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ORIGINAL PAPER

# Potential use of *Sorghum bicolor* and *Carthamus tinctorius* in phytoremediation of nickel, lead and zinc

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**Abstract** Metals are very common contaminants in the soil. High-yielding biomass crops offer good potential for the phytoremediation of soils contaminated with heavy metals. Biomass fuel crops grown on contaminated land have several advantages as site remediation combined with bioenergy production. In this context, two energy crops, *Sorghum bicolor* and *Carthamus tinctorius*, were grown hydroponically to assess their potential use in phytoremediation of nickel (Ni), lead (Pb) and zinc (Zn) and biomass production. The experiment was carried out in a growth chamber using half-strength Hoagland's solution spiked separately with five concentrations for Ni, Pb and Zn (between 5 and 100 mg $L^{-1}$). Shoot and root biomass were determined and analyzed for their metals contents. Results showed that the tested plants were able to uptake Ni, Pb and Zn. Furthermore, roots accumulated more metals than shoots. Ni seems to be more toxic than Zn and Pb. In fact, both species were unable to grow at Ni concentration above 10 mg $L^{-1}$. Metal toxicity ranked as follows: Ni > Zn > Pb. High toxicity symptoms and biomass reduction were observed at concentrations of Pb and Zn above 25 mg $L^{-1}$ for both species. *S. bicolor* was more efficient than *C. tinctorius* in metal uptake due to the high biomass production and the relatively high shoot concentration of metal. *S. bicolor* could be successfully used in phytoremediation applications in marginal soils with moderately heavy metal contamination. However, results obtained through the hydroponic experiment need to be confirmed by field experiments.

**Keywords** Biomass · Energy crops · Heavy metals · Hydroponics · *S. bicolor* · *C. tinctorius*

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## Introduction

Heavy metals are very common contaminants in the environment. The adverse environmental impacts from excessive heavy metals include contamination of water and soil, phytotoxicity, soil degradation and pose serious risks to human health (Adriano 2001). Their negative impacts on the environments are causing increasing concern in scientists, politicians and general public worldwide.

Current remediation techniques of heavy metals are classified in biological (biodegradation by living organisms), chemical (chelators, chemical immobilization, oxidation, etc.) and physical (electrokinetic remediation, incineration technologies, soil washing, stabilization/solidification, thermal desorption, etc.) remediation techniques (Hamby 1996). However, all of them are expensive, time-consuming and environmentally destructive. Therefore, effective cleanup requires their removal/immobilization to reduce or remove toxicity (Henry 2000).

In recent years, scientists generated cost-effective technologies, including the use of immobilizing soil amendments such as compost (Al Chami et al. 2013), biochar (Hmid et al. 2014), bagasse fly ash (Gupta and Ali 2004; Gupta et al. 2003; (Gupta and Ali 2000) and/or plants to clean polluted areas. Various additives were applied as soil amendments to reduce metal plant uptake (Vangronsveld et al. 2009).

Phytoremediation is the direct use of living plants for in situ remediation of contaminated soil, sludges, sediments and groundwater through contaminant removal, degradation or containment (EPA 1999). Phytoremediation is an emerging technology for cleaning up contaminated sites. It is cost-effective and offers esthetic advantages and long-term applicability. The main phytoremediation techniques implemented for heavy metal-contaminated land are phytostabilization and phytoextraction. Phytostabilization is applied by using root-accumulating plants in order to reduce the mobility or bioavailability of metals, which are stabilized in the substrate and/or accumulated in root tissue (Salt et al. 1995). Phytoextraction is a method of using plants with high shoot-accumulation ability to extract metals from soils/sediments/water, and it has been demonstrated to be an economically feasible method of treating polluted land (Fritioff and Greger 2003). Many plant species were tested for their ability to accumulate toxic metals to high extent in the aboveground biomass. Most hyperaccumulator plants, such as *Thlaspi caerulescens* or *Alyssum bertolonii*, are characterized by slow growth and low biomass production, which make them less effective for use in phytoextraction in the field. For this reason, more recent research projects on phytoextraction have focused on high biomass crop species (Luo et al. 2005). High biomass crop species are characterized by their lower ability to accumulate toxic metals, but the total uptake (TU) of elements is comparable to hyperaccumulating plants due to high yield of aboveground biomass. In this context, plants belonging to *Brassica* spp. seem to be more effective for removing Zn from the contaminated soil compared to Zn hyperaccumulator *T. caerulescens* producing one order lower amount of shoot biomass (Ebbs et al. 1997). *Nicotiana tabacum* accumulating predominantly Cd and Cu, and *Zea mays* are considered effective plants because of their high production of aboveground biomass with a relatively high content of metals (Wenger et al. 2002). EPA recommendations (EPA 2000) include metal accumulator plants such as *Z. mays*, *Sorghum bicolor* and *Medicago sativa* among plants that are able to remove a greater amount of metals, but more research is necessary to verify it.

In addition to the above-mentioned environmental threat caused by heavy metal contamination, recently, another problem has arisen: the fossil fuels use and the consequent greenhouse gas emission. The European energy policy promotes a gradual substitution of fossil fuels with renewable sources. This is motivated by increasing oil costs, the need to achieve partial energy independence, and the need to reduce greenhouse gas emissions. European countries are committed themselves to achieve, by 2020, the following targets: cutting greenhouse gases emission by 20 %; reducing overall energy consumption by 20 % through increased energy efficiency; meeting 20 % of energy needs from renewable resources (EU 2008). In this context, the use of biomass feedstock from agricultural sources for bioenergy production might play a key role. Growing crops for bioenergy production has often been criticized as it would compete with food crops. Bioenergy can affect food security both positively and negatively. Nevertheless, the success of phytoextraction combined with bioenergy production depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amounts of biomass (Begonia et al. 1998; Clemens et al. 2002). Bioenergy crops grown on contaminated land offer real opportunities for stabilization, bioremediation and phytoremediation of heavy metal-contaminated soils. Bioenergy crops can remove heavy metals from soils and biomass produced can be used for fuel production. In addition, bioenergy crops grown on heavy metal-contaminated sites or marginal lands may provide additional income for land owners.

The full development of phytoremediation combined with bioenergy production needs a great deal of scientific work, which still remains to be done in the field of research and experimentation.

In this study, we investigated the possible use of two energy crops, *S. bicolor* and *Carthamus tinctorius*, grown hydroponically, coupling a good phytoremediation potential and biomass production for bioenergy purposes. These plants can provide a number of benefits such as: (1) optimal use of marginal land and industrial sites providing an economic advantage, (2) bioenergy production from renewable sources, (3) restoration of damaged land and reduction in the risk associated with heavy metal contamination and (4) economically advantageous cultivation of energy crops on contaminated lands.

*Sorghum bicolor* (sweet sorghum), belonging to the family Poaceae, is a hardy C4 grass widely used as a forage crop (Unger 2001), and it is considered as a great promising energy plant, due to its fast-growing and high biomass production. *S. bicolor* is relatively inexpensive to grow and gives high yields. Some studies showed that *S. bicolor* has the ability to accumulate heavy metals showing the greatest removal of Pb by leaves and the greatest removal of Cd and Zn by stems. Cropping of *S. bicolor* plants facilitated by agronomic practices may be a sustainable technique for partial decontamination of heavy metal-contaminated soils (Ping et al. 2009). *S. bicolor* can produce approximately 30 tons $ha^{-1}$ $year^{-1}$ of dry biomass on low-quality soils with low inputs of fertilizer and limited water (Renewable Energy World 2000). In addition, it can be processed into various high value-added commodities, such as bio-ethanol, pyrolysis oil, electricity/heat, charcoal, hydrogen, activated coal, methanol and pulp for paper (Shoemaker and Bransby 2010). Cropping of *S. bicolor* plants facilitated by agronomic practices may be a sustainable technique for partial decontamination of heavy metal-contaminated soils (Zhuang et al. 2009). Contaminated biomass resulting from phytoremediation could be used for bioenergy production such us pyrolysis technology (Al Chami et al. 2014). In this context, contaminated biomass will be converted into energy and the remained biomass (char) will be reduced in weight and volume. This will allow us to add economic value to contaminated soils. *C. tinctorius* (safflower), an oilseed crop, belonging to the family Compositae. It is cultivated mainly for the production of edible oil. *C. tinctorius* is a highly branched, annual herbaceous plant, with a deep root system that enables it to draw water and nutrients from a considerable depth, conferring on it the ability to survive in areas with little surface moisture (Dajue and Mündel 1996). In addition, oil from *C. tinctorius* is considered to be a potential alternative fuel for diesel engines, thanks to its chemical and physical properties which are similar to those of commercial-grade diesel fuel (Dorado et al. 2004). Industrial processing will make *C. tinctorius* economically interesting crops for farmers of phytoremediation technology. *C. ticnctorius* is tolerant to metals, can be grown on contaminated soils and can be successfully used in the phytoremediation. It possesses interesting characteristics in terms of Cd accumulation. It has been reported that *C. ticnctorius* is capable of accumulating high levels of Cd in roots and leaves without showing symptoms of toxicity (Shi et al. 2010).

Hydroponic screening experiment using Hoagland's solution with a mixture of heavy metals can be suitable for the purposes of a rapid metal tolerance screening test and enables the differentiation between species and clones (Zacchini et al. 2009; Watson et al. 2003). Relative performance of the species tested hydroponically broadly corresponded to those observed in the field (Watson et al. 2003). Recently, many experiments were conducted using hydroponic experiment as a rapid plant screening test for metal tolerance and phytoremediation purposes (Amer et al. 2013). Hydroponic screening is a rapid test that could reflect the performance of the tested plant species in the field (Utmazian et al. 2007).

The main objective of this work is to evaluate the performance of two energy crops *S. bicolor* and *C. tinctorius* in heavy metal phytoremediation of low/moderate contamination, through a hydroponic study as rapid metal tolerance screening test. The heavy metals chosen for this work were Pb, Ni and Zn. Those metals are widely spread and are listed in the EU sewage sludge Council Directive (86/278/EEC) (1986) covers the almost entire legislation relating to soil contamination by heavy metals within the EU.

## Materials and methods

### Plant materials, chemicals and growth conditions

Seeds of *S. bicolor* were purchased from Syngenta Seeds S.A.S. (France) and *C. tinctorius* seeds from Nunhems S.A. (Paraje la Cumbre-Spain). Seeds were sterilized using 5 % NaOCl (v/v) for 5 min, washed with distilled water and germinated over moistened filter papers with 2 mL distilled water in closed Petri dishes for 4–5 days at 23 °C, until the primary roots reached 4–5 cm. Three uniform seedlings were then transferred to 300 mL glass bakers wrapped with aluminum papers to prevent evaporation, light penetration and algae growth. Glass bakers were filled with half-strength Hoagland's nutrient solution (Millner and Kitt 1992). The composition of the nutrient solution was: 2.5 mM $Ca(NO_3)_2$; 2.5 mM $KNO_3$; 1 m $MMgSO_4$; 0.2 μM $KH_2PO_4$; 50 μM NaFeEDTA; 0.2 μM $Na_2MoO_4$; 10 μM $H_3BO_3$; 2 μM $MnCl_2$; 0.5 μM $CuSO_4$; 1.0 μM $ZnSO_4$; and 0.2 μM $NiSO_4$. The solution was buffered with 0.5 mM MES (2-[N-morpholino]ethanesulfonic acid), adjusted to pH 6.0 and spiked with heavy metals. The nutrient solutions were replaced entirely twice a week to prevent nutrient and metal depletion.

The treatments were as follows: (a) control treatment (CTR), half-strength Hoagland's solution, (b) half-strength Hoagland's solution spiked with single metals in the following concentrations: 5, 10, 25, 50 and 100 mg $L^{-1}$ for Ni, Pb and Zn. The treatments were identified by the chemical symbol of the metal followed by a number indicating the metal concentration in mg $L^{-1}$.

This experiment was conducted in a growth chamber (FDM mod. C1500S; F.lli Della Marca S.r.l.; Italy) with 16-h day and 8-h night photoperiod, a thermoperiod of 25 and 23 °C, respectively, and relative humidity of 60 % (day) and 70 % (night).

Chemicals used for the nutrient solution [i.e., $Ca(NO_3)_2$, $KNO_3$, $MgSO_4$, $KH_2PO_4$, NaFeEDTA, $Na_2MoO_4$, $H_3BO_3$, $MnCl_2$, $CuSO_4$, $ZnSO_4$, $NiSO_4$] were purchased from Sigma Aldrich (Germany). $PbNO_3$, $NiSO_4$ $6H_2O$ and $ZnSO_4.7H_2O$ were supplied by MerckKgaA (Germany). Deionized water (Elix; Millipore Corporation) was used to prepare the plant nutrient solution. For sample mineralization and chemical analysis, $HNO_3$, $H_2O_2$ TraceSelect and certified heavy metal standard solution were bought from Sigma Aldrich (Germany). Ultrapure water (18.2 MΩ $cm^{-1}$) was obtained with a Milli-Q purification system from Millipore (Bedford, MA, USA).

### Determination of growth parameters and metal concentration

*S. bicolor* and *C. tinctorius* were grown in the growth chamber on the metal-enriched nutrient solution for 30 days. Plants were then collected and separated into shoots and roots. Roots were exposed for 2 h to a solution of 0.05 M $CaCl_2$ acidified at pH 3 with HCl in order to remove adsorbed metals on root surface (Stolt et al. 2003). Roots were then washed repeatedly with distilled water. Shoot and root length were measured. Shoot and root dry weights were determined after incubation in an oven at 60 °C till constant weight was reached.

Dried plant materials were homogenized by means of Mixer Mill (MM 200-Retsch GmbH-Germany), and humidity at 105 °C was determined. Total heavy metals of the plant tissues were extracted by wet digestion of the dried sample (1 mL $H_2O_2$ and 5 mL $HNO_3$ for 20 min at 190 °C) using a microwave digester (CEM model, MARS Xpress). Samples were then filtered using a Whatman No. 42 filter paper, and the leachates were diluted (1:25) with ultrapure water. Metal content in the extracts was determined by inductively coupled plasma optical emission spectrometry (ICP–OES) (Thermo Electron ICAP 6300), after calibration with certified standard solutions. Total metal concentrations in plant tissues are expressed in mg $kg^{-1}$ dry weight at 105 °C.

### Translocation factor and total uptake index

Translocation factor (TF) is defined as the ratio of metal concentration in shoots to that in roots (McGrath and Zhao 2003) and is used to evaluate and quantify the translocation of heavy metals from roots to the harvestable aerial parts (Barman et al. 2000; Gupta et al. 2008). TU is defined as the product of metal concentration in shoots or roots (μg $g^{-1}$ dw) and shoots or roots biomass (g $plant^{-1}$) (Utmazian et al. 2007). The indexes were calculated according to the following equations:

1.

$$\mathrm{TF} = \frac{\mu\mathrm{g\ metal/g\ shoot\ dw}}{\mu\mathrm{g\ metal/g\ roots\ dw}}$$

2.

$$\mathrm{TU}(\mu\mathrm{g\ plant}^{-1}) = \mu\mathrm{g\ metal}^{-1}\mathrm{shoots\ or\ roots\ dw} \times \mathrm{g\ shoots\ or\ roots\ dw\ perplant}$$

### Statistical analysis

Complete randomized design with five replications for each treatment was adopted. Values are means of five measurements reported for each of the studied parameters. One-way analysis of variance (ANOVA) was carried out, and separation of means was performed using LSD test at $P = 0.05$ significance level. All statistics were computed using SAS software version 9 (SAS Institute, Cary, NC).

## Results and discussion

### Growth performances

Different response of *S. bicolor* and *C. tinctorius* to metal exposure was found, ranging from death, severe toxicity and biomass reduction to metal tolerance. The level of toxicity and biomass reduction as result of metal effect depended on metal type, metal concentration and plant species.

The visual symptoms of Ni, Pb and Zn toxicity observed on *S. bicolor* and *C. tincotorius* shoots and roots are shown in Figs. 1 and 2. The plants at high metal concentration were stunted, growth was reduced, and leaves showed interveinal chlorosis or became dark red. At high metal concentration, roots exhibited blackening, blunting stunted growth and reduced biomass. Nickel was more toxic than Pb and Zn on the studied plant species. In fact, no growth was observed at Ni concentration above 10 mg $L^{-1}$ for both species, while no growth was observed at Pb and Zn concentration above 50 mg $L^{-1}$ on *S. bicolor* and above 25 mg $L^{-1}$ on *C. tinctorius*.

Growth performance of *S. bicolor* and *C. tincotorius* as shoot and root dry weight (SDW and RDW), expressed both in g $plant^{-1}$ for SDW and in cm for shoot and root length (SL and RL) and both as percentage of the control, as affected by Ni, Pb and Zn is shown in Table 1. In most treatments, more inhibition was observed on roots than on shoots. Jadia and Fulekar (2008) reported that heavy metals are found to be more toxic for root growth because they accumulate on root and retard cell division and elongation. The greater impact of heavy metals was observed on the root growth as compared to shoot and led to greater reduction in plant length and weight (Elloumi et al. 2007).

Despite its toxicity, Ni at concentration of 5 mg $L^{-1}$ seemed to have a slight stimulating effect on *S. bicolor* shoots. In fact, Ni5 treatment significantly increased *S. bicolor* SDW by 11 % and SL by 20 %. Conversely, Ni5 treatment decreased *C. tinctorius* SDW and SL by 67 and 64 %, respectively. Ni5 and Ni10 treatments reduced root growth in both studied species. Ni seems more toxic than Pb and Zn. In fact, the toxic effect of Ni was more pronounced and the plants were not able to grow at Ni concentration above 10 mg $L^{-1}$. Severe toxicity symptoms and biomass reduction were observed in Ni10 treatments for both species. At the concentration 5 mg $L^{-1}$, Ni has no effect on growth for *S. bicolor*, while a significant biomass reduction was observed for *C.*

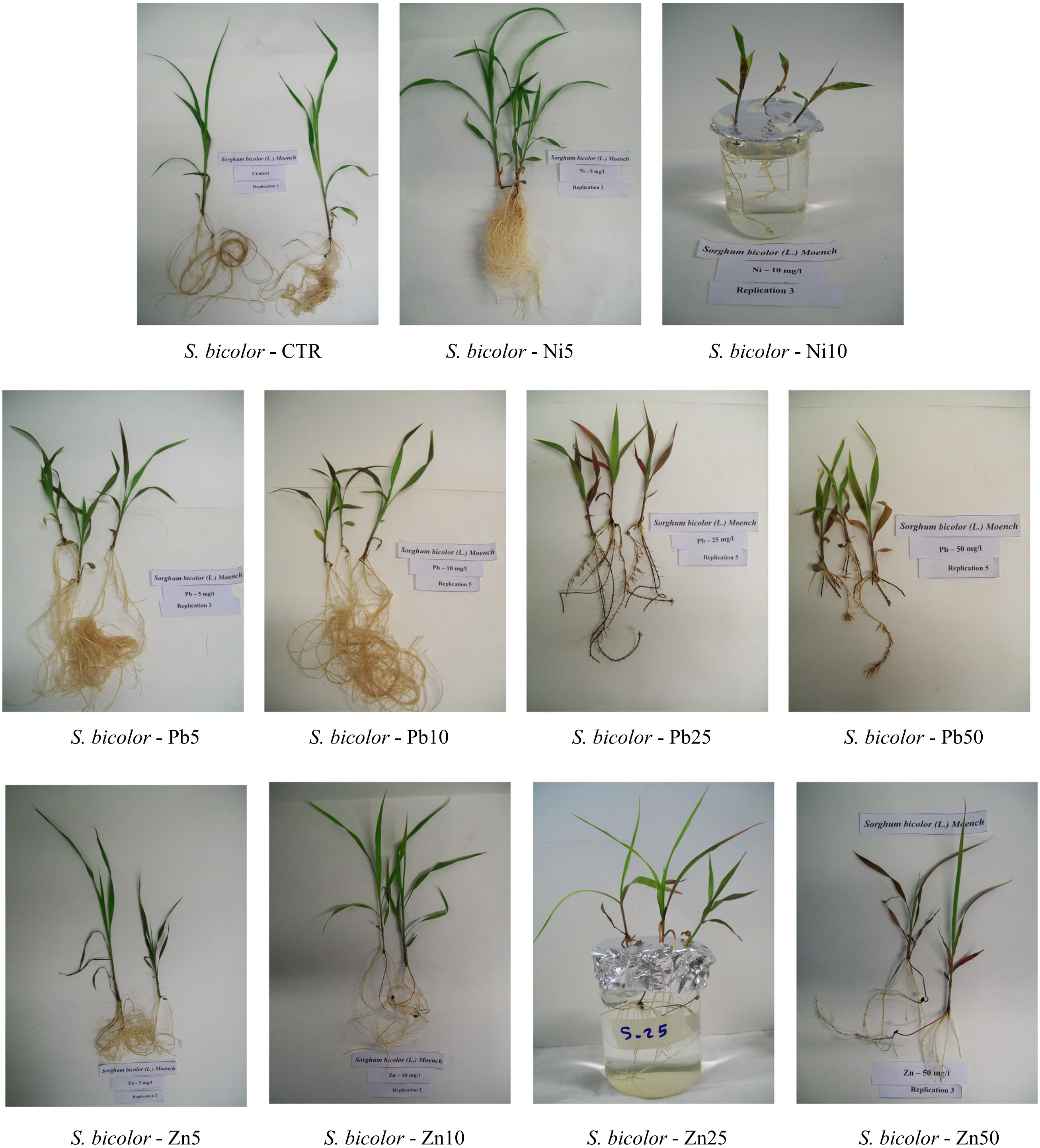


**Fig. 1** Growth performance and toxicity symptoms on *S. bicolor*

*tinctorius*. These results are in accordance with Kachout et al. (2009) who reported that even if Ni is an essential element for plants at low concentrations, it is, however, extremely toxic at high concentrations on the annual halophytes *Atriplex hortensis* and *Atriplex rosea*. These results were confirmed by Amer et al. (2013) who studied the effect of Ni on *Atriplex halimus*, *Portulaca oleracea* and *Medicago lupulina*. Moreover, several enzyme activities depend on the presence of Ni ion, which can explain the promoting effects of low Ni concentrations on plant growth and development (Gerendas and Sattelmacher 1999). In addition, Carlson et al. (1991) found that

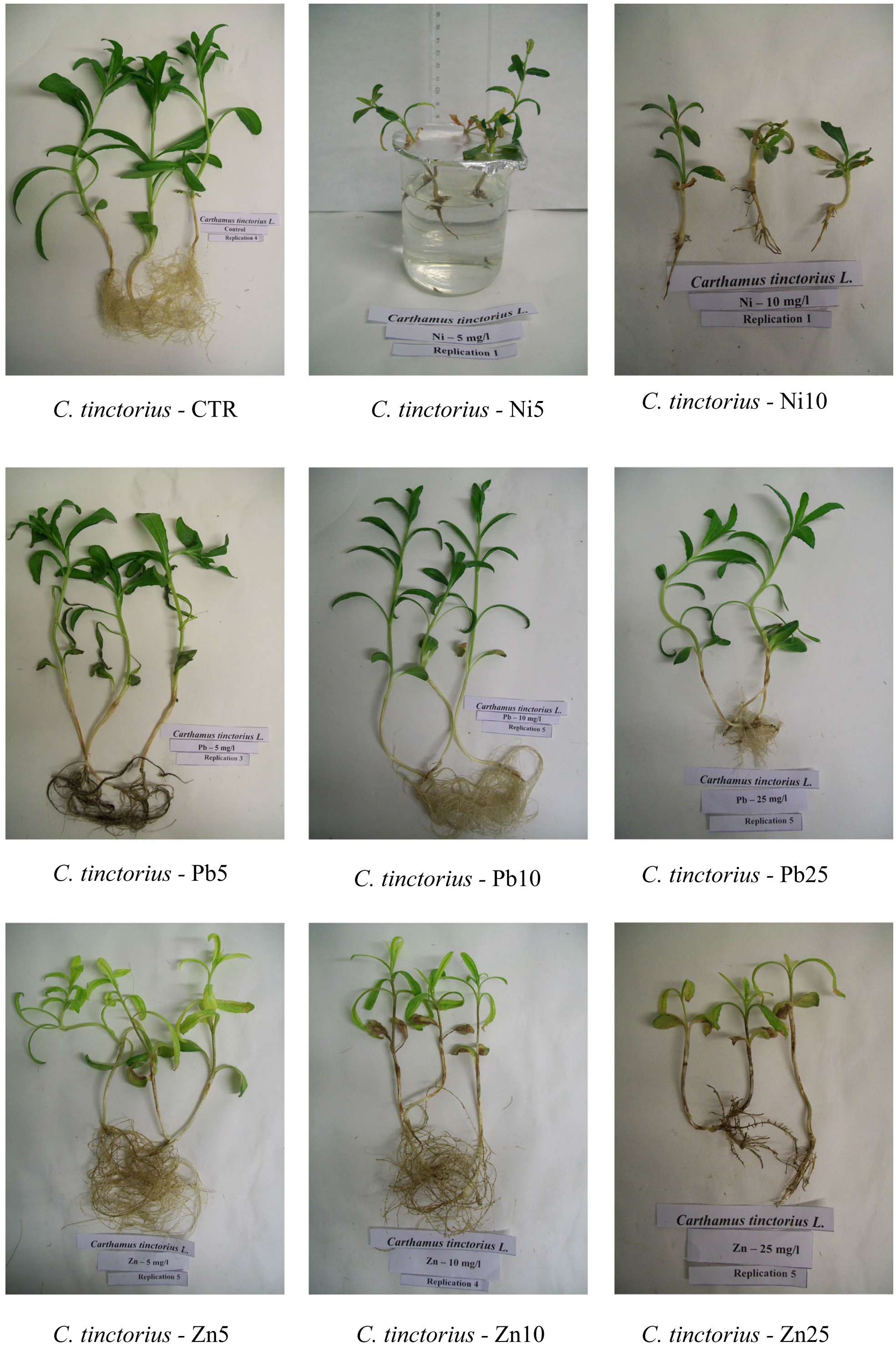


**Fig. 2** Growth performance and toxicity symptoms on *C. tinctorius*

concentration of Ni >1 mg $L^{-1}$ was enough to cause 50 % reduction in root elongation for several vegetable crops. There is a narrow concentration range between beneficial effects of Ni as micronutrient and its toxic effects on plant species as contaminated metal.

Lead was less toxic than Ni. *S. bicolor* plants continued to grow till a concentration of 100 mg $L^{-1}$ in solution, while *C. tinctorius* was less tolerant and was not able to resist a concentration above 25 mg $L^{-1}$ in solution. Pb5 and Pb10 treatments had no effects on *C. tinctorius* growth. In fact, no

**Table 1** Shoot and root dry weight expressed in g plant$^{-1}$, shoot and root length expressed in cm, and both expressed as percentage of the control

| Treatments | *S. bicolor* | | | | | | | | *C. tinctorius* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SDW | | RDW | | SL | | RL | | SDW | | RDW | | SL | | RL | |
| | g plant$^{-1}$ | % | g plant$^{-1}$ | % | cm | % | cm | % | g plant$^{-1}$ | % | g plant$^{-1}$ | % | cm | % | cm | % |
| CTR | 0.0849$^{b}$ | 100 | 0.1090$^{a}$ | 100 | 26.1$^{b}$ | 100 | 49.2$^{a}$ | 100 | 0.3698$^{a}$ | 100 | 0.0547$^{a}$ | 100 | 26.6$^{a}$ | 100 | 19.3$^{a}$ | 100 |
| Ni5 | 0.0941$^{a}$ | 111 | 0.0755$^{b}$ | 69 | 31.2$^{a}$ | 120 | 33.3$^{b}$ | 68 | 0.0841$^{b}$ | 23 | 0.0140$^{b}$ | 26 | 9.7$^{b}$ | 36 | 2.9$^{b}$ | 15 |
| Ni10 | 0.0171$^{c}$ | 20 | 0.0123$^{c}$ | 11 | 7.1$^{c}$ | 27 | 8.2$^{c}$ | 17 | 0.0610$^{b}$ | 17 | 0.0099$^{b}$ | 18 | 6.1$^{b}$ | 23 | 1.6$^{c}$ | 8 |
| Ni25 | ng | ng | ng | ng | ng | ng | ng | ng | | | | | | | | |
| CTR | 0.0849$^{a}$ | 100 | 0.1090$^{a}$ | 100 | 26.1$^{a}$ | 100 | 49.2$^{a}$ | 100 | 0.3698$^{a}$ | 100 | 0.0547$^{a}$ | 100 | 26.6$^{a}$ | 100 | 19.3$^{a}$ | 100 |
| Pb5 | 0.0627$^{b}$ | 74 | 0.1141$^{a}$ | 105 | 19.5$^{b}$ | 75 | 51.8$^{a}$ | 105 | 0.3108$^{b}$ | 84 | 0.0618$^{a}$ | 113 | 27.5$^{a}$ | 103 | 22.8$^{a}$ | 118 |
| Pb10 | 0.0454$^{c}$ | 53 | 0.0741$^{b}$ | 68 | 16.4$^{b}$ | 63 | 36.7$^{b}$ | 74 | 0.2224$^{c}$ | 60 | 0.0600$^{a}$ | 110 | 21.3$^{b}$ | 80 | 21.2$^{a}$ | 110 |
| Pb25 | 0.0386$^{c}$ | 45 | 0.0489$^{c}$ | 45 | 10.9$^{c}$ | 42 | 13.1$^{c}$ | 27 | 0.0859$^{d}$ | 23 | 0.0129$^{b}$ | 23 | 20.7$^{b}$ | 78 | 5.8$^{c}$ | 30 |
| Pb50 | 0.0402$^{c}$ | 47 | 0.0384$^{d}$ | 35 | 12.6$^{c}$ | 48 | 14.6$^{c}$ | 30 | ng | ng | ng | ng | | | | |
| Pb100 | 0.0358$^{c}$ | 42 | 0.0365$^{d}$ | 33 | 10.4$^{d}$ | 10 | 9.6$^{d}$ | 19 | ng | ng | ng | ng | | | | |
| CTR | 0.0849$^{a}$ | 100 | 0.1090$^{a}$ | 100 | 26.1$^{a}$ | 100 | 49.2$^{a}$ | 100 | 0.3698$^{a}$ | 100 | 0.0547$^{a}$ | 100 | 26.6$^{a}$ | 100 | 19.3$^{a}$ | 100 |
| Zn5 | 0.0801$^{a}$ | 94 | 0.0734$^{b}$ | 67 | 25.1$^{a}$ | 96 | 53.4$^{a}$ | 109 | 0.1184$^{b}$ | 32 | 0.0293$^{b}$ | 54 | 17.1$^{b}$ | 64 | 18.4$^{a}$ | 95 |
| Zn10 | 0.0488$^{b}$ | 57 | 0.0403$^{c}$ | 37 | 17.6$^{b}$ | 67 | 32.3$^{b}$ | 66 | 0.0572$^{c}$ | 15 | 0.0122$^{c}$ | 22 | 12.4$^{c}$ | 47 | 12.6$^{b}$ | 65 |
| Zn25 | 0.0301$^{c}$ | 35 | 0.0299$^{d}$ | 27 | 11.2$^{cd}$ | 43 | 17.0$^{c}$ | 35 | 0.0462$^{c}$ | 13 | 0.0083$^{c}$ | 15 | 8.6$^{c}$ | 32 | 5.8$^{c}$ | 30 |
| Zn50 | 0.0307$^{c}$ | 36 | 0.0196$^{e}$ | 18 | 12.8$^{c}$ | 49 | 13.6$^{d}$ | 28 | ng | ng | ng | ng | | | | |
| Zn100 | 0.0156$^{d}$ | 18 | 0.0098$^{f}$ | 9 | 9.8$^{d}$ | 38 | 7.3$^{e}$ | 15 | ng | ng | ng | ng | | | | |

Values are mean ± standard deviation ($n = 5$)

Means with different letters within the column of the same species indicate significant difference between values; LSD test ($P < 0.05$)

*SDW* shoot dry weight, *RDW* root dry weight, *SL* shoot length, *RL* root length, *ng* no growth

significant difference in comparison with control was observed on all growth parameters. Conversely, severe toxicity symptoms were observed in Pb25 treatment, whereas no growth was observed at Pb50 and Pb100 treatments. *S. bicolor* behaved differently than *C. tinctorius*. In fact, slight reduction in growth started to be observed at Pb5. Growth reduction started to be severe at higher Pb concentration. However, *S. bicolor* continued to grow even in Pb100 treatment. Pb is a toxic metal and is not considered an essential element for plant growth, but it may stimulate the growth of some plants at very low concentration (Dou 1988). However, in our case, no stimulation effect of Pb at low concentration was observed on our investigated plants. Our findings are in disagreement with many studies showing that small amounts of Pb in plant tissues may have a stimulation effect on *Brassica juncea* (L.) (Liu et al. 2000). In addition, our results are in contrast with Amer et al. (2013) who found that Pb at low concentration has a stimulation effect on *A. halimus*, *P. oleracea* and *M. lupulina*. Toxicity symptoms and biomass reduction are some of the physiological responses to metals exposure exhibited by plants. In this study, Pb concentration at 10 mg L$^{-1}$ exhibited a severe growth reduction and reduced shoot and root biomass. Wierzbicka et al. (2007) confirmed that Pb affects water potential that causes dehydration in plant tissues, thus influencing plant development and resulting in growth reduction. According to Sharma and Dubey (2005), Pb affects plant physiology by inhibition of enzymatic activities, alteration of mineral nutrition and membrane permeability.

Zinc toxicity symptoms and growth reduction were observed on *S. bicolor* at concentration above 10 mg L$^{-1}$, whereas severe Zn toxicity symptoms and growth reduction started to be observed on *C. tinctorius* at low Zn concentration. In Zn10 treatment, *S. bicolor* plants had toxicity symptoms, and SDW, RDW, SL and RL were lower than the CTR by 43, 66, 33 and 34 %, respectively. However, *S. bicolor* continued to grow even in Zn100 treatment, while no growth of *C. tinctorius* in Zn50 and Zn100 treatments. Zn did not show any promoting effect within our concentrations, which is in contrast with the findings of Grifferty and Barrington (2000), who showed that the increased Zn concentration from 25 to 50 mg kg$^{-1}$ had a significant positive effect on the dry biomass yield of wheat plants. In addition, Sridhar et al. (2007) reported that Zn acts as a growth promoting micronutrient at low concentrations on barley plants. According to Jadia and Fulekar (2008), soil with low concentration of Pb, Ni and Zn from 5 to 20 mg kg$^{-1}$ was observed to stimulate the root and shoot length and increase biomass of the *M. sativa* plants. In our study, low-to-moderate toxicity was observed for both species in treatments with Zn below 10 mg $L^{-1}$, while severe toxicity symptoms were observed in treatments with Zn concentration above 10 mg $L^{-1}$. Ni and Zn in elevated concentration can lead to the formation of reactive oxygen species and improper protein binding, which can alter the protein structure (Yang et al. 2006). Our study points out that *S. bicolor,* the most Ni resistant species among the tested plants, could be used for Ni phytoremediation in case of low levels of soil contamination.

It is worth noting that Ni and Zn were unable to induce a stimulating effect of the studied plant species, despite their important functions for plant growth. Ni and Zn, as redox-active metals, play a role of cofactors in many metalloenzymes, being Zn also active as protein stabilizer (Hansch and Mendel 2009). Ni and Zn are typically present in plant cells at concentrations of 15–22 and 15–50 mg $kg^{-1}$, respectively (Hansch and Mendel 2009).

## Heavy metal content, uptake and translocation factor

Total metal concentrations in shoots and roots are shown in Fig. 3. Large amounts of metals were accumulated in shoots and roots of *S. bicolor* and *C. tinctorius*. This amount significantly varied among plant species, metals and treatments. Within our experimental conditions, Ni, Pb and Zn content in shoots and roots increased as the concentrations of these metals in the growing media increased.

Our results showed that *S. bicolor* and *C. tinctorius* do not belong to Ni, Pb and Zn hyperaccumulating plants because metal content was higher in the roots. Similar results were found by Soudek et al. (2014) who studied the accumulation of Cd and Zn on *Sorgum* sp. According to Baker and Brooks (1989) criterion, *S. bicolor* and *C. tinctorius* cannot be considered as Pb, Ni or Zn hyperaccumulators as the concentration of Ni, Pb and Zn were $<1000$ µg $g^{-1}$ dw for Ni and Pb, and $<10{,}000$ µg $g^{-1}$ dw for Zn in their leaves. However, the concentrations of metals achieved in plant tissues together with their high biomass production lead to the suggestion that the studied species could be used for phytoremediation applications combined with biomass production for bioenergy purposes.

Zinc was the dominant metal in shoots followed by Pb. The highest Zn concentration (4395 mg $kg^{-1}$ dw) was observed in *S bicolor* shoots in the Zn100 treatment. The highest Pb concentration (2292 mg $kg^{-1}$ dw) was found in *C. tinctorius* shoots in the Pb25 treatment. Lead was the dominant metal in roots followed by Zn. The highest Pb concentration (36,229 mg $kg^{-1}$ dw) was found in *C. tinctorius* roots in the P25 treatment. Similar amounts of Zn concentration were found in *C. tinctorius* roots in the Zn25 treatment (9273 mg $kg^{-1}$ dw) and *S. bicolor* roots in the Zn100 treatment (9230 mg $kg^{-1}$ dw).

Yoon et al. (2006) studied the accumulation of Pb, Cu and Zn in native plants (17 species) growing on a contaminated Florida site. Total Pb concentrations in the plants ranged from non-detectable to 1183 mg $kg^{-1}$, while Zn content ranged from 17 to 598 mg $kg^{-1}$. Most of the plant samples, the root Pb and Zn concentrations were much greater than those of the shoot Pb contents, indicating low mobility of Pb from the roots to the shoots. Similar results were obtained by Pitchtel et al. (2000) who studied the distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. Also, Stoltz and Greger (2002) reported a Pb range from 3.4 to 920 mg $kg^{-1}$ and Zn concentrations range from 68 to 1630 mg $kg^{-1}$, while Zn concentration found by Shu et al. (2002) ranged from 66 to 7607 mg $kg^{-1}$ in plant biomass. In our study, metal concentration in the shoots was in the order Zn $>$ Pb $>$ Ni, whereas the metal concentration in the roots was in the order Pb $>$ Zn $>$ Ni. Similar results were obtained by Meers et al. (2005) who noted that *Helianthus annuus* and *Z. mays* showed more uptake of than Ni. This could be due to the fact that Pb uptake does not require any energetic expense, and it was deposited in large amounts in the roots (Wierzbicka et al. 2007). Brown and Slingsby (1972) showed that the high tolerance of Pb in plants results from Pb accumulation only in the cell wall without penetrating into the protoplast. Root growth decreased progressively with increasing concentration of Pb in solutions. Meers et al. (2005) reported that shoot selectivity of *H. annuus* was in the order of Zn $>$ Cu $>$ Ni $>$ Cd $=$ Pb, while in Indian mustard, shoot selectivity was in the order of Zn $>$ Cd $>$ Ni $>$ Cu $>$ Pb (Do Nascimento et al. 2006). Jadia and Fulekar (2008) reported that plant growth was adversely affected by heavy metals at higher concentration (40 and 50 mg $kg^{-1}$). Furthermore, metals were efficiently taken up mainly by roots; the order of uptake was: Zn $>$ Ni $>$ Pb. Bonfranceschi et al. (2009) found that the increases in metal concentration in the hydroponic solution lead to a great accumulation at the root level in *M. sativa* and *S. bicolor.*

Several plant species are able to accumulate higher concentrations of Zn in the shoots (e.g., red beet, field pumpkin, chicory), whereas other plants accumulate zinc in the roots (e.g., barley, white cabbage, maize) (Sekara et al. 2005). Baker (1981) reported that metal uptake and transport of indicator plants were regulated in such a way that the ratio of the concentration of element in the plant to that in the soil is close to 1. Thus, in our study, *S. bicolor* and *C. tinctorius* could be considered excluders for Ni, Pb and Zn. It has been shown that Pb is mainly located in roots. Kabata-pendias (2011) reported that plant uptake of Pb increased with the increasing concentration in the solution and the translocation from root to shoot was greatly limited. According to Wallace and Romney (1977), the accumulation of Pb occurs mostly in the root tissues of *S.*

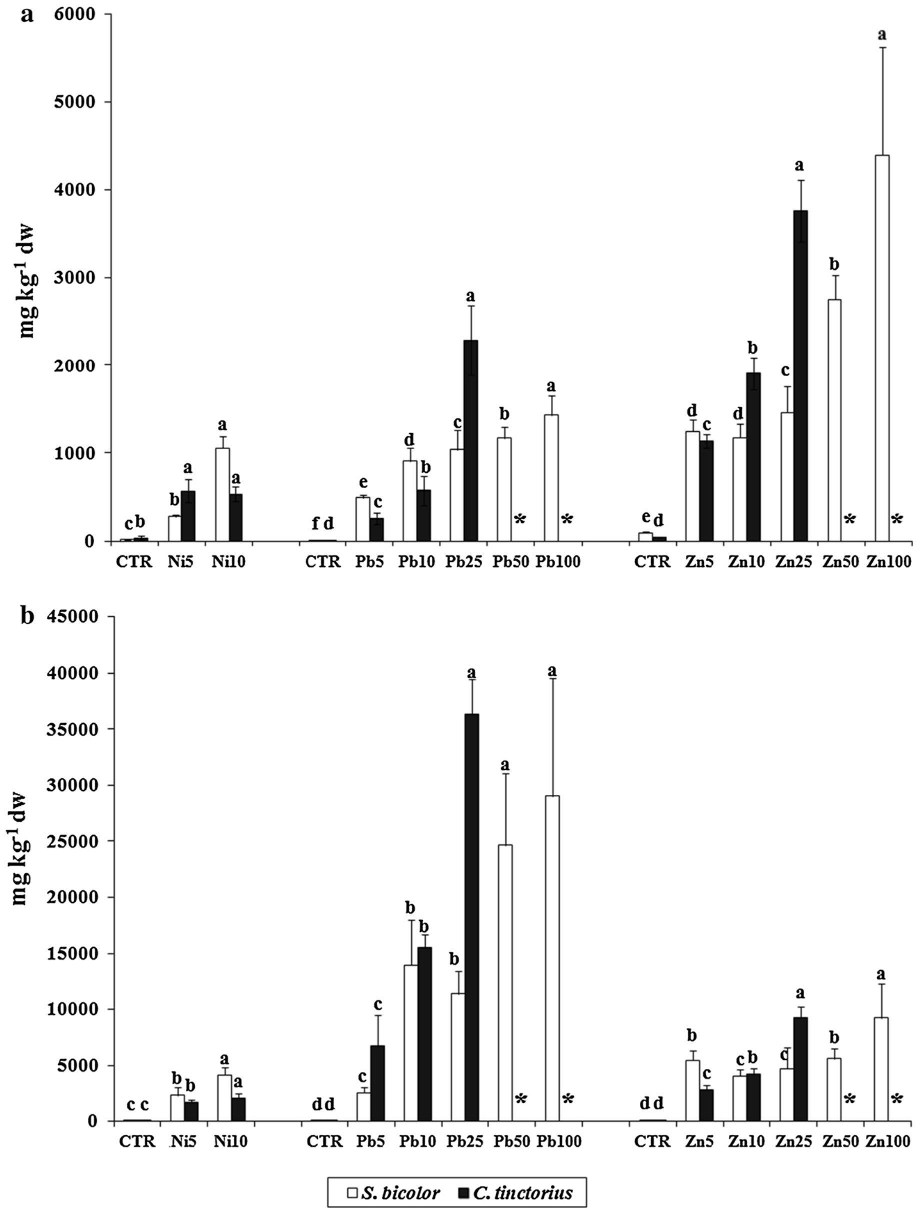


**Fig. 3** Total Ni, Pb and Zn contents in shoots (**a**) and roots (**b**). Means with *different letters* within the same species and metal indicate significant difference between values; LSD test ($P < 0.05$). *C tinctorius*: no growth

*bicolor* and *H. annuus*. Root tissues act as barriers to apoplastic and symplastic Pb transport, and therefore, Pb transport to shoot gets restricted (John et al. 2009). Metal tolerance is often associated with enhanced metal retention in roots, but that does not necessarily mean that increased root retention itself could be the cause of tolerance (Harmens et al. 1993). However, metal tolerance and root to shoot metal transport are often negatively correlated. High content of Pb was found in the roots for all species under our conditions. Similar results were obtained by Amer et al. (2013) who conducted similar experiment on *A. halimus*, *M. sativa* and *P. oleracea*, and they found similar concentrations that were accumulated in plant roots. This high Pb concentration could be due to the low biomass of the roots, which results in high concentration of the metal in the roots, or could be due also to the precipitation of the metal on the root surface. However, before analysis, roots were exposed for 2 h to a solution of 0.05 M $CaCl_2$ acidified at pH 2–3 with HCl in order to remove adsorbed metals on root surface. Roots were subsequently washed with distilled water. However, similar concentration of Pb was reported by Sahi et al. (2002) who found that *Sesbania drummondii*, a leguminous shrub, can tolerate Pb levels up to 1500 mg $L^{-1}$ and accumulate up to 40 g $kg^{-1}$ shoot dw. In addition, Kumar et al. (1995) found that *B. juncea* can accumulate Pb up to 34.5 g $kg^{-1}$ shoot dry weight. Variation of the TF from roots to other organs might be due to the interaction between different metals occurring at the root surface and also within the plant (Ashraf et al. 2011; Sharma et al. 2007).

**Table 2** Total metal uptake in shoots and roots

| Treatments | *S. bicolor* | | *C. tinctorius* | |
|---|---|---|---|---|
| | Shoot uptake µg/plant | Root uptake | Shoot uptake | Root uptake |
| Ni5 | 26 | 312 | 48 | 23 |
| Ni10 | 18 | 29 | 33 | 21 |
| Ni25 | ng* | ng | ng | ng |
| Pb5 | 31 | 294 | 78 | 415 |
| Pb10 | 41 | 1033 | 129 | 932 |
| Pb25 | 40 | 557 | 197 | 467 |
| Pb50 | 47 | 945 | ng | ng |
| Pb100 | 51 | 1060 | ng | ng |
| Zn5 | 100 | 399 | 135 | 83 |
| Zn10 | 57 | 164 | 110 | 51 |
| Zn25 | 44 | 141 | 174 | 77 |
| Zn50 | 85 | 109 | ng | ng |
| Zn100 | 68 | 90 | ng | ng |

CTR: control; Ni5, Ni10 and Ni25: nickel concentration of 1, 2 and 5 mg $L^{-1}$, respectively; Zn5, Zn10, Zn25, Zn50 and Zn100: zinc concentration of 5, 10, 25, 50 and 100 mg $L^{-1}$, respectively; Pb5, Pb10, Pb25, Pb50 and Pb100: lead concentration of 5, 10, 25, 50 and 100 mg $L^{-1}$, respectively

* ng = no growth

*S. bicolor* and *C. tinctorius* potential for phytoremediation can be evaluated by both TU and TF. Total metal uptake (TU) in shoots and roots is shown in Table 2. Ni TU was the lowest in all tested plants in all treatments, followed by Zn; the highest uptake was found for Pb. *C. tinctorius* showed higher TU than *S. bicolor* for all tested metals. Root uptake was higher than shoot uptake for all tested plants in all treatments. Metal uptake was higher in roots than in shoots. These results agree with Amer et al. (2013) who found that roots are the main accumulation site for Ni, Pb and Zn, while only a limited amount was translocated to shoots. In contrast, Zn was very mobile and a significant amount was translocated to shoots. In fact, Al Chami et al. (2013) and Terzano et al. (2008) found that Zn was translocated to shoots with a TF between 0.7 and 1.1. High shoot biomass produced can compensate for the low metal concentration and translocation. An important amount of Zn was translocated to shoots in comparison with Ni and Pb. These results are in agreement with Adesodun et al. (2010) who found that the translocation of Zn from root to shoot for *Tithonia diversifolia* and *H. annuus* was higher than Pb. Baker and Brooks (1989) also discussed restriction of metal uptake by plants from contaminated soils and the presence of exclusion mechanisms in such plant species. Since Zn and Ni are essential nutrients for plant systems, higher translocation from roots to shoots in comparison with Pb is understandable. Considering that roots are the main accumulation site of Ni, Pb and Zn for all studied species in all used metal concentrations, these plant species are potential candidates to be used in phytoremediation process, but in phytostabilization and not phytoextraction. In highly polluted areas, where the removal of metals by phytoextraction using hyperaccumulating plants is not efficient due to the slowness of the process (Ernst 1996), the most suitable method is phytostabilization (Arthur et al. 2005). TF is shown in Table 3. TF was very low for all tested plants in all treatments (<0.5), being the roots the tissues in

**Table 3** Translocation factor

| Species | Treatments | TF | Species | Treatments | TF |
|---|---|---|---|---|---|
| *S. bicolor* | Ni5 | 0.07 | *C. tinctorius* | Ni5 | 0.34 |
| | Ni10 | 0.45 | | Ni10 | 0.26 |
| | Ni25 | ng* | | Ni25 | ng |
| | Pb5 | 0.19 | | Pb5 | 0.04 |
| | Pb10 | 0.07 | | Pb10 | 0.04 |
| | Pb25 | 0.09 | | Pb25 | 0.06 |
| | Pb50 | 0.05 | | Pb50 | ng |
| | Pb100 | 0.05 | | Pb100 | ng |
| | Zn5 | 0.23 | | Zn5 | 0.40 |
| | Zn10 | 0.29 | | Zn10 | 0.45 |
| | Zn25 | 0.31 | | Zn25 | 0.41 |
| | Zn50 | 0.49 | | Zn50 | ng |
| | Zn100 | 0.48 | | Zn100 | ng |

CTR: control; Ni5, Ni10 and Ni25: nickel concentration of 1, 2 and 5 mg $L^{-1}$, respectively; Zn5, Zn10, Zn25, Zn50 and Zn100: zinc concentration of 5, 10, 25, 50 and 100 mg $L^{-1}$, respectively; Pb5, Pb10, Pb25, Pb50 and Pb100: lead concentration of 5, 10, 25, 50 and 100 mg $L^{-1}$, respectively

* ng = no growth

which heavy metals mostly accumulated. The highest TF value was found for Zn, followed by Ni, and both were much greater than Pb. TF values for *S. bicolor* were higher than TF values for *C. tinctorius*. Based on the average TFs of all plant samples, Yoon et al. (2006) found that the plants were most efficient in translocating Cu (TF = 1.2) followed by Zn (TF = 0.98) and Pb (TF = 0.58). Goni et al. (2014) studied the uptake and translocation of metals in different parts of rice plants irrigated with metals contaminated water and found that the TF for all metals was below 1, which indicates that most of the metals were confined in the roots after rice plant uptake. However, the highest TF was observed for Zn in edible parts. In our case, TF for Zn and Ni ranged between 0.2 and 0.5, while TF for Pb was below 0.1. The differences in root and shoot uptake in our study can possibly be explained by the fact that one of the normal functions of roots is to selectively acquire ions from the soil solution, whereas shoot tissue does not normally play this role (Salt et al. 1997). Many metal-tolerant species have restricted translocation of metals to the shoot (Baker and Walker 1990). The reason for restricted shoot metal uptake could be the presence of exclusion mechanisms, maybe for the protection of photosynthesis from toxic levels of heavy metals (Baker 1981; Stoltz and Greger 2002). A higher metal uptake in roots comparing to shoots was reported in grasses, semi-resistant, sensitive and resistant plants including sorghum (Pinto et al. 2004). In this experiment, shoot selectivity was in the order of Zn > Pb > Ni. Heavy metals are transported from root to shoot in terrestrial plants to different extents. Different metals are differently mobile, and within a plant, Zn is more mobile than Pb (Greger 2004). Zn may be translocated extensively as it is essential to the plant metalloenzymes (Delhaize et al. 1985; Van Assch and Clijsters 1990) and photosynthesis (Hsu and Lee 1988). However, many factors including anatomical, biochemical and physical factors might also contribute to metal uptake, accumulation and distribution in the different plant parts (Salt et al. 1995; Singh et al. 2011).

## Conclusion

Results showed that both plant biomass and metal accumulation varied with the metals considered, their concentrations and the plant species. Metals induced a number of physiological changes, such as growth reduction, chlorosis.

Ni seems to be more toxic than Zn and Pb, though physiological changes were more pronounced for Ni in the studied species. High toxicity symptoms were observed in all studied species at Pb and Zn concentrations above 25 mg $L^{-1}$. Heavy metal content in root was much higher than shoot in all treatments. Heavy metal toxicity ranked as follows: Ni > Zn > Pb.

Based on metal concentration in shoots and root and on TF, none of the plant species was identified as hyperaccumulator. Due to the high shoot biomass production and high concentration of metal in *S. bicolor* and *C. tinctorius* roots, these plants could be successfully used in phytostabilization and biomass production in marginal soils with moderately heavy metal contamination. Growing plants on contaminated soil will eventually improve the chemical, physical and biological properties of the contaminated soils. In this context, it is essential to point out that the final purpose of any soil remediation process must not be only to remove the contaminants from the polluted soil or to reduce their toxicities, but to restore and ameliorate its overall ecosystems.

In conclusion, our results from hydroponic experiment cannot be directly extrapolated to the phytostabilization performance in the field. However, the results of hydroponic tests about heavy metals tolerance can confirm that tested plant species were found to be tolerant to heavy metals, performed well in hydroponic experiment and could perform better under field conditions. Further field experiments are necessary to confirm our hydroponic experiment results and to quantify precisely, the growth inhibition, biomass production, plant uptake and translocation rates on a long-term basis and in real metal-polluted field conditions.

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