

## THE EFFECT OF SODIUM HYDROGEN PHOSPHATE/ CITRIC ACID MIXTURES ON PHYTOREMEDIATION BY ALFALFA & METALS AVAILABILITY IN SOIL

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### ABSTRACT

The effect of sodium hydrogen phosphate/citric acid mixtures on enhancing phytoremediation of heavy metal-contaminated soil by alfalfa (*Medicago sativa L.*) and the changes of metal availability in soils were investigated. Alfalfa plants grew healthily in heavy metals-contaminated soils (collected from molybdenum mine) for 30 days. The heavy metal content was determined by ICP-OES. In this paper, it was found that: the phytoextraction efficiency of alfalfa plants was enhanced after the sodium hydrogen phosphate/citric acid mixtures were added into soil. As usual, the phosphorus can promote plants to accumulate more biomass (increasing 0.35%-24.62%) even if the availability of metals to extraction were increased by citric acid in the soil. With the treatment of sodium hydrogen phosphate/citric acid mixtures, the bioconcentration factor (BCF) values of alfalfa plants and the acid soluble fractions of heavy metals in soils were increased. The translocation factor (TF) values of all alfalfas in heavy metals were low, but the TF of As, Cr, Hg, Mo tended to increase with sodium hydrogen phosphate/citric acid mixtures. In other words, both the availability and the biological-availability of heavy metals in soils were increased after sodium hydrogen phosphate/citric acid mixtures were added into soils.

**Key words:** sodium hydrogen phosphate/citric acid, alfalfa, heavy metal, phytoremediation, availability.

### INTRODUCTION

Anthropogenic activities such as mining and smelting of metal ores have increased the prevalence and occurrence of heavy metal contamination at the Earth's surface. Mining and smelting activities have contaminated soil and water resources with heavy metals throughout the world (Bhattacharya *et al.*, 2006). Heavy metal in the soil requires the implementation of appropriate remedial

measures because it represents a serious threat for the ecosystem and human health (Vaxevanidou *et al.*, 2008). Soil remediation can reduce the contamination and preserve soil as a non-renewable natural resource.

Among many technologies used to remediate soils with pollutants, soil extracting has been one of the most-used treatments because of its rapid

remediation process (Tuin and Tels, 1990), low cost in comparison to other remediation techniques (Kuhlman and Greenfield, 1999). The extractants usually employed were acids, chelating agents, electrolytes, oxidizing agents and surfactants (Schramel *et al.*, 2000; Reddy and Chinthamreddy, 2000). The successful application of this technique depends mainly on the use of the extracting agent. The most used extracting solutions for extracting also have deficiencies: ethylene diamine tetraacetic acid (EDTA) is expensive, toxic and presents a low level of biodegradability (Dirilgen, 1998; Finžgar, 2006); nitrilotriacetic acid (NTA) is also toxic and considered a class II carcinogen (Peters, 1999); nitric acid (HNO<sub>3</sub>) is lethal to soil microflora and destructive to the physical and chemical properties of soil (Neilson *et al.*, 2003); hydrochloric acid (HCl) can alter soil properties (Di Palma and Mecozzi, 2007); citric acid is a nontoxic extracting extractant that forms relatively strong complexes and it is easily biodegradable, but it presents a lower effectiveness in the removal of metal ions (Salt *et al.*, 1995).

The problem with soil extraction is the production of a complex solution that requires an additional treatment. The use of plant species for phytoremediation is a potential strategy to remove the heavy metals from the contaminated habitat (Lasat, 2002; Niu *et al.*, 2007). Phytoremediation was defined as the use of plants to remove pollutants from the environment or to render them harmless (Queirolo and Valenta, 1987; Salt *et al.*, 1995). Alfalfa plants have also been shown to be extremely resistant to contaminants as well as a bioaccumulator (Carrillo and Cajuste, 1992). Alfalfa may be a good source of plant tissues because it is tolerant of heavy metals and grows well in contaminated soils (Baligar *et al.*, 1993; Gardea-Torresdey *et al.*, 1997).

Phosphates (P) are of special interest because P is not only an important nutrient but can also precipitate heavy metals, making them less available for either plant or microbial uptake; citric acid is known to enhance metals solubility and plant uptake via formations of soluble citrate-metal complexes. The single effect of either phosphates or citric acid in soils on extracting and phytoremediation is entirely different, practically opposite. But the combined action of phosphates and citric acid on extracting and phytoremediation is not known. Herein, the objective of this study was to investigate the effect of sodium hydrogen phosphate/citric acid mixtures on enhancing phytoremediation of heavy metal-contaminated soil by alfalfa (*Medicago sativa L.*) and the changes of metal availability in soils.

## **MATERIALS AND METHODS**

### **Soil preparation and analyses**

The soil was the black soil, collected (0-20 cm deep) randomly from farmland at a distance of 100 m from the molybdenum mine (N 40°45'15", E 120°51' 45") located in Liaoning province, China. The soils were mixed homogeneously and sieved through 2 mm mesh size after being air-dried. The physical and chemical properties of the soils are shown in Table 1.

### **Pot experiments**

Alfalfa seeds were surface sterilized by immersion in 2% (v/v) H<sub>2</sub>O<sub>2</sub> and shaken at 144 rpm on an orbital shaker in sterile deionized water for 6 h (Ma *et al.*, 2007). Then they were sown onto stainless plates with aseptic gauze in incubator, keeping the temperature and humidity at 25°C and

**Table 1.** The physicochemical properties and patterns of pollution.

| Parameter         | Soil                               |
|-------------------|------------------------------------|
| pH                | 6.51                               |
| Organic matter    | 17.94 g·kg <sup>-1</sup>           |
| CEC               | 7.06 c mol·kg <sup>-1</sup>        |
| CaCO <sub>3</sub> | 2.46 g·kg <sup>-1</sup>            |
| As                | 150.36±41.58 mg kg <sup>-1</sup>   |
| Cd                | 75.29±17.20 mg kg <sup>-1</sup>    |
| Cr                | 182.65±60.69 mg kg <sup>-1</sup>   |
| Cu                | 31.84±27.11 mg kg <sup>-1</sup>    |
| Hg                | 4.56±1.71 mg kg <sup>-1</sup>      |
| Mo                | 710.98±56.23 mg kg <sup>-1</sup> * |
| Ni                | 47.35±12.91 mg kg <sup>-1</sup>    |
| Pb                | 398.26±49.68 mg kg <sup>-1</sup> * |
| Zn                | 290.67±36.32 mg kg <sup>-1</sup> * |

Data are average ± S.E, n=20. \* Significant at  $p < 0.05$ . CEC means the cation exchange capacity. pH measured at a 1:2.5 ratio (soil-to-water). The metal contents in soil were determined by inductively coupled plasma-optical emission spectrometer (ICP-OES, Varian) after the soils were digested with HNO<sub>3</sub>-HF-HClO<sub>4</sub> in a Teflon plastic crucible (the digestion methods were shown in fraction of soil analysis in materials and methods).

60%. Seedlings grew to a length of 1 cm 30 seedlings were then transplanted in plastic pots which were filled with 5 kg of previously analyzed soil in a green house.

40 ml different pHs and proportion mixtures of sodium hydrogen phosphate + citric acids, the collocation methods and treatment No. are shown as Table 2, were diluted with double distilled water to 200 ml and added into pots separately after alfalfa plants have grown 10 days in the green house. The pots were migrated outdoor and the alfalfa plants grew in natural conditions. Pots were irrigated with deionized water every four days (keeping at 60% equivalent humidity) and the leachates were collected and irrigated in the next time, respectively. In the experiments, three replicates were made

and the controls were carried out with deionized water.

### Plant analysis

The plants were harvested by clipping the shoots and pulling out the roots after alfalfas have grown 30 days. The roots and shoots were separated and washed with double deionized water to remove soil and dust. The plants' parts (both shoot and root) were dried in an oven at 70°C for 72 h, and the dry weights were recorded by electronic balance (the limit is 0.1 mg). Parts of plants including root and shoot were digested at 150°C for 200 min with 10 ml of mixture of HNO<sub>3</sub> and HClO<sub>4</sub>, the proportion of HNO<sub>3</sub>/HClO<sub>4</sub> was 4:1 (Qu *et al.*, 2008). The digestion was accomplished by using the simple setting-furnace digestion and big cuvettes (Žemberyová *et al.*, 2006). Subsequently, the digested solution passed through 0.45 μm mesh size filter, and the volume of digested samples were adjusted to 20 ml with double deionized water and determined by ICP-OES.

### Soil analysis

1 g of surface soils (0-20 cm) in every pot were weighed and placed in a Teflon plastic crucible, after alfalfa plants were harvested. The soils were ground with agate mortar and sieved to < 0.15 mm after being air-dried. 5 ml HNO<sub>3</sub>, 10 ml HF and 12 ml HClO<sub>4</sub> were added to soils and oscillated for 10 hours under constant temperature (80°C). When the digestants were almost dried, 10ml nitric acid (the proportion was 1:1) were added to dissolve the soils continually and transferred constantly to 50 ml volumetric flask to determine the total contents of heavy metals in the soil (Lin, 1997).

An extraction procedure for the acid soluble fraction of heavy metals, proposed by the Commission of the European

Communities Bureau of Reference (BCR), has been applied to the analysis of heavy metals in the soil (Stromberg and Banwart, 1994). The acid soluble fractions of heavy metals in soils were availability and biological-validity (it means more metals could be absorbed and uptake by plants). The acid soluble fractions of heavy metals in soils were used to represent availability of heavy metals for plants (Papoyan and Kochian, 2004). The extraction procedures of acid soluble fraction in heavy metals were described in details as follow: 40 ml volume of acetic acid ( $0.11 \text{ mol}\cdot\text{l}^{-1}$ ) was added to 1 g of soil in a PTFE vessel. The vessel was shaken at room temperature in a mechanical shaker operating at  $300 \text{ motions}\cdot\text{min}^{-1}$  for 16 h (overnight), the shaking speed was adapted in order to ensure a continuous suspension of the mixture. The extract was separated from the solid residue by centrifugation for 20 min and decanted into a polyethylene container and stored in a refrigerator at  $4^{\circ}\text{C}$  for analysis. The residue was washed with 20 ml of distilled water by shaking for 20 min, centrifuged and the washings discarded (Žemberyová *et al.*, 2006).

After digestion and extraction of the soil samples, the contents of total mass and acid soluble fraction in heavy metals were analyzed by ICP-OES.

#### **Statistical analysis**

Two metal accumulation factors, BCF (bioconcentration factor, defined as  $C_{\text{plants}}/C_{\text{culture}}$ ) and TF (translocation factor, defined as  $C_{\text{shoot}}/C_{\text{root}}$ ), which were computed from the treatments contents, were used to discuss the results from this study. Controls and treatments were in triplicates for analysis.

All the data obtained from this study were analyzed by SPSS 13.0. The differences among treatment means were

compared by least significant difference ( $p < 0.05$ ).

## **RESULTS**

### **Influence of alfalfa**

#### *Biomass of alfalfa*

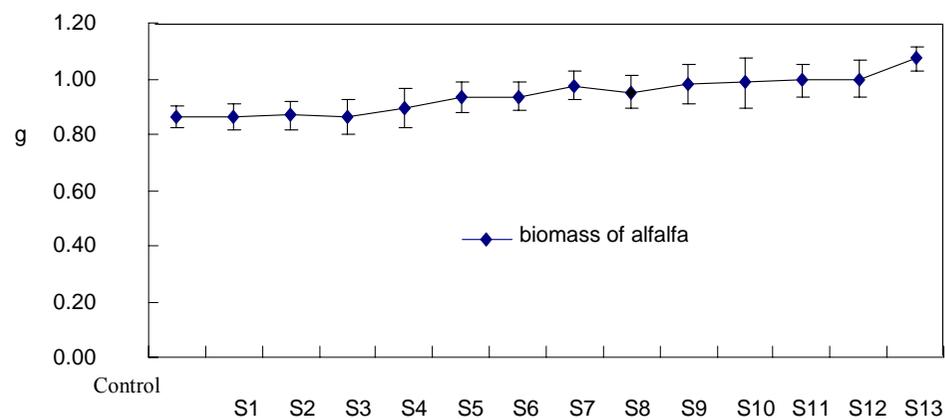
The weight of oven dried alfalfa plants was recorded by electronic balance (Figure 1). Alfalfa had the highest biomass ( $1.073 \text{ g pot}^{-1}$ , dry weight) in S13 treatment and the lowest biomass ( $0.861 \text{ g}\cdot\text{pot}^{-1}$ , dry weight) in control ( $p < 0.05$ ). The results indicated that sodium hydrogen phosphate/citric acid mixtures could promote plants to accumulate biomass (increasing 0.35%-24.62%), but the differences between the biomass of controls and treatments were insignificant. This increasing can be the result of nutritive properties of P, because the S13 solution had the highest P contents. The biomass can express the tolerance of plants to toxic metals indirectly, but most of metal hyper accumulators were small and slow growing (Gardea-Torresdey *et al.*, 1998). All alfalfa plants grew healthily without showing any intoxication phenomenon. It is hypothesized that: to a certain degree, phosphorus can promote plant to accumulate biomass and tolerance to metals even if the availability of metals were increased by citric acid in the soil.

In this experiment, biomass of alfalfa plants with sodium hydrogen phosphate/citric acid mixtures was higher than control. So, sodium hydrogen phosphate/citric acid mixtures can be used for enhancing alfalfa plants to accumulate biomass as a remediation strategy for soil contaminated with heavy metals (Renella *et al.*, 2004).

**Table 2.** the experimental design for the treatments of pot experiments.

| Treatments   | Control | S1    | S2    | S3    | S4    | S5    | S6    | S7    | S8    | S9    | S10   | S11   | S12   | S13   |
|--|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.2 mol l <sup>-1</sup><br>sodium hydrogen<br>phosphate (ml) | 0.00    | 21.44 | 22.30 | 23.20 | 24.18 | 25.26 | 26.44 | 27.70 | 29.10 | 30.90 | 32.94 | 34.78 | 36.34 | 37.76 |
| 0.1 mol l <sup>-1</sup><br>citric acid (ml)                  | 0.00    | 18.56 | 17.70 | 16.80 | 15.82 | 14.74 | 13.56 | 12.30 | 10.90 | 9.10  | 7.06  | 5.22  | 3.66  | 2.54  |
| pH of mixtures   | 7.0     | 5.2   | 5.4   | 5.6   | 5.8   | 6.0   | 6.2   | 6.4   | 6.6   | 6.8   | 7.0   | 7.2   | 7.4   | 7.6   |

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**Figure 1.** The biomass of alfalfa (g pot<sup>-1</sup> dry weight).

*Bioaccumulation of heavy metal by alfalfa*

After 30 days, alfalfa plants could uptake heavy metals from the soil. The contents of heavy metals in the roots compared with the shoot were higher, which may be related both to heavy metal uptake from the soil and xylem translocation from root to shoot. The contents of heavy metals in treatment alfalfa plants were higher than control. Similarly, the contents of Zn and Mo in alfalfa plants were significantly higher than other metals, the results agreed with the previous results found about alfalfa (Krishnamurti *et al.*, 1997). Bioaccumulation depends not only on the characteristics of the organism itself, but also the characteristics of the substance and the environmental factors. The sodium hydrogen phosphate/citric acid mixtures have the potential to enhance metals mobility in soils profiles by reducing soil pH and forming complexes with heavy metals (Schwab *et al.*, 2005), the presence of citric acid may affect heavy metal desorption, solubility and mobility (Duarte *et al.*, 2007). Dissolved organic matter can compete with As for sorption sites and displace both As (V) and As (III) from iron oxides (Redman *et al.*, 2002). But the reduction of Cr in soils is accelerated by the presence of organic matter and divalent iron (Kumpiene *et al.*, 2008). Results of some studies indicated that Zn was immobilized as metal-phosphate precipitates with low solubility and high resistance to soil acidification (Brown *et al.*, 2005). The maximum contents of heavy metals in alfalfa plants appeared at lower pH for the treatments of sodium hydrogen phosphate/citric acid mixtures except for Hg and Pb. This may be due to the metal binding affinities for ligand in the soils, the antagonism of ions, chemical competition for multi-metal and physicochemical properties of Hg and Pb. The treatment of S1 is better application to phytoremediation for As, Cd, Cr, Cu,

Mo, Ni, and Zn by alfalfa plants, and the treatment of S10 is better application to phytoremediation for Hg and Pb by alfalfa plants.

As pH is a key factor in regulating the sorption-desorption and leachability of trace metals in many types of contaminated materials (Van der Sloot *et al.*, 1996). The increase of soil pH can change the oxidation of Cr (III) to Cr (VI) and cause a higher Cr mobility and uptake by vegetation (Pranav *et al.*, 2007). Stability of Cu in soil is strongly pH dependent, and the mobility decrease with increasing of pH. The stabilization of Cd in soil increased with increasing pH for the absorption of soil, but the bioavailability of Zn increased with increasing pH due to competition for sorption sites (Rai *et al.*, 2004). Bioaccumulation depends not only on the characteristics of the organism itself, but also on the characteristics of the substance and the environment factors. In general, with sodium hydrogen phosphate/citric acid mixtures added into the soil, cationic metals tend to become more mobile as the pH decreases due to a decrease in binding sites and enhanced proton competition.

*BCF and TF of alfalfa in heavy metal*

BCF and TF values were studied in each sample (Table 3). The average BCF values of alfalfa with sodium hydrogen phosphate/citric acid mixtures were higher than control, which meant that their ability of bioaccumulation of heavy metal was better than alfalfa without sodium hydrogen phosphate/citric acid mixtures, the result agreed with other findings about citric acids (Evangelou *et al.*, 2007). The BCF values differed with kinds of heavy metals, the accumulation ability and physiological factors of plants and environmental conditions. The average BCF values of alfalfa's shoot were 2.11, 0.94, 1.83, 1.18, 2.30, 5.26,

1.83, 1.32, and 2.54-fold higher than the control in As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn, respectively, the average bioconcentration factors of alfalfa's root were 2.00, 1.37, 1.19, 1.32, 1.32, 3.17, 2.09, 1.45, and 2.36-fold higher than the control in As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn, respectively. This may be due to four reasons: citric acid complexation leading to an increase in the mobility of heavy metals; the pH was changed; excess of heavy metal mobilization was turning from a nutrient state to a phytotoxic form (Pushenreiter *et al.*, 2001); heavy metals produced dissolvable or indissolvable phosphates.

The higher values of TF indicate that plants could move and distribute more heavy metals. In this experiment, it was shown that the TF values of heavy metals in alfalfas were low, it showed that there were less heavy metals moved into the shoots; the TF values of As, Cr, Hg, Mo, and Zn in alfalfa tended to increase with sodium hydrogen phosphate/citric acid mixtures, but TF values of Cd, Cu, Ni, and Pb in alfalfa decreased after sodium hydrogen phosphate/citric acid mixtures were added into the soil. This may be explained by the fact that Cd, Cu, Ni, and Pb was retained in the cation exchange sites of the vessel walls of xylem parenchyma cells in roots and immobilized in the vacuoles of the root cells. The capacity of cell walls for binding metals allows the plant to tolerate heavy metals (Seregin and Kozhevnikova, 2006).

#### **Influence of availability of heavy metals in the soil**

Refer to Table 4 for the acid soluble fractions of heavy metals in soils by the treatment of sodium hydrogen

phosphate/citric acid mixtures and control.

With the treatment of sodium hydrogen phosphate/citric acid mixtures, the average contents of acid soluble fraction were higher than the control. In other words, the availability and biological-validity of heavy metals in soils were increased after sodium hydrogen phosphate/citric acid mixtures were added into soils. With different treatments, the maximums of As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn were 4.09, 1.90, 1.91, 6.76, 1.56, 58.45, 7.92, 7.44, and 102.85 mg·kg<sup>-1</sup>, respectively. The average contents of acid soluble fraction in As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn were 2.08, 1.86, 3.05, 1.18, 1.81, 1.43, 2.51, 1.64, and 1.65-fold higher than control, respectively. The result agreed with the previous results found about citric acid (Chen *et al.*, 2002). The citric acid has a high affinity for metals, which may be due to the competition of citric acid with heavy metals on the surface sites (Jean *et al.*, 2007). The results agreed with those found about citric acid (Jean *et al.*, 2008). There are lots of reports on metals being immobilized as metal-phosphate precipitates by phosphorus amendments, but in this experiment the average contents of acid soluble fraction were increased by citric acid even if phosphate were added into soils.

The acid soluble fractions of heavy metals in soils were increased by the treatment of sodium hydrogen phosphate/citric acid mixtures, which could be ascribed to: the competition of high contents of cations for adsorption sites in soils (Yuan *et al.*, 2007); the increase of activities of heavy metal due to sodium hydrogen phosphate/citric acid mixtures; the formation of ionic pairs or chelating compounds (Chen, 1996).

**Table 3.** The average BCF and TF of alfalfa plants.

| Heavy metal                        | As   | Cd   | Cr   | Cu   | Hg   | Mo   | Ni   | Pb   | Zn   |
|------------------------------------|------|------|------|------|------|------|------|------|------|
| Average BCF of shoot in control    | 1.66 | 2.01 | 1.18 | 1.42 | 2.08 | 1.03 | 1.04 | 0.81 | 1.81 |
| Average BCF of root in control     | 2.55 | 5.32 | 2.17 | 3.52 | 4.47 | 2.25 | 1.70 | 1.02 | 2.96 |
| Average TF of control              | 0.65 | 0.38 | 0.54 | 0.40 | 0.47 | 0.46 | 0.61 | 0.79 | 0.61 |
| Average BCF of shoot with mixtures | 3.51 | 1.88 | 2.16 | 1.67 | 4.78 | 5.42 | 1.90 | 1.07 | 4.59 |
| Average BCF of root with mixtures  | 5.11 | 7.29 | 2.58 | 4.63 | 5.90 | 7.13 | 3.55 | 1.48 | 6.99 |
| Average TF of mixtures             | 0.69 | 0.26 | 0.84 | 0.36 | 0.81 | 0.76 | 0.54 | 0.72 | 0.66 |

**Table 4.** The acid soluble fractions of heavy metals in soils after pot experiments (mg kg<sup>-1</sup>).

| No.       | Control         | S1              | S2              | S3              | S4              | S5              | S6              | S7              | S8              | S9              | S10             | S11              | S12             | S13             |
|-----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|
| <b>As</b> | 1.83<br>±0.32   | 3.60<br>±0.36*  | 3.68<br>±0.29*  | 3.95<br>±0.29*  | 3.91<br>±0.32*  | 4.03<br>±0.36*  | 4.09<br>±0.40*  | 3.53<br>±0.32*  | 3.74<br>±0.38   | 3.69<br>±0.23*  | 3.88<br>±0.59   | 3.94<br>±0.44    | 3.75<br>±0.41   | 3.5<br>±0.33*   |
| <b>Cd</b> | 0.86<br>±0.12   | 1.73<br>±0.14*  | 1.86<br>±0.17*  | 1.88<br>±0.13*  | 1.81<br>±0.14*  | 1.72<br>±0.15*  | 1.39<br>±0.12*  | 1.16<br>±0.13*  | 1.03<br>±0.09*  | 1.25<br>±0.10*  | 1.90<br>±0.16*  | 1.68<br>±0.13*   | 1.60<br>±0.12*  | 1.75<br>±0.11*  |
| <b>Cr</b> | 1.26<br>±0.10*  | 4.79<br>±0.36*  | 5.27<br>±0.48*  | 5.64<br>±0.44*  | 5.91<br>±0.43*  | 4.61<br>±0.40*  | 4.07<br>±0.38*  | 3.57<br>±0.30*  | 3.41<br>±0.27*  | 3.26<br>±0.26*  | 3.09<br>±0.24*  | 2.50<br>±0.23*   | 2.16<br>±0.11*  | 1.63<br>±0.15*  |
| <b>Cu</b> | 5.20<br>±0.53   | 6.70<br>±0.60*  | 6.59<br>±0.48*  | 6.43<br>±0.33*  | 6.64<br>±0.60*  | 6.72<br>±0.55*  | 6.69<br>±0.51*  | 6.76<br>±0.40*  | 6.13<br>±0.42*  | 5.8<br>±0.39*   | 5.55<br>±0.47*  | 5.48<br>±0.49*   | 5.35<br>±0.34*  | 5.23<br>±0.36*  |
| <b>Hg</b> | 0.53<br>±0.09   | 1.34<br>±0.11*  | 1.40<br>±0.08*  | 1.56<br>±0.13*  | 1.57<br>±0.10*  | 1.27<br>±0.11*  | 1.00<br>±0.06*  | 0.86<br>±0.05*  | 0.70<br>±0.06*  | 0.60<br>±0.04*  | 0.62<br>±0.05*  | 0.59<br>±0.04*   | 0.49<br>±0.03*  | 0.45<br>±0.02*  |
| <b>Mo</b> | 35.12<br>±3.32* | 53.81<br>±5.00* | 54.60<br>±5.33* | 56.54<br>±4.94* | 52.03<br>±4.62* | 50.97<br>±3.99* | 48.10<br>±4.53* | 47.65<br>±3.21* | 41.43<br>±3.85* | 45.32<br>±2.96* | 46.01<br>±4.37* | 47.21<br>±3.80*  | 50.84<br>±3.27* | 58.55<br>±4.09* |
| <b>Ni</b> | 2.70<br>±0.19*  | 6.76<br>±0.57*  | 6.90<br>±0.65*  | 6.08<br>±0.33*  | 7.27<br>±0.27*  | 7.19<br>±0.50*  | 7.43<br>±0.64*  | 7.55<br>±0.53*  | 7.79<br>±0.62*  | 7.92<br>±0.75*  | 6.66<br>±0.39*  | 5.77<br>±0.40*   | 5.12<br>±0.47*  | 5.61<br>±0.54*  |
| <b>Pb</b> | 4.07<br>±0.18*  | 7.14<br>±0.60*  | 6.09<br>±0.46*  | 7.26<br>±0.71*  | 6.43<br>±0.55*  | 6.37<br>±0.46*  | 5.84<br>±0.57*  | 7.44<br>±0.37*  | 6.73<br>±0.58*  | 6.54<br>±0.40*  | 6.40<br>±0.42*  | 6.67<br>±0.53*   | 7.03<br>±0.62*  | 6.81<br>±0.35*  |
| <b>Zn</b> | 58.61<br>±4.09* | 96.10<br>±7.73* | 98.96<br>±4.97* | 98.06<br>±6.98* | 98.15<br>±6.85* | 98.57<br>±8.42* | 97.92<br>±6.07* | 98.23<br>±7.44* | 97.73<br>±9.07* | 88.34<br>±8.14* | 92.85<br>±8.63* | 103.01<br>±8.48* | 91.17<br>±5.29* | 95.69<br>±7.17* |

## CONCLUSIONS

This study investigated the influence of sodium hydrogen phosphate/citric acid mixtures on the enhancement of accumulation of biomass by alfalfa and the availability of heavy metals in the soil. The main conclusions were drawn as follows: accumulation of biomass by alfalfa with sodium hydrogen phosphate/citric acid mixtures was higher. It is hypothesized that phosphorus can promote plant to accumulate biomass and tolerance to metals even if the availability of metals were increased by citric acid in the soil. The contents of heavy metals in alfalfa plants under treatment were higher than control. So, the sodium hydrogen phosphate/citric acid mixtures have the potential to enhance metal mobility in soil profiles by forming complexes with heavy metals and enhance the efficiency of phytoremediation; the average BCF values of alfalfa's shoot were 2.11, 0.94, 1.83, 1.18, 2.30, 5.26, 1.83, 1.32, and 2.54-fold higher than the control in As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn, respectively, the average BCF values of alfalfa's root were 2.00, 1.37, 1.19, 1.32, 1.32, 3.17, 2.09, 1.45, and 2.36-fold higher than the control in As, Cd, Cr, Cu, Hg, Mo, Ni, Pb, and Zn, respectively; the TF values of alfalfa were low and those of As, Cr, Hg, Mo, and Zn tended to increase with sodium hydrogen phosphate/citric acid mixtures. However, TF values of Cd, Cu, Ni, and Pb in alfalfa decreased after sodium hydrogen phosphate/citric acid mixtures were added into soil; with the treatment of sodium hydrogen phosphate/citric acid mixtures, the average contents of acid soluble fraction were higher than control. In other words, the availability and biological-validity of heavy metals in soils were increased after sodium hydrogen phosphate/citric acid mixtures were added into soils.

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