

## INVESTIGATING LEAD AND ZINC UPTAKE AND ACCUMULATION BY *STIPA HOHENACKERIANA* TRIN AND RUPR. IN FIELD AND POT EXPERIMENTS

### INVESTIGAÇÃO SOBRE A CAPTAÇÃO E ACÚMULO DE CHUMBO E ZINCO POR *Stipa hohenackeriana* TRIN. & RUPR. EM EXPERIMENTOS DE CAMPO E EM VASO

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**ABSTRACT:** This study was conducted to evaluate Pb and Zn uptake, mobility, and accumulation in *Stipa hohenackeriana* using field soil in pot and field experiments. Moreover, the effects of Municipal Solid Waste Compost (MSWC) (0, 1, and 2%) and Nano-Silica (NS) (0, 250, and 500 mg/kg) on *Stipa* biomass, Pb and Zn availability in the soil, and Pb and Zn uptake and accumulation were studied using pot experiments. Samples of soil, root, and shoots of *Stipa* were collected from field and greenhouse and after drying, extraction of Pb and Zn was done by acid digestion. Bio-Concentration Factor (BCF) and Translocation Factor (TF) were calculated to determine Pb and Zn phytoremediation efficiency. The amount of Zn and Pb remediation by *Stipa* from soil was determined by remediation factor (RF). The results of field experiments showed the Pb and Zn level decreased in the order of: soil >shoot>root. Results of the pot experiments also showed that plants grown in NS500-amended pots had 33% and 32% higher Pb in roots and shoots compared to control pots, respectively. In comparison, roots Pb concentration in pots amended with MSWC1% and MSWC2% decreased 22.4% and 1.7%, respectively. Roots and shoots Zn concentration in NS500-amended pots was 5.6% and 6.5% higher, respectively. However, root Zn concentration in treatments of MSWC1% and MSWC2% decreased 52.3% and 39.4%, respectively. Shoots Zn concentration decreased 52.5% and 40.0%, respectively. Although MSWC decreased the uptake and accumulation of Pb and Zn in *Stipa* roots and shoots, it improved the plant growth and consequently increased RF and soil remediation compared to the NS. Thus, it seems that applying MSWC and NS simultaneously can be a suitable strategy for the purpose of improving phytoremediation capability of *Stipa* in the Pb and Zn contaminated soils. In general, *Stipa* can be a suitable candidate for the accumulation of heavy metals, especially for Pb and Zn contaminated soils.

**KEYWORDS:** MSWC. NS. phytoremediation. heavy metal.

## INTRODUCTION

Heavy metals pollution in soils, water body, and the air is one of the world's major environmental problems, posing significant risks to human health and ecosystems. Therefore, the development of remediation strategies for contaminated soils is required for human health and ecosystems conservation. Most of the traditional methods are either highly expensive (i.e., solidification, excavation, and burial) or simply involve the isolation of the contaminated sites. Some methods, such as soil washing, can pose an adverse effect on biological activity, soil structure, and fertility (PULFORD; WATSON, 2003). In general, traditional land remediation techniques are very expensive, and result in deterioration of soil quality. Phytoremediation methods cost almost one-fourth of the other traditional methods of contaminating treatment. The main benefits of phytoremediation are that it is cost effective and

technically feasible, improves soil quality, promotes high rhizosphere activity, and allows restoration in a reasonable period of 2 to 3 years. Moreover, using this technique, plants serve as sufficient biomass for rapid remediation (SABEEN et al., 2013).

Lead (Pb), as one of most widespread metal contaminants in soil, has limited bioavailability due to complexation with organic and inorganic soil colloids, sorption on clays, oxides, and precipitation as hydroxides, phosphates, and carbonate. Zn concentrations are generally low in soils their and total concentration is around 50 mgZn/kg (KIEKENS, 1995). At these amounts, Zn is sufficiently taken by plants as an essential element (MARSCHNER, 1995). Zn is the most widespread heavy metal pollutants in wastes arising from industrialized communities (MERTENS et al., 2004).

Using amendments to contaminated soils may be useful because they can change the behavior of heavy metals, their accessibility to plants,

toxicity, immobilization/mobilization and leaching potential (ADRIANO et al., 2004). Sabir et al. (2015) reported that compost and farmyard manure increased the ammonium bicarbonate diethylene triamine penta acetic acid (AB-DTPA) extractable Ni and Mn in the soil and decreased Cu and Zn. Alvarenga et al., (2009) reported that mixed MSWC could be successfully used in the remediation of a contaminated mine soils. Besides, it can correct soil pH and increase soil OM, total N, available P and K to levels that improved the establishment of perennial ryegrass.

Nanomaterials form another group of effective soil amendment candidates. Due to the size of nanomaterials, they can change physicochemical soil properties and have a greater surface area more than their bulk materials. Also, considering their larger surface area, they have a higher solubility and surface reactivity (CASTIGLIONE; CERMONINI, 2009). Plants generally require silica to control biotic stress. NS is an important metal oxide that it is used in the major fields of technology and sciences including electronics, industries, and biomedicine (PAULKUMAR et al., 2012). It has gained greater attention because of its highly reactive surface-to-volume ratio property. Application of nanoparticles in agriculture is currently an interesting field of study. The introduction of nanoparticles into plants might have a significant impact and, thus, it can be used for agricultural applications for a better growth and yield. Different researchers have shown that the application of NS in the soil enhances the growth of maize (YUVAKKUMAR et al., 2013). Karunakaran et al., (2013) reported that the silica and protein content of bacterial biomass clearly showed an increase in uptake of silica with an increase in NS concentration in maize. NS promotes seed germination percentage (100%) in maize and has a favorable effect on beneficial bacterial population and nutrient value of soil.

National Iranian Lead & Zinc Company-Zanjan is located at 10 km east of Zanjan city, Iran. It has been operating since 1981. So, the surrounding soils of this Company have been contaminated with some of the heavy metals. As a result, it has caused the risks for natural ecosystems and people health of Zanjan city. *Stipa* is a dominant species with a wide distribution in the study area that can provide suitable biomass for absorption heavy metals. Therefore, the objective of this study is to evaluate plant uptake, Pb and Zn mobility, and accumulation of lead and zinc in *Stipa* using field soil in the pot with amendments of MSWC and NS were added to the soil.

## MATERIAL AND METHODS

### Field experiment

#### Study area description

National Iranian Lead and Zinc Company of Zanjan is located between 36°36'40" and 36°38'25"N latitudes and 48°37'33" and 48°38'54"E longitudes in Zanjan province, Iran. Rangelands around covers an area of approximately 500 ha. The mean elevation of the region is 1700 m above sea level. The climate of the region is semi-arid and cold (SHARIFI et al., 2012). The annual rainfall ranges from 200 to 400 mm, with an average of 300 mm. The Average maximum temperature is 32.1 (August), while the average minimum is -7.5 (January) (MOAMERI et al., 2017).

#### *Stipa hohenackeriana* description

*Stipa hohenackeriana* Trin and Rupr. (Poaceae) is a species of perennial grass. The plant is a bunchgrass with culms length of 30-80 cm. It has a strong, branched, and dense root system. The penetration depth of its roots in the soil varies from 24 to 60 cm. In cases where the canopy cover is wider, root penetration depth also increases. It is a grass in Asia-temperate, Siberia, Soviet Middle Asia, Caucasus, western Asia, Arabia, China, and Iran (MOGHIMI, 2005). *Stipa* is a dominant species and with a wide distribution in the study area that can provide suitable biomass for absorption heavy metals.

#### Plant sampling in field

As the first step, to evaluate Pb and Zn uptake by the *Stipa*, Pb and Zn mobility, and accumulate of Pb and Zn in *Stipa*, field experiment was conducted. Plant samples were collected around the National Iranian Lead and Zinc Company-Zanjan. Sampling was conducted at 11 sites with an area of 10×10 m<sup>2</sup>. The first site was randomly selected at the Company margins. Samples of root and shoots of *Stipa* from an area of 1 m<sup>2</sup> along one of the diagonals sites 10×10 m<sup>2</sup> were collected by cutting all plants collected. So, from each site, 3 plant samples were taken.

#### Plant analysis for field studies

After transferring the plant samples to the laboratory of the faculty of natural resources (University of Tehran), they were divided into shoots and roots, washed gently with deionized distilled water for approximately 3 min to remove soil particles adhered to the plants. After washing, the samples were then oven-dried at 70 until the samples were completely dry (MOAMERI et al.,

2017). After drying, all plant samples were preprocessed for Pb and Zn determination by finely grinding and passing the samples through a 2mm sieve. Extraction of Pb and Zn from plant samples was done by acid digestion. Plant samples digested in the di-acid mixture (3:1) of nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>) (MOAMERI et al., 2017; Ebrahimi; Madrid Diaz, 2014). The digested plants were analyzed for Pb and Zn concentrations using Inductively Coupled Plasma Optical Emission Spectroscopy "ICP-OES" (GBC Avanta, Australia).

### Soil sampling in field

Three soil samples along one of the diagonals were taken from each site within the plots. In addition, other sites were systematically selected at a distance of 100 m from the first site. Soil samples were taken from the rooting zone (0-20 cm) on May 2014. At each profile, 1 kg of soil was collected. Samples were air-dried at room temperature for two weeks and passed through a 2 mm sieve to remove gravels and debris.

### Soil analysis for field studies

Some soil properties including Electrical Conductivity (EC), pH, texture, Cation Exchange Capacity (CEC), Calcium carbonate equivalent (CCE) percentage, organic matter, N%, available Potassium, and available phosphorus (Table 1) were measured by pursuing standard methods (MOAMERI et al., 2017). Then, soil samples were analyzed for total Pb and Zn. The total concentration of Zn and Pb in soil samples was determined after digesting soil with 4M HNO<sub>3</sub> acid (1:10 ratio) at 60°C water bath for 14 hours (AMACHER; SELIM, 1994). For estimating the available concentrations of Pb and Zn, soil samples were extracted by the standard DTPA-extraction method according to Lindsay and Norwell (1978) (SOLTANPOUR, 1991). Metals of Zn and Pb extracted from soil samples were determined using Inductively Coupled Plasma Optical Emission Spectroscopy "ICP-OES" (GBC Avanta, Australia).

### Phytoremediation Efficiency

The mobility of the heavy metals from the soil contaminated into the roots of the plants and the ability to translocate the metals from roots to shoots were evaluated respectively by BCF and TF (LORESTANI et al., 2011). BCF and TF were calculated to determine the heavy metal phytoremediation efficiency (YOON et al., 2006). BCF expresses the ability of a plant to accumulate metal from soils and TF is the capacity of a plant to transfer metal from its roots to shoots. In this study, the TF and BCF values for Pb and Zn are given by:

$$TF = (M_{shoot}) / (M_{root})$$

$$BCF_{shoot} = (M_{shoot}) / (M_{soil})$$

$$BCF_{root} = (M_{root}) / (M_{soil})$$

where  $M_{shoot}$  and  $M_{root}$  are the metal concentrations in the shoots and roots, respectively, and  $M_{soil}$  is the metal concentration in the soil (YOON et al., 2006).

### Pot experiment

The purpose of this step was to assess the effectiveness of *Stipa* to accumulation Pb and Zn in its biomass from metals contaminated soil and to evaluate the effect of MSWC and NS on Pb and Zn uptake by *Stipa*. The soil for the greenhouse experiment was taken from the rooting zone in rangelands surrounding National Iranian Lead & Zinc Company-Zanjan (field) and transferred to the greenhouse of Department of Agriculture and Natural Resources, University of Tehran, Iran. This soil was air-dried for two weeks and passed through 2mm sieve to remove gravels and debris. In addition, *Stipa* seeds were collected from the study area.

The compost amendment tested were conducted using compost obtained from the unsorted municipal solid waste MSWC. MSWC, in turn, was obtained in a composting plant near Tehran, Iran. Three replicates of MSWC were analyzed using the methodology (Table 1) previously described by Alvarenga et al., (2007). Powder of NS was supplied by Iran Polymer and Petrochemical Institute. The characteristics of NS are given in Table 1.

**Table 1.** Soil and amendments' properties

Properties	Soil	Municipal Solid Waste Compost	Properties	Nano-Silica
pH	7.76	6.9	Structural nature	Amorphous
EC (dS/m)	0.2	3.66 (1:10)	Purity (%)	98
Organic matter (%)	0.85	16.77	Morphology	Spherical
CCE (%)	3.35	-	Special surface area (m <sup>2</sup> /g)	400
CEC (meqg/100g)	16.53	25.8	Total pore volume	200-2000

N (%)	0.063	1.4	(mL/100g)	
Exchangeable P (mg/kg)	87.51	0.35	Average pore diameter (nm)	30
Exchangeable K (ppm)	200	0.63	Mass size (µm)	1-40
Clay (%)	13.4	-	Pore diameter distribution	Very wide
Silt (%)	14.81	-	Average primary particle size (nm)	5-50
Sand (%)	71.6	-		
Soil texture	Silty	-		
	Sand			
Pb (mg/kg)	4509.9	20.1		
Cd (mg/kg)	50	2.6		
Zn (mg/kg)	1031.4	174		

MSWC and NS were added to the soil. Treatments of MSWC were 0, 1, and 2 wt.% of MSWC that were thoroughly mixed with soil and then 4kg of this soil was added to plastic pots (diameter 15 × height 20 cm). Pots size was selected based on the morphology of *Stipa*. A filter of paper was placed at the bottom of each pot to prevent soil, Pb, Zn, MSWC, and NS escaping from the drainage holes. Then, 15 seeds were buried evenly throughout each pot at least 1 to 2cm from the edge with five replicates for each treatment (ABBASI KHALAKI et al., 2016).

After growing for 14 days, different doses of NS were added to the soil in the different pots based on experimental design. These treatments included soil samples amended with NS using solutions at doses of 0mg/kg NS, 250mg/kg NS, and 500mg/kg NS. In general, pot experiment was performed in 25 pots, including control (5 pots), MSWC1% (5 pots), MSWC2% (5 pots), NS 250mg/kg (5 pots), and NS 500mg/kg (5 pots).

The pots were watered with deionized distilled water from the top and the soil moisture content was maintained at 70% water-holding capacity. The light needed for the growth of the plants was supplied from the solar radiation. The samples were put behind the glass windows of the greenhouse and received the solar light during the experiment. The minimum and maximum temperature were 18 and 25°C, respectively. Throughout the growing season, plants were fertilized once a month with 300 mg/1L of NPK fertilizer. Plants were grown for a period of 180 days from December 2014 to May of 2015.

#### Plant harvesting and metals analysis

In May 2015, after five months of growth, plants were harvested and separated into shoots and roots. The roots were rinsed with distilled water to remove the loosely bound soil. Plant samples were dried in an oven for 48 hours at 70°C. After recording dry shoots (SDW), roots (RDW), and total (TDW) weights, samples were ground to form a powder using Wiley Mill and digested in the di-acid mixture (3:1) of nitric acid (HNO<sub>3</sub>) and perchloric acid (HClO<sub>4</sub>) (MOAMERI et al., 2017). The digested plants were analyzed for Pb and Zn concentrations using Inductively Coupled Plasma Optical Emission Spectroscopy “ICP-OES” (GBC Avanta, Australia).

After harvesting the plants, the soil samples were taken out of the pots and their physicochemical properties were determined (EC, pH, texture, CEC, CCE%, O.M%, N%, available potassium and available phosphorus) using standard methods (MOAMERI et al., 2017). Next, soil samples were analyzed for total and DTPA-exchangeable metals (Pb and Zn).

#### Remediation factor (RF)

RF is defined as the ratio of an element accumulation in the shoot to that in the soil, which is calculated as follows:

$$RF (\%) = (M_{\text{shoot}} \times W_{\text{shoot}}) \times 100 / (M_{\text{soil}} \times W_{\text{soil}})$$

where  $M_{\text{shoot}}$  is the metal concentration in shoots of the plant (mg/kg),  $W_{\text{shoot}}$  is the plant dry above ground biomass (g),  $M_{\text{soil}}$  is the metal content of soil (mg/kg), and  $W_{\text{soil}}$  is the weight of soil in the pot (g). The RF values reflect the amount of a metal remediation by a plant from soil (SUN et al., 2011).

#### Data analysis

The statistical processing was conducted using analysis of variance (ANOVA) technique. Before performing the analysis, data were checked for their normality with the Kolmogorov-Smirnov test and for homogeneity of variance with the Levene's test ( $p < 0.05$ ), and were long-transformed where necessary. For one-way ANOVA, Duncan's test between means was calculated only if the F-test was significant at the 0.05 level of probability. A  $P \leq 0.05$  value was considered statistically significant. All statistical calculations were performed using SPSS ver.22.0. Data in the text were expressed as the mean  $\pm$  standard error.

## RESULTS AND DISCUSSION

### Pb and Zn Concentration in the soil and *Stipa hohenackeriana* in field experiment

The concentrations of Pb and Zn in soil samples collected from rangelands around the National Iranian Lead & Zinc Company-Zanjan and in shoots and roots of *Stipa* are presented in Table 2. In addition, TF and BCF are provided. BCF and TF are two factors used to estimate a plant's potential for phytoremediation purpose. As shown in Table 2,  $TF > 1$  for shoots and roots tested in this work. It has to be noted that when  $TF > 1$ , it shows that the accumulation of heavy metals in shoots is higher than that in the roots. The mean of TF values

measured for Pb (1.13) indicates that *Stipa* may be effective as an accumulator and it is able to translocate Pb from roots to the shoots. BCF was  $< 1$  for Pb of *Stipa*, implying that its concentration in the *Stipa* shoots was greater than the roots. In general, the Pb level decreased in the order of soil  $>$  shoot  $>$  root. The mean accumulation of Zn in the shoots ( $235.8 \text{ mg kg}^{-1}$ ) of the *Stipa* was more than that of roots ( $202.01 \text{ mg kg}^{-1}$ ). The mean TF values measured for Zn (1.15) indicates that *Stipa* would be effective for phytoremediation of Zn. The BCF was  $< 1$  for Zn of *Stipa*, suggesting that Zn concentration in *Stipa* shoots was greater than that in its roots. In general, the Zn level decreased in the order of: soil  $>$  shoot  $>$  root. This plant is able to translocate Zn to shoots. According to Pais and Jones (1997), toxic concentrations of heavy metals for various plant species are 30 and  $100 \text{ mg/kg}$  for Pb and Zn, respectively. Although, heavy metal contents in the *Stipa* were higher than toxic levels, the toxic-symptoms were not so severe. Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is toxic to other plants and is manifested by an interaction between a genotype and its environment (MACNAIR et al., 2000). Chelation of metals in the cytosol by high affinity ligands is potentially a very important mechanism of heavy metal detoxification and tolerance (CLEMENS, 2001).

**Table 2.** Pb content in soil, Shoots and Roots of the *S. hohenackeriana* in field ( $\text{mg kg}^{-1}$ )

Metals	Soil		Plant		Phytoremediation indexes		efficiency
	Total	Exchangeable	Shoots	Roots	TF	$BCF_{\text{shoot}}$	$BCF_{\text{root}}$
Pb	$4509.9 \pm 1030.8$	$18.35 \pm 7.2$	$149.9 \pm 10.2$	$132.4 \pm 10.0$	1.13	0.033	0.03
Zn	$1031.4 \pm 162.9$	$13.95 \pm 5.1$	$235.8 \pm 15.5$	$202.01 \pm 11.2$	1.15	0.2	0.17

Values shown are the means  $\pm$  SE. Table numbers are an average of 33 numbers.

### Effects of MSWC and NS on *Stipa* biomass in pot experiment

MSWC and NS significantly ( $P < 0.001$ ) affected SDW, RDW, and TDW of *Stipa* compared to those of the control (Table 3). In this regard, MSWC increased SDW, RDW and TDW of *Stipa*, while NS decreased them with respect to the control. SDW was maximum ( $14.6 \text{ g/pot}$ ) for plants grown in pots amended with MSWC2% while it was minimum ( $4.82 \text{ g/pot}$ ) in the NS500 pots. RDW and TDW were the maximum for pots treated with MSWC2% and also were the minimum for plants amended with NS500. As shown in Table 4, MSWC due to having nutrient enhanced soil NPK increased the growth and biomass (SDW, RDW, and TDW). Moreover, MSWC increased organic matter on pots

and thus enhanced plant biomass. This is consistent with the results of Moslehi et al., (2014) who reported that MSWC chelate increases plant growth and biomass and thus enhances dry weight. Sharifian et al., (2014) reported that compost as a soil amendment has a considerable effect on plant growth and yield (*Marigold (Calendula officinalis Moench.)* and *Daisy (Bellis Perennis L.)*) and increase plants growth. Wilson et al., (2004) found that in the plants cultivated in MSW compost all growth parameters are improved compared to the plants cultivated in pit. NS decreased the growth and biomass (SDW, RDW, and TDW) of *Stipa*. An explanation for this decrease might be the fact that NS increases Pb and Zn uptakes; as a result, the plant may be under stress and consequently, its

growth and biomass are decreased. Le et al., (2014) reported that NS decreased significantly the plant height, shoot, and root biomasses; the SiO<sub>2</sub>

nanoparticles also affected the contents of Cu, Mg in shoots and Na in roots of transgenic cotton.

**Table 3.** Effect of MSWC and NS on dry weights of shoots (SDW), roots (RDW) and total (TDW) (g pot<sup>-1</sup>)

Treatments	SDW	RDW	TDW
Control	11.4 ± 1.14b	7.2 ± 1.48ab	18.6 ± 0.89b
MSWC 1%	13.2 ± 1.64ab	7.4 ± 1.14ab	19.6 ± 1.81b
MSWC 2%	14.6 ± 2.3a	8.2 ± 0.83a	22.8 ± 2.86a
NS250 (mg/kg)	7.56 ± 0.93c	6.0 ± 1.58bc	13.56 ± 1.47c
NS500 (mg/kg)	4.82 ± 1.32d	5.2 ± 1.4c	10.02 ± 1.24d
Duncan	34.39**	3.7*	129.34**

\*\* = significant at P < 0.01, \* = significant at P = 0.01- 0.05; Mean values are reported with SE (Standard Error). Values within a column followed by the same letter do not differ significantly (p < 0.05, post hoc Duncan test)

### Effect of MSWC and NS on EC, pH, N, K, P, OM, and CEC in pot experiment

The characteristics of the soils amended with MSWC and NS were analyzed after 5 months of the experiment (Table 4). By the end of the experiment, in all experimental pots, soil pH values decreased as compared with control and their differences were significant (p < 0.01). Soil pH was the maximum (7.57) in control while it was the minimum (6.97) in the NS500 treatment. The reason for the decrease in the pH is probably that hydrated silica is formed through binding the Si-O to water molecules and hence reduces pH. Karunakaran et al. (2013) and Iler (1979) found that nanosilica and microsilica shows a slight decrease in pH as they both are slightly soluble in water at ppm range, which is converted to silicon colloids because of hydrogen bonding, making them biologically active molecules. EC was low in MSWC treatments, increased in NS250 treatment it was increased as compared with control and decreased in NS500 treatment.

The total nitrogen was increased as a result of the addition of MSWC (Table 4) probably due to its nitrogen in urban waste compost. Sallami et al., (2013) reported that the total nitrogen was increased as a result of the addition of Hoagland solution and compost. In comparison, the addition of NS led to the slight increase in the soil N, which was not significantly difference compared to the control.

MSWC and NS treatments increased soil K content and a significant difference was observed between these treatments with control (p < 0.01). Soil K was the maximum (287.4 ppm) in MSWC2%, while it was the minimum (204.2 ppm) in the control treatment. MSWC treatments increased soil

P content, while it did not show a significant difference between NS treatments with control (p < 0.05). Karunakaran et al. (2013) reported that the availability of silicon sources (sodium silicate, micron silica, silicic acid, and tetraethyl silicate) might increase available nitrogen, phosphorus, and potassium (NPK) value to some extent, which is not comparable with NS. Thus, the incorporation of NS into soil enhances soil NPK values.

MSWC and NS application positively affected soil organic matter (OM) content that increased from 1.31% in control to 1.83, 1.87, 1.67 and 1.83% in MSWC1%, MSWC2%, NS250 and NS500 treatments, respectively (Table 4). In addition, a significant difference was observed between treatments with control (p < 0.01). OM is mainly composed of humic and fulvic substances. The complexation reaction between heavy metals and organic complexants is usually recognized as the most important reaction pathway, since this reaction largely determines the bioavailability of metals and then affects the mobility of heavy metals in the soils (PENG et al. 2009). In addition, NS affects the root rhizosphere and increases the total soil bacterial population. So, these bacteria will increase decomposition of plant material, and consequently, soil OM will could increase. MSWC application positively affected soil CEC content. Soil CEC was the maximum (19.27) in MSWC2% while it was the minimum (17.34) in the control treatment. The CEC of different soils varies widely both in quantity and quality and can range from 1 to 100 meq/100 g of soils (this value of most soils does not exceed 30 meq/100 g). The results showed that MSWC had a high CEC (25.8 meq/100 g) and thus increased soil CEC in the pots.

**Table 4.** Influence of MSWC and NS on soil characteristics

Treatments	pH	EC (ds/m)	N (%)	K (ppm)	P (ppm)	OM (%)	CEC(meq/100grsoil)
Control	7.57 ± 0.02a	2.14 ± 0.15b	0.07 ± 0.0c	204.2 ± 3.7d	107.6 ± 5.86c	1.31 ± 0.03d	17.34 ± 0.15c
MSWC 1%	7.29 ± 0.12b	0.79 ± 0.05d	0.23 ± 0.03a	283.6 ± 2.7a	118.2 ± 5.4b	1.83 ± 0.05b	18.56 ± 0.16b
MSWC 2%	7.09 ± 0.09c	0.88 ± 0.03d	0.1 ± 0.0b	287.4 ± 1.82a	143.8 ± 4.76a	1.87 ± 0.07a	19.27 ± 0.1a
NS250 (mg/kg)	7.13 ± 0.12bc	3.12 ± 0.13a	0.09 ± 0.0c	224.2 ± 1.92c	107.42 ± 7.9c	1.67 ± 0.02c	17.36 ± 0.06c
NS500 (mg/kg)	6.97 ± 0.2c	1.97 ± 0.09c	0.09 ± 0.01c	255.0 ± 9.3b	105.83 ± 7.1c	1.83 ± 0.03ab	17.37 ± 0.07c
Duncan	17.25**	451.72*	0.96*	286.2**	31.9**	229.8**	279.9**

\*\* = significant at  $P < 0.01$ , \* = significant at  $P = 0.01 - 0.05$ ; EC: Electrical Conductivity N: Nitrogen P: Phosphorus K: Potassium OM: Organic Matter CEC: Cation Exchange Capacity

### DTPA extractable metals in Soils in pot experiment

Soil metals are majority found as insoluble compounds unavailable for the transport into roots that consequently affects the metal uptake by accumulating plants. MSWC and NS indicated a significant ( $P < 0.01$ ) effect on DTPA extractable Pb and Zn in the post experimental soil (Table 5). In general, the addition of MSWC and NS leads to an increase in the available Pb and Zn in soil compared to the control. The maximum extractable Pb ( $444.95 \text{ mg kg}^{-1}$ ) was recorded in the pots treated with NS500 that was 37% higher compared to the control. In addition, the maximum extractable Zn ( $129.6 \text{ mg kg}^{-1}$ ) was recorded in the pots treated with NS500 that was 20% higher compared to the

control. Extractable Pb and Zn concentration in the soils increased due to the application of both MSWC and NS, irrespective of application rates. Perhaps the most important explanation for this result is the formation of a stable complex of chelates with the heavy metals and preventing their deposition in the soil. Another cause might be the increase in Pb and Zn concentrations in the soil by the use of MSWC and NS that decrease the pH. Moslehi et al. (2014) reported that application of MSWC and EDTA (together) in the soil increased solubility and availability of Pb and Cd. Peng et al. (2009) reported that a decrease in the soil pH results in an increase in the competition between  $\text{H}^+$  and dissolved metals for ligands such as  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{OH}^-$ ,  $\text{S}^{2-}$ , and phosphates.

**Table 5.** Effect of MSWC and NS on extractable Pb in post-experiment soils

Treatments	Pb	Zn
Control	$277.8 \pm 1.92d$	$102.8 \pm 1.9e$
MSWC 1%	$380.0 \pm 3.8c$	$107.4 \pm 1.14d$
MSWC 2%	$390.5 \pm 1.92b$	$111.0 \pm 1.5c$
NS250 (mg/kg)	$380.6 \pm 1.51c$	$124.8 \pm 1.9b$
NS500 (mg/kg)	$444.95 \pm 2.26a$	$129.6 \pm 1.1a$
Duncan	$311.8^{**}$	$265.8^{**}$

\*\* = significant at  $P < 0.01$ , \* = significant at  $P = 0.01 - 0.05$ ; Mean values are reported with SE (Standard Error). Values within a column followed by the same letter do not differ significantly ( $p < 0.05$ , post hoc Duncan test)

### Pb and Zn concentration in shoots and roots in pot experiment

Results showed that NS significantly ( $P < 0.01$ ) increased Pb and Zn concentrations in Stipa roots and shoots compared to the control (Table 6). Plants grown in NS500-amended pots had the maximum Pb ( $614.6 \text{ mg kg}^{-1}$ ) in roots and ( $672.0 \text{ mg kg}^{-1}$ ) in shoots. There was a significantly ( $P < 0.01$ ) effect of NS500 on Zn concentration in roots with maximum ( $1708.0 \text{ mg kg}^{-1}$ ) and the

minimum Zn concentration ( $768.8 \text{ mg kg}^{-1}$ ) recorded with the MSWC1%, respectively. In addition, the maximum Zn in shoots of plants grown in NS500 amended pots was  $1769.2 \text{ mg kg}^{-1}$  while the minimum value ( $784.4 \text{ mg kg}^{-1}$ ) occurred in MSWC1% amended pots. Perhaps the most important reason for the increased Pb and Zn concentration in Stipa organs (in the treatment NS) is the reduced soil pH. The solubility of heavy metals is often shown as a function of pH affected

by the amount and kind of OM. In soil, the solution concentrations of metal contaminants tend to increase with decreasing pH, mainly due to their displacement from exchangeable sites on solid surfaces by increasing the activity of hydrogen ions as there is a decrease in pH. Accordingly, an increase in the availability of the contaminant for plant uptake may occur (RASKIN; ENSLEY, 1999). Addition of MSWC enhanced soil CEC and also reduced the absorption and accumulation of Pb and Zn in *Stipa* roots and shoots. Cations of heavy metals are often bonded to soil particles because of soil CEC. The negative charges are supplied by clay and OM of the soil. The binding affinity of cations reduces cation movement in vascular plants. Thus, a higher CEC of the soil leads to the greater the sorption and immobilization of the metals (GUPTA et al., 2013). In addition, the addition of MSWC

enhanced OM, which could bind Pb and Zn thus decreasing their solubility in the soil (SABIR et al., 2015). In the other words, an increase in the concentration of compost results in a subsequent decrease in heavy metal solubility. The release of phosphates, carbonates, and other salts during the decomposition of OM may lead to the formation of insoluble metal compounds and limit metal solubility (WALKER et al., 2003). Using organic amendments to soils may decrease the accessibility of heavy metals by transforming available fractions into the fractions associated with metal carbonates, oxides, and OM (WALKER et al., 2004). In general, organic amendments immobilized heavy metals through adsorption reactions which may be due to an increase in surface charge and the presence of metal binding compounds (GONDAR; BERNAL, 2009).

**Table 6.** Effect of MSWC and NS on concentrations of Pb and Zn in root and shoots of *Stipa*

Treatments	Pb		Zn	
	Root	Shoot	Root	Shoot
Control	410.0 ± 9.06c	455.2 ± 18.03b	1612.0 ± 6.1c	1652.8 ± 29.3c
MSWC 1%	318.0 ± 13.7d	399.6 ± 48.8c	768.8 ± 8.3e	784.4 ± 10.8e
MSWC 2%	402.8 ± 22.7c	422.4 ± 21.8bc	976.8 ± 13.7d	990.4 ± 23.08d
NS250 (mg/kg)	494.8 ± 276.6b	645.6 ± 9.2a	1638.0 ± 8.0b	1737.2 ± 23.1b
NS500 (mg/kg)	614.6 ± 16.3a	672.0 ± 8.7a	1708.0 ± 34.9a	1769.2 ± 13.3a
Duncan	4.82**	125.19**	2984.0**	2417.0**

### Soil remediation

RF is the ratio of metal accumulation in plants to that in the soils and indicates how efficiently metals are extracted from the soils and indirectly indicating mobilization or immobilization of metals in the soil. Higher RF indicates extraction efficiency of the metals from the soil due to increased solubilization of metals in the soil and vice versa (SABIR et al., 2015). Mean RF for Pb with MSCW was higher compared to the RF with NS500, suggesting that Pb was extracted more efficiently by the plants grown with MSCW (Table 7). Mean RF for Zn with MSCW2% was higher

compared to the RF with NS250, NS500, and control treatments indicating that Zn was extracted more efficiently by the plants grown with MSCW2% (Table 7). RF for Zn was decreased with levels of MSWC1% (0.29%) and NS500% (0.24%) compared to that of the control (0.4%). MSCW2% due to having more nutrient and organic matter increased the growth and above-ground biomass. As a result, RF was increased, as well. The main reason for such a declined RF in plants grown in NS500 amended pots might be the high absorption of Pb and Zn, which poses some stresses to the plants and thus lowers growth and dry weights of shoots.

**Table 7.** RF% for metals studied with MSWC and NS applied at different levels in post-experiment soils

Treatments	Pb	Zn
Control	0.022 ± 0.004ab	0.4 ± 0.045b
MSWC 1%	0.026 ± 0.005a	0.29 ± 0.043c
MSWC 2%	0.03 ± 0.007a	0.5 ± 0.083a
NS250 (mg/kg)	0.024 ± 0.005a	0.39 ± 0.048b
NS500 (mg/kg)	0.016 ± 0.005b	0.24 ± 0.066c
Duncan	4.18*	13.35**

\*\* = significant at  $P < 0.01$ , \* = significant at  $P = 0.01 - 0.05$ ; Mean values are reported with SE (Standard Error). Values within a column followed by the same letter do not differ significantly ( $p < 0.05$ , post hoc Duncan test)



In order to study the metal mobility in *Stipa*, TF and BCF were calculated (Table 8). TF represents the ability of metal transferring from roots to shoots of a plant. Plants with TF>1 are classified as high-efficiency plants for metal translocation from the roots to shoots (MA et al., 2001). The ability of a plant to accumulate Pb and Zn is evaluated by the BCF, where plants with BCF>1 are accumulators, while those with BCF<sub>shoot</sub><1 are excluders (YOON et al., 2006). In all treatments, the recorded values of TF for *Stipa* were greater than 1 for Pb and Zn, indicating that the accumulation of Pb and Zn in the shoots is higher than that in the roots, probably due to the phytoremediation potential of *Stipa*. *Stipa* had BCF<sub>shoot</sub><1 for Pb, indicating that Pb accumulation in the soil is more than that in shoots. BCF<sub>shoot</sub>>1 for Zn (except treatment MSWC1%). On the subject of phytoremediation strategies, plants with TF and BCF<sub>shoot</sub> values >1 are suitable for phytoextraction. Thus, *Stipa* can be considered as a good candidate

for phytoextraction of Zn from contaminated soils. Some reasons for the proportionality of this plant for Zn phytoextraction: 1) this plant is able to tolerate high soil Zn concentration and can be potentially used for phytoremediation of heavy-metal-contaminated soils (PULFORD; WATSON, 2003). 2) The TF of this plant was higher than 1. 3) This plant is the most common species with a wide distribution on these Pb- and Zn-polluted soils. 4) This plant has a large aboveground biomass and thus it can extract considerable amounts of Zn from the soil at each harvest. Sharifi et al. (2012) reported that plants including phytoremediation strategies, *Acantholimon brachystachyum*, *Astragalus gossypinus*, *Stipa hohenackeriana*, and *Ephedra major* a high TF can be potentially used for phytoextraction. Results were revealed BCF<sub>root</sub>>1 for Zn (except treatment of MSWC1%). Therefore, *Stipa* can be considered as a suitable candidate for phytostabilization of Zn from contaminated soils, as well.

**Table 8.** Translocation factor (TF) and bioaccumulation factor (BCF) for *S. hohenackeriana*

Metals	Treatments	TF	BCF <sub>Root</sub>	BCF <sub>Shoot</sub>
Pb	Control	1.11 ± 0.04b	0.068 ± 0.004ab	0.06 ± 0.00c
	MSWC 1%	1.25 ± 0.15a	0.066 ± 0.008a	0.05 ± 0.004d
	MSWC 2%	1.05 ± 0.10b	0.07 ± 0.00a	0.068 ± 0.004b
	NS250 (mg/kg)	1.04 ± 0.015b	0.11 ± 0.004a	0.1 ± 0.005a
	NS500 (mg/kg)	1.04 ± 0.03b	0.11 ± 0.005b	0.11 ± 0.00a
	Duncan	5.24**	106.46**	259.0**
Zn	Control	1.02 ± 0.017b	1.47 ± 0.05ab	1.44 ± 0.036c
	MSWC 1%	1.02 ± 0.023b	0.78 ± 0.036a	0.76 ± 0.03d
	MSWC 2%	1.01 ± 0.028b	1.01 ± 0.053a	1.0 ± 0.034b
	NS250 (mg/kg)	1.06 ± 0.017a	1.82 ± 0.049a	1.72 ± 0.024a
	NS500 (mg/kg)	1.03 ± 0.017ab	1.78 ± 0.026b	1.72 ± 0.041a
	Duncan	3.7*	501.9**	798.8**

\*\* = significant at P < 0.01, \* = significant at P = 0.01- 0.05; Mean values are reported with SE (Standard Error). Values within a column followed by the same letter do not differ significantly (p<0.05, post hoc Duncan test)

## CONCLUSION

Different amendments are widely used to increase heavy metals mobilization/immobilization and their bioavailability to plants, to improve plant growth, and enhance soil fertility. In the present study, MSWC and NS were applied at different ratios to evaluate their effects on lead and zinc uptake and accumulation in *Stipa*. Plants grown in NS500-amended pots had 33% and 32% higher Pb in roots and shoots compared to those grown in control pots, respectively. In comparison, in plant roots grown on MSWC1%- and MSWC2%-amended pots it decreased to 22.4% and 1.7% compared to the control, respectively. Zn

concentration in roots and shoots of plants grown in NS500-amended pots was 5.6% and 6.5% higher than those grown in control pots, respectively. However, for roots treated with MSWC1% and MSWC2%, Zn concentration decreased 52.3% and 39.4% compared to the control, respectively. In addition, in shoots Zn concentration decreased 52.5% and 40.0% compared to the control, respectively. Although MSWC decreased the uptake and accumulation of Pb and Zn in *Stipa* roots and shoots compared to the control, it improved the plant growth and dry weight and consequently increased RF% and soil remediation compared to the NS. RF with MSWC was higher than that with NS, indicating that plants grown with MSWC,

extracted more metals from the soil compared to NS amended plants. This difference might be due to the higher biomass production of the plants with MSWC. *Stipa* shoots contained a higher concentration of Pb and Zn compared to the roots irrespective of amendment applied.

In both pot and field experiments, *Stipa* showed tolerance to high Pb and Zn concentrations in the soil, and was able to take up and translocate Pb and Zn into roots and above-ground plant tissue and consequently be effective in Pb and Zn remediation in contaminated soils. *Stipa* in the pot experiment treated with MSWC and NS amendments accumulated much more Pb and Zn than those in the

field experiment. In general, plants grown in pot experiments may contain higher concentrations of heavy metals than those grown in the field (FISCHEROVA et al., 2006). In general, the *Stipa* can be a suitable candidate for phytoremediation of heavy metals, especially for Pb and Zn contaminated soils. Although *Stipa* showed a high accumulation of Pb and Zn in plant tissues in the pot experiment, long-term field trials and the use of amendments MSWC and NS in the field are required to further investigate the potential of *Stipa* to remediate soils highly contaminated with Pb and Zn.

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**RESUMO:** Este estudo foi conduzido para avaliar a absorção, mobilidade e acumulação de Pb e Zn em *Stipa hohenackeriana* em experiências usando solo de campo em vaso e em campo. Além disso, os efeitos do Composto Municipal de Resíduos Sólidos (MSWC) (0, 1 e 2%) e de nanopartículas de sílica (NS) (0, 250 e 500 mg/kg) na biomassa de *Stipa*, na disponibilidade de Pb e Zn no solo, e na absorção e acúmulo de Pb e Zn foram estudados usando experiências em vaso. Amostras de solo, raiz e brotos de *Stipa* foram coletadas do campo e da estufa e, após a secagem, a extração de Pb e Zn foi feita por digestão ácida. O Fator de Bioconcentração (BCF) e o Fator de Translocação (TF) foram calculados para determinar a eficiência de fitorremediação de Pb e Zn. A quantidade de remediação de Zn e Pb pela *Stipa* a partir do solo foi determinada pelo Fator de Remediação (RF). Os resultados das experiências de campo mostraram que o nível de Pb e Zn diminuiu na seguinte ordem: solo > broto > raiz. Os resultados das experiências em vaso também mostraram que as plantas cultivadas em vasos corrigidos com NS500 apresentaram teores de Pb 33% e 32% maiores em raízes e brotos em comparação com vasos de controle, respectivamente. Em comparação, a concentração de Pb em raízes em vasos corrigidos com MSWC1% e MSWC2% diminuiu 22,4% e 1,7%, respectivamente. A concentração de Zn em raízes e brotos em vasos corrigidos com NS500 foi de 5,6% e 6,5% maior, respectivamente. No entanto, a concentração de Zn da raiz nos tratamentos de MSWC1% e MSWC2% diminuiu 52,3% e 39,4%, respectivamente. A concentração de Zn nos brotos diminuiu 52,5% e 40,0%, respectivamente. Embora o MSWC tenha diminuído a absorção e acumulação de Pb e Zn nas raízes e brotos de *Stipa*, melhorou o crescimento da planta e conseqüentemente aumentou o RF e a remediação do solo em relação ao NS. Assim, parece que aplicar MSWC e NS simultaneamente pode ser uma estratégia adequada com o objetivo de melhorar a capacidade de fitorremediação de *Stipa* nos solos contaminados com Pb e Zn. Em geral, a *Stipa* pode ser um candidato adequado para a acumulação de metais pesados, especialmente para solos contaminados com Pb e Zn.

**PALAVRAS-CHAVE:** MSWC. NS. Fitorremediação. Metal pesado.

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