considerably depended on the available metal concentrations in the soils (24). As a result, it is supposed that possibly due to low bioavailable Mn concentration in the soil of *S. soda* in our work, no acceptable extent of Mn was detected in plant tissues. Although the evaluation of total heavy metals concentration in the soils provides some information about exerted pressures on environment by these metals, it is not a completely proper parameter to determine amount of metals availability in soil solution for root uptake. Therefore, in order to carry out research on accumulation of heavy metals in plants, available concentration, as a fraction of total concentration, is more pivotal and proper for evaluation of BF index and identification of accumulator plant (25). The soil factors such as total metal concentration, pH and so forth. are able to change soil heavy metal concentrations and effect indirectly on plant concentrations (26). It has been reported that soil pH has a significant effect on bioavailability of metals such as Mn and Zn, and plant uptake increases as soil pH decreases (27). In our study, soil acidic relatively pH (6.5±0.2, the data have not been showed) probably increases solubility of mentioned metals and consequently plant uptake. Total metal concentration, as maximum pool of a metal in the soil, has a clarified role in amount of bioavaiable metal form (26). Thus, higher total Mn concentrations would cause more solubility and availability of Mn in most soil samples of this research. It has been suggested that low bioavailable concentration of Zn is largely due to binding to Fe-Mn oxides and organic compounds in the soils (24). In present study, different plant species growing on the same soil have displayed a variety of uptake and accumulation for a special metal which is in consistent with many field studies (12, 13, 14, 15, 16 and so on). It has been proposed that an exact evaluation of plant available metals will not be achieved unless the amount of soil exploitation by the roots is determined (28). Therefore, in addition to soil factors, plant factors are thought to influence on the bioavailability of metals for uptaking and accumulation in the plants.

There are four definitions to characterize a hyperaccumulator plant: 1) metal concentration in shoot parts is equivalent to hyperaccumulating limit in connection with a special metal for example, Mn and Zn > 10000 mg $kg^{-1}$ in dry matter (29), 2) the ratio of heavy metal concentration in the shoots to the roots (TF) and the ratio metal concentration in the plant to the soil (BF) are higher than 1 (29) and 4) hyperaccumulators have high ability to tolerate large amounts of heavy metals (5).

*Austromyrtus bidwillii* (30), *Phytolacca americana* (31) and *Phytolacca acinosa* (32) have been introduced as Mn hyperaccumulators. In this study, Mn concentrations in shoots parts of *H. strobilaceum*, *T. kotschyi*, *M. parviflora* and *S. nigrum* were found greater than those in the roots. Although, Mn accumulation was less than the threshold value of Mn hyperaccumulator (*e.g.*, < 10000 mg $kg^{-1}$), but TF and shoot BF parameters were much higher than 1 in mentioned species. Thus, it is though that these species are able to uptake bioavailable portion of Mn from the soil and transport and accumulate in their aboveground parts and may be effective for revegetation of contaminated soils. There are some plant species for instance, some *Salix* clones which are not classified as hyperaccumulator, but are able to transfer heavy metals effectively into aboveground organs (22). Some studied plants from an area affected by miming activities have been suggested these plants as useful tools for metal immobilization and reduction of soil erosion (15).

Involved mechanisms in heavy metals tolerance in excluder plants are included (5): binding of heavy metals to the root cell walls, a decreased translocation to the shoots and metal chelating in cytosol or sequestration in vacuoles of root cells. Therefore, consistent with definition of excluder species, it seems that *L. temulentum* and *C. arvensis* would be the members of excluders and restrict Mn in the root tissues. Moreover, because of minimum root-to-shoot translocation, these species may be beneficial to decline movement of Mn toxic concentrations into the food chains. Such case was suggested by Del Rio-Celestino *et al*. (2006) in Pb immobilizing by the roots of *Chenopodium album*. Moreover, the *Populus* clones such as Eridano, have been reported which have relatively high ability for Mn accumulation in the roots and may be a useful plant for rhizofilteration metal-polluted waters in industrial areas (33).

Some plant species have very high potential to concentrate Zn in harvestable parts which are presented as Zn hyperaccumulators such as *Arabidopsis halleri* (34), *Thlaspi caerulescens* and some species from this genus, *Viola calaminaria* (29) and *Corydalis davidii* (35). Regarding to biomass value and TF index, four plant species *Cynondon dactylon*, *Hirsfeldia incana*, *Malva nicaeensis* and *Sylibum marianum* have been proposed which may be functional for Zn phytoextraction (12). In agreement with Oropeza *et al*. (2014), translocation of heavy metals from the roots to the shoots reveals an important role in phytoextraction and probably reduces the soil pollution with plant harvesting. In this study, *T. kotschyi*, *M. parviflora*, *S. nigrum* and *H. strobilaceum* showed an increased level of Zn in the shoots compared to the roots, while TF and BF parameters were higher than 1 for these species. These results illustrate that mentioned species not only absorb Zn from soil but also efficiently transport Zn from the roots to the aerial parts. However, the shoot Zn concentrations of these plants were lower than the threshold levels in Zn hyperaccumulator plants. As a consequence, screened species are not categorized as Zn hyperaccumulators in this study. Among studied species, *L. temulentum*, *C. arvensis* and *S. soda* presented almost similar concentrations of Zn in both of the shoots and the roots. With regard to TF which is nearly 1 and BF>1, it is postulated that these plants may not be able to translocate effectively Zn from the roots to the shoots, but have relative capacity to uptake this metal from the soil. Therefore, this species may use some mechanisms, like excluder plants, to prevent more translocation of Zn to aboveground parts.

In summary, our main aim was to evaluate Mn and Zn content in investigated plants through a field screen. In spite of low concentrations in the soils, metals' value in most screened plants were more elevated than the soils in this research. Therefore, with regard to proper adaptability of the studied plant species to grow in region climate, this result can be significant physiologically and environmentally. The data showed that although most investigated plants are able to effectively uptake and transport Mn and Zn to shoot parts, concerning description of hyperaccumulators and normal concentrations of Mn and Zn in plant dry matter, 10-100 and 15-50 mg $kg^{-1}$, respectively (1), are not defined as hyperaccumulator plants, at least in field conditions. However, Mn and Zn contents in their tissues can be considered as a value of bioavalable content for plant uptake and efficient translocation. Based on these results, it is thought that *H. strobilaceum*, *T. kotschyi*, *M. parviflora* and *S. nigrum* have Mn and Zn translocation and accumulation capacity in the aerial parts. These species may be useful to reduce the leaching of Mn and Zn and soil erosion in metal-rich areas. On the other hand, obtained results indicated that *L. temulentum* and *C. arvensis* are capable to concentrate Mn in the roots and reduce metal translocation to the shoots. Therefore, these species, especially *L. temulentum*, may be used to prevent entrance of metal into the food chains and to reduce metal mobility in zones with industrial activities. In experimental conditions, the response and behavior of the plants for the metal uptake and accumulation may be different. As a result, it is required to perform complementary experiments under controlled conditions to precisely evaluate metal accumulation potential and biomass production in all investigated plants.

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