Phyto-extraction of zinc, lead, nickel, and cadmium from a zinc leach residue

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Abstract
Zinc leach residue is one of the substantial industrial wastes containing large quantities of heavy metals, releasing toxic elements into the environment. Phyto-extraction was applied to extract zinc, lead, nickel, and cadmium by Amaranthus retroflexus plant. Through three stages of screening, soil amendment, and modification, the bio-concentration factor, weight of biomass, and lifetime of Amaranthus retroflexus were enhanced. Due to the high salinity of the residue, following screening tests, mixture of 25 wt% zinc leach residue +75 wt% soil, was determined as the optimum condition. For further reduction of the salinity, the soil amendment was carried out both by draining and leaching of the residue with distilled water. In the leached condition, the plant illustrated better results. Finally, soil modification was performed by adding two levels of citric acid (0.005 M and 0.1 M) to the previous optimum conditions. Using the lower level of citric acid, bio-concentration factor of zinc, lead, nickel, and cadmium were obtained as 0.6838, 0.3042, 1.1027, and 2.3621, respectively and the plant survived 5 weeks. Phyto metal extraction index (ratio of concentrations of metal in dried plant to concentration of metal in tailing) for zinc, lead, nickel, and cadmium at the final optimum condition were obtained as 17.09%, 7.60%, 27.57%, and 59.05%, respectively. The separation factors showed that Amaranthus retroflexus's tendency to extract heavy metals was in ascending order for lead, zinc, nickel, and cadmium.

1. Introduction
Currently, 80–85% of zinc (Zn) is generated by hydrometallurgical processes—oxidative roasting, acid leaching, purification, elecrowinning—worldwide (Tang et al., 2018; Yan et al., 2014). The most critical disadvantage of this approach is the substantial amount of zinc leach residue—ZLR—that is produced in the leaching stage and dumped around hydrometallurgical plants. Approximately, 0.5–0.9 ton of ZLR is generated per ton of zinc (Sethurajan et al., 2016), which includes high concentration of heavy metals such as zinc, lead (Pb), nickel (Ni), and cadmium (Cd) (Çoruh and Ergun, 2010; Jiang et al., 2017; Tang et al., 2018; Ye et al., 2017). Based on the leachability and bioavailability of ZLR metal contents, ZLR is considered as a hazardous waste, and therefore exposure of this hazardous waste to acid rains and winds can cause groundwater contamination and soil pollution which is a critical threat to ecosystem. (Çoruh and Ergun, 2010; Moameri et al., 2017; Osmolovskaya et al., 2018; Sethurajan et al., 2016; Shakoor et al., 2014). In addition, disposing this huge amount of ZLR occupies massive land and results in huge loss of valuable elements (Tang et al., 2018). In this regard, a method for ZLR recycling is required to remove hazardous heavy metals from the environment and also to recycle valuable elements from contaminated areas (Jiang et al., 2017; Yan et al., 2014).

For ZLR recycling, pyrometallurgical and hydrometallurgical processes are the two conventional methods that are being used (Yan et al., 2014). Pyrometallurgy methods which are used for recycling heavy metals from ZLR—Waelz and Ausmelt—have some considerable drawbacks: high energy consumption as a result of high temperature (1100–1300 °C) and producing a high amount of iron-bearing residue (Jiang et al., 2017; Li et al., 2012a; Roshanfar et al., 2019; Yan et al., 2014). Hydrometallurgical processes are more economically and environmentally acceptable in comparison with pyrometallurgy because of lower capital and operation costs as well as considerably lower temperature; however, severe leaching conditions such as relatively high temperature and high acid concentrations are required for decomposition of high resistant chemical compositions such as zinc ferrites (Jiang et al., 2017; Li et al., 2012a, b; Yan et al., 2014). Furthermore, some hydrometallurgy processes require roasting as a pretreatment that can
produce hazardous gases (Yan et al., 2014). In general, it is necessary to find an eco-friendly and economical method to recover and manage the huge amount of stockpiled ZLR.

Phyto-extraction is an in-situ, cost-effective, and environmentally friendly technique which benefits from the ability of plants for metal extraction from low grade ores and tailings, bringing about environmental sustainability and a green landscape (Wang et al., 2017; Yang et al., 2017; Ye et al., 2017). Table 1 shows the qualitative comparison of pyrometallurgy, hydrometallurgy, and phyto-extraction regarding recovery of metals. Organic and inorganic contaminants of soil, tailing, and water are absorbed by the root of plants, and then transported into harvestable plant tissues (Aderholt et al., 2017; Ali et al., 2013; Rahman and Boyce, 2013; Moameri et al., 2017). The success of phyto-extraction depends on many factors, the most important of which are: soil composition; contaminant bioavailability; and using a suitable plant by the features of high growth rate, excessive biomass production, great accumulation of the target heavy metal, tolerance to the target heavy metal toxicity, as well as easy cultivation and harvest (Ahmad et al., 2015; Attinti et al., 2017; Mohammadzadeh et al., 2014; Yang et al., 2017). Many plants are able to tolerate heavy metal concentration in their tissues to some extent (Shahid et al., 2012). However, only particular species of plants are suitable for phyto-extraction which are known as hyper-accumulators. Hyper-accumulators are the plants that are able to tolerate an extreme level of heavy metals in their aerial tissues even 100 times more than normal plants (Ali et al., 2013; Sheoran et al., 2016). Shoot to root metal concentration ratio or translocation factor (TF) for hyper-accumulator plants is more than 1 (Ali et al., 2013):

$$\text{TF} = \frac{\text{Concentration of metal in dried shoot} (\text{mg/kg})}{\text{Concentration of metal in dried root} (\text{mg/kg})}$$

(1)

Although this factor is helpful to support distinguishing hyper-accumulators, it is not sufficient to ascertain that a plant is hyper-accumulator (Ali et al., 2013). To calculate the efficiency of phyto-extraction, a bio-concentration factor (BCF), or an accumulation factor, is more applicable (Ali et al., 2013; Li et al., 2017):

$$\text{BCF} = \frac{\text{Concentration of metal in dried shoot} (\text{mg/kg})}{\text{Concentration of metal in soil mixture} (\text{mg/kg})}$$

(2)

BCF is a vital factor to define a plant as hyper-accumulator. The plants with BCF greater than one are known as hyper-accumulators. Regardless of the mentioned definition, BCF can be less than one for a metal with high concentration in soil (Ali et al., 2013).

The main restrictive factor in phyto-extraction is low bioavailability and solubility of some metals in soil, such as lead (Aderholt et al., 2017; Mousavi et al., 2018; Muhammad et al., 2009). In this regard, phyto-extraction of heavy metals is divided into two categories, natural and induced. In natural phyto-extraction, there is no soil modification. In induced or chelated assisted phyto-extraction, however, soil is modified by different chelating agents in order to enhance heavy metal bioavailability in soil for plant uptake that results in more heavy metal extraction by the plants. (Ali et al., 2013; Ghosh and Singh, 2005; Muhammad et al., 2009; Ningyu et al., 2016). There are different chelating agents such as ethylenediaminetetraacetic acid (EDTA), citric acid (CA), oxalic acid, and malic acid that have been used in phyto-extraction processes (Ali et al., 2013; Khan et al., 2016; Muhammad et al., 2009). A suitable chelating agent should increase metallic compound solubility and metallic ion uptake. Moreover, it should be easily biodegradable. Although, EDTA has a significant effect on metal uptake, this agent exists in the environment for a long time due to its poor biodegradation. EDTA is also able to leach hazardous metals into groundwater. Hence, the use of EDTA is limited today (Ali et al., 2013; Attinti et al., 2017).

Generally, organic acids form complexes with heavy metals and increase their bioavailability (Tatar et al., 1999; Wang et al., 2017). Among organic acids, citric acid is a desirable chelating agent by virtue of its natural origin, simple biodegrading, proper complex binding with many elements, and non-toxicity (Ali et al., 2013; Wang et al., 2017). There are extensive studies that have investigated the effects of different citric acid concentrations on the heavy metal extraction of diverse plants. Wang et al. (2017) investigated the effect of citric acid on the cadmium uptake of Festuca arundinacea and Poa pratensis plants and found that citric acid increases the uptake of cadmium in both the root and the shoot of Festuca arundinacea as well as in the root of Poa pratensis. In the investigations of Muhammad et al. (2009), the effects of different concentrations of EDTA and citric acid on the accumulation of cadmium, copper, lead, and chromium in Typha angustifolia tissues were studied. According to their results, citric acid is an efficient chelating agent increasing the accumulation of cadmium and chromium in the Typha angustifolia tissues. In another study, the efficiency of EDTA and citric acid for the accumulation of copper, lead, zinc, and cadmium in Helianthus annuus tissues was investigated. The results showed that EDTA enhanced the metal concentrations in the Helianthus annuus shoot, but citric acid was an inefficient chelating agent in increasing the metal concentrations in the Helianthus annuus tissues due to the citric acid mineralization (precipitation of heavy metal species in the organic matrix) and high soil buffering capacity (Lesage et al., 2005).

**Amaranth (A.)** plants—nich high tolerance and cadmium hyper-accumulators—grow in many parts of sub-tropical and tropical Asia, Africa, and Central America. These plants have a great potential to adapt to new environments and different climates. Also, **Amaranths** are capable of being farmed with minimum crop management (Feine et al., 1979). Many kinds of **Amaranth** are weedy types and a limited number of them are used as crops. The weedy types such as A. viridis, A. spinosus, A. retroflexus, and A. hybridus tend to be indeterminate and robust in growth habit (NRC, 1984). In addition, they grow rapidly and have large biomass (Fan and Wei, 2009; Ningyu et al., 2016; Osmolovskaya et al., 2018). Due to these traits, A. retroflexus as a species of **Amaranth** is a suitable plant for a phyto-extraction process.

Table 1: Qualitative comparison of pyrometallurgy, hydrometallurgy, and phyto-extraction regarding recovery of metals from ZLR (Ali et al., 2013; Ghosh and Singh, 2005; Jackson, 1986; Jha et al., 2001; Li et al., 2012a, b; Luo et al., 2017; Sethurajan et al., 2017; Tamás and Martínoia, 2006; Yan et al., 2014).

<table>
<thead>
<tr>
<th>Method</th>
<th>Metal Recovery</th>
<th>Energy Consumption</th>
<th>Hazardous Residue/ Effluent</th>
<th>Chemicals</th>
<th>Cost (capital and operating)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrometallurgy</td>
<td>High</td>
<td>High</td>
<td>Solid and Gas</td>
<td>Toxic/Non-toxic</td>
<td>High</td>
</tr>
<tr>
<td>Hydrometallurgy</td>
<td>High</td>
<td>Moderate</td>
<td>Liquid and Solid</td>
<td>Toxic/Non-toxic</td>
<td>Moderate</td>
</tr>
<tr>
<td>Phyto-extraction</td>
<td>Moderate (need time to full remediation)</td>
<td>Low</td>
<td>None</td>
<td>Non-toxic</td>
<td>Low (more than 10 times lower than the other two methods)</td>
</tr>
</tbody>
</table>
To the best of our knowledge, in the case of ZLR, no study has been performed on the phyto-extraction of heavy metals from this waste. With respect to the aforementioned discussions, in the present study, the phyto-extraction and recovery of zinc, lead, nickel, and cadmium from ZLR—as a metallurgical waste—have been investigated. *A. retroflexus* was used for uptake and extraction of heavy metals. Also, the effect of two concentrations of citric acid (0.005 and 0.1 M)—as a chelating agent—was investigated on the phyto-extraction process. Also, a parameter called phyto metal extraction index (PME) was suggested to quantify the degree of metal extraction from tailings, ores, and contaminated soil.

2. Materials and method

2.1. Materials and preparations

*Amaranthus retroflexus* from the *Amaranthaceae* family and the *amaranth* genus was chosen by virtue of its great features mentioned in the literature. Citric acid monohydrate (C₆H₈O₇⋅H₂O) supplied by Merck Company was used as the chelating agent and the levels of the citric acid concentration (0.005 and 0.1 M) were selected based on the previous studies (Farid et al., 2017; Mihalík et al., 2010; Najaeb et al., 2009; Sinhal et al., 2010). Selecting these two levels was considered to make an estimation of the citric acid concentration that the plant can sufficiently grow and accumulate heavy metals to benefit from in the subsequent experiments. It should be mentioned that in the previous studies some paradoxical conclusions were reported regarding the effect of citric acid concentration on the plants (Aderholt et al., 2017; Lesage et al., 2005; Muhammad et al., 2009; Sinhal et al., 2010). Citric acid was diluted by distilled water. The ZLR tailing used in this study was provided by Khalessazan Industrial Group, Zanjan, Iran. The soil used for diluting the ZLR tailing was regular soil with no fertilizer and modifier provided by Gardens and Green Spaces Organization, University of Tehran. Both the ZLR and soil were initially air-dried and then the ZLR was ground by a jar mill. After homogenizing by a riffle, the ZLR was sieved and the −2.36 mm size fraction was separated.

2.2. ZLR characterization

The physical and chemical properties of the ZLR were analyzed. To determine the pH and electrical conductivity of the soil (Ec), a pH meter (Metrohm–620) and a conductivity meter (JENWAY–4320) were applied (Thomas, 1996). The ZLR texture was analyzed by the Bouyoucos method (Bouyoucos, 1962). Inductively coupled plasma optical emission spectrometry (ICP-OES, 7300DV, Optima Co.) was employed to quantitatively determine the amount of zinc, lead, nickel, and cadmium in the ZLR head sample as well as the dried plants after the experiments. The heavy metal samples, both the ZLR head sample and the plants, were leached in *aqua regia* (3HCl : HNO₃) to be prepared for ICP analysis. X-ray diffraction (XRD, Philips-3040/60 PW) was utilized to investigate the existing phases of the ZLR and the plants after the experiments.

2.3. Experimental procedure

The pot experiments were carried out under greenhouse conditions (Lesage et al., 2005; Mihalík et al., 2010). The temperature was set at 27 ± 2 °C and the day/night cycle was maintained at a 12 h/12 h. Plastic pots with 14.5 cm height and 16 cm mouth opening diameter were applied. In order to avoid soil loss, a filter paper was placed at the bottom of each pot. Because of the high Ec of the ZLR, the experiments were performed at three stages:

1. Screening: Determining the practical weight percentage of the ZLR to the soil so that the plants can live longer and grow.
2. Soil amendment: Improving the plant life and BCF by soil amendment.
3. Modification: Modification of the plants BCF by adding citric acid to the soil.

At the first stage, screening experiments were carried out to determine the suitable weight percentage of the ZLR to the soil. Four weight percentages of the ZLR to the soil (100 wt%, 75 wt%, 50 wt%, 25 wt%) were selected. Firstly, the ZLR and the soil at a specific ratio were mixed and then all the mixtures were homogenized. All the experimental pots were filled with 1.5 kg of each soil mixture. Each experiment was repeated three times (4 ZLR percentages × 3 repeats = 12 experimental pots). Then, *A. retroflexus* seedlings were transported to the experimental pots.

At the second stage, the plant lifetime and BCF were improved. Leaching of the ZLR and draining with distilled water were used as two separate treatments to reduce the ZLR salinity and enhance the plant lifetime and BCF. Leaching of the ZLR was performed by a liquid to solid (L/S) ratio of 5; i.e., 5 L distilled water was mixed with 1 kg of ZLR in a bucket at room temperature for about 1 h. To perform the draining stage, a certain weight of the ZLR was placed in a pot and washed with five times of the saturation capacity of the ZLR with distilled water. The treated ZLR (leached or drained) was mixed with the soil at the optimum percentage. Six pots (3 leached ZLR + 3 drained ZLR) were filled with 1.5 kg of the optimum mixture. Then, *A. retroflexus* seedlings were transferred to the pots.

At the last stage, citric acid was added to the pot to reach the optimum BCF of the plants. The effect of citric acid concentration was investigated at the optimum conditions obtained from the previous stages. After transferring the seedlings to the pots, two levels of citric acid concentrations (0.005 and 0.1 M) were added to separate pots. The lower level of the acid was added to the pots at once; while, the higher concentration was introduced to the soil mixture in 4 times by 2 days interval. Each experiment was repeated 3 times (2 levels of citric acid × 3 repeats = 6 experimental pots).

In all the experiments, the pots were daily watered with distilled water to maintain humidity at 70% water holding capacity. Soil moisture was daily checked by Smart Plant Monitor Sensor (SPMS, Global, Xiaomi Mi). After 5 weeks, the plants were harvested. The shoots of the plants were separated, washed with distilled water, and then dried in an oven at 80 °C for 48 h until the plant’s weight became constant (Luo et al., 2017; Wang et al., 2017). Then, the shoot samples were crushed in a mortar, leached by *aqua regia*, and analyzed by ICP. Finally, to evaluate the efficiency of phyto-extraction, BCF (Eq. (2)) was calculated. All data were reported as the mean of three replicates.

2.4. Statistical analysis

Statistical analysis was carried out by Statistical Analysis System (SAS, version 9.4) using the general linear model (GLM) procedure followed by the least significant difference (LSD) at a 0.05 significant level (α = 0.05). All data are presented as means of three replicates ±2 × standard error (SE).

3. Results and discussion

3.1. ZLR characterization

Table 2 shows the physical and chemical properties of the ZLR. The pH of the ZLR was 6.10. Based on the Ec analysis, ZLR is a saline soil with Ec of 48.20 ds/m. Also, the soil texture was determined as sandy loam with 55% sand, 15% clay, and 32% silt.
Table 3 shows the metal concentrations in the ZLR analyzed by ICP. As shown, zinc and lead were the main metal contents of the ZLR, with 141600 and 12900 mg/kg, respectively. The concentrations of cadmium and nickel were also considerable due to their high toxicity and environmental drawbacks (Osmolovskaya et al., 2018; Pietrini et al., 2015). Also, the soil was analyzed by ICP and the results showed that heavy metal concentration (zinc, lead, nickel, and cadmium) were negligible and not considered in the calculations.

Fig. 1 shows the XRD patterns of soil, ZLR, leached ZLR, and the mixture of 25 wt% leached ZLR + 75 wt% soil. According to Fig. 1(a), the main phases of the soil are calcium oxide (CaO), silicon oxide (SiO₂), calcium carbonate (CaCO₃), and Hematite (Fe₂O₃). Fig. 1(b) shows XRD pattern of the ZLR where zinc exists as zinc sulfide (ZnS) and zinc ferrite (ZnFe₂O₄) phases as well as lead and cadmium as lead sulfate (PbSO₄) and cadmium sulfide (CdS). According to the XRD pattern of the water leached ZLR (Fig. 1(c)), the main phases and their relative intensities were remained unchanged after the leaching treatment. According to Fig. 1(d), the main phases of mixtures (25 wt% leached ZLR + 75 wt% soil) are compatible with the results of the ZLR pattern and soil pattern.

3.2. Plant growth

Fig. 2 shows the mean lifetime of the plants in the performed experiments. As mentioned in the experimental section, the experiments were performed at three stages of screening, soil amendment, and modification. At the first stage (screening), in 100 wt%, 75 wt%, and 50 wt% of the ZLR, the plants lived for 1 week. This observation is attributed to the high toxicity and high Ec of the ZLR. Using 25 wt% ZLR, the lifetime of A. retroflexus was enhanced to 4 weeks. The Ec of the mixture of 25 wt% ZLR + 75 wt% soil was 8.79 dS/m that was remarkably lower than the pot containing 100 wt% ZLR (48.20 dS/m). Also, the concentrations and toxicity of heavy metals were decreased by the ZLR reduction in the mixtures. Therefore, 25 wt% ZLR was selected as the optimum condition for the next stages.

At the second stage (soil amendment), leaching or draining was used for soil treatment. The results showed that the mean lifetime of the plants was increased to 5 weeks in the pots that the leached ZLR had been used, probably due to the Ec reduction. Table 4 shows the Ec of the 100 wt% ZLR, 100 wt% drained ZLR, and 100 wt% leached ZLR. The results showed that leaching the ZLR reduced the Ec from 48.20 dS/m to 7.92 dS/m. The Ec of the mixture of 25 wt% ZLR (leached) + 75 wt% soil (8.79 dS/m) is much lower than that of 100 wt% ZLR (48.20 dS/m). In other words, by mixing the treated ZLR with the soil, the Ec of the mixture was reduced to even lower amounts. Therefore, the leached ZLR was selected as the optimum condition for the third stage.

At the third stage (modification), citric acid, at two concentrations, was used, and the plants were harvested after 5 weeks. The duration of the phyto-extraction process was selected as 5 weeks, since the results were comparable with the results of the second stage.

Table 5 shows the mean dry weight (DW) of the A. retroflexus plants (shoot) at the three abovementioned stages. In the screening experiments, by decreasing the weight percentage of the ZLR, the dry weights of the plants' biomass enhanced, as a result of the higher mean lifetime of the plants as well as lower toxicity and Ec of the mixtures. Moreover, in the higher ZLR percentages (100 wt%, 75 wt%, and 50 wt%) changes in the ultrastructural plants' organs can be the cause of the reduction in plant growth and lifetime (Afshan et al., 2015). At the second stage, the dry weights of the
plants' biomass increased in the pots that either the leached or the drained ZLR had been used, as a result of the Ec reduction and the higher mean lifetime of the plants (Shrivastava and Kumar, 2015). At the third stage, by adding citric acid, the dry biomass of the plants were significantly increased. Many studies (Afshan et al., 2015; Lesage et al., 2005; Muhammad et al., 2009), were reported that citric acid can increase the plant nutrient uptake (especially sulfur) (Afshan et al., 2015), causes the plant phytochelatin synthesis (Afshan et al., 2015; Muhammad et al., 2009), and also can change the physicochemical structure of the soil (Lesage et al., 2005). Thus, they suggested these factors can improve the plant growth rate and produce more biomass.

3.3. Phyto-extraction of heavy metals

3.3.1. Screening results

Fig. 3 shows the mean BCF of *A. retroflexus* for zinc, lead, nickel, and cadmium for the plants harvested from the pots containing four different ZLR weight percentages at the first stage. In the case that the plants grew in the pots containing 25 wt% ZLR, the BCF of *A. retroflexus* for zinc, lead, nickel, and cadmium extraction were determined to be 0.3452, 0.1797, 0.7268, and 1.7360, respectively. The results were greater than the ones for the pots with the other ZLR percentages. Thus, 25 wt% ZLR was selected as the optimum condition for the soil mixture at the first stage. With respect to the zinc extraction, in the pots containing 100 wt%, 75 wt%, 50 wt%, and 25 wt% ZLR, the BCFs of the plants were less than one. This can be due to the high zinc concentration in the ZLR (141600 mg/kg) (Ali et al., 2013). The lead BCFs are also less than one due to the low bioavailability of the lead (Lesage et al., 2005; Shakoor et al., 2014). It is reported that amaranth is a high tolerant plant for nickel (Osmolovskaya et al., 2018; Pietrini et al., 2015). The nickel BCFs in 100 wt%, 75 wt%, 50 wt%, and 25 wt% ZLR were increased, respectively. This trend might occur as a result of the high Ec of the soil and short lifetime of the plants at higher ZLR percentages (100 wt%, 75 wt%, and 50 wt%). In the case of cadmium, at the optimum condition (25 wt% ZLR), the BCF was greater than one (1.7360). This result confirms that *A. retroflexus* is a practical plant for cadmium accumulation in the shoot (Li et al., 2012b).

Table 6 illustrates the concentrations of the heavy metals in the dried biomass of *A. retroflexus* in the four weight percentages of the ZLR. The results provided in Table 6 are compatible with those in Fig. 3.

3.3.2. Soil amendment

After the screening experiments, for increasing the lifetime of *A. retroflexus* and the higher accumulation of zinc, lead, nickel, and cadmium in the plant tissues, the modification experiments were performed. According to Eq. (2), to calculate the BCF of the metals, the metals concentrations in the soil are required; therefore, the calculations for both the leached and drained ZLR were conducted according to the reference samples. The reference sample for draining is the ZLR that was firstly drained, and then mixed with the soil at the optimum condition (25 wt% treated ZLR + 75 wt% soil). The leaching reference sample is the sample that the ZLR was first leached, and then mixed with the soil at the optimum condition. It should be mentioned that the concentrations of the heavy metals in the draining and leaching reference samples were not changed significantly (140400, 12900, 266, and 1020 mg/kg for zinc, lead, nickel, and cadmium), because the main phases of the heavy metals in the ZLR (ZnS, ZnFe₂O₄, PbSO₄, and CdS) were not water soluble.

Fig. 4 shows the BCFs of the heavy metals in *A. retroflexus* at the soil amendment stage. For the plants harvested from the pots containing drained ZLR (25 wt%), the BCFs of the metals increased in comparison with the plants harvested from the pots with 25 wt% ZLR + 75 wt% soil, i.e., no treatment. However, the results from the pots with the leached ZLR illustrated a slight increase comparing with the drained case and reached to 0.5870, 0.2552, 0.9474, and 2.0091 for zinc, lead, nickel, and cadmium, respectively. The increase in the case of using drained ZLR can be due to the Ec reduction and for the leached ZLR can be attributed to both the Ec reduction and the longer lifetime of the plants. Table 7 shows the concentration of the heavy metals in this stage.

### Table 5

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean dry weight of plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1.18 ± 0.09 D**</td>
</tr>
<tr>
<td>75</td>
<td>1.34 ± 0.09 C**</td>
</tr>
<tr>
<td>50</td>
<td>1.65 ± 0.12 B**</td>
</tr>
<tr>
<td>25</td>
<td>1.85 ± 0.12 A**</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leached ZLR</td>
<td>2.08 ± 0.02 A</td>
</tr>
<tr>
<td>Drained ZLR</td>
<td>1.97 ± 0.03 B</td>
</tr>
</tbody>
</table>

### Table 7

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low level of citric acid</td>
<td>3.49 ± 0.02 A*</td>
</tr>
<tr>
<td>High level of citric acid</td>
<td>3.36 ± 0.02 A*</td>
</tr>
</tbody>
</table>

Note: Mean values labeled with different letters are significantly different (α = 0.05) in each stage. (Non-star – p > 0.05), (* – p ≤ 0.05), (**) – p ≤ 0.01.

Fig. 3. The mean (M±SE) BCFs of Zn, Pb, Ni, and Cd in *A. retroflexus*, screening stage. Note: Mean values labeled with different letters are significantly different (α = 0.05) in each metal. (Non-star – p > 0.05), (* – p ≤ 0.05), (**) – p ≤ 0.01.
Table 6

Mean (M ± 2 × SE) concentration of heavy metals in dry weight of plant at screening stage.

<table>
<thead>
<tr>
<th>ZLR (wt.%)</th>
<th>Zn (mg/kg DW)</th>
<th>Pb (mg/kg DW)</th>
<th>Cd (mg/kg DW)</th>
<th>Ni (mg/kg DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>4022.67 ± 8.51 D**</td>
<td>442.67 ± 8.51 C**</td>
<td>159.67 ± 10.97 D**</td>
<td>30.33 ± 9.82 D</td>
</tr>
<tr>
<td>75</td>
<td>8620.67 ± 4.67 C**</td>
<td>459.33 ± 10.41 C**</td>
<td>242.33 ± 5.70 C**</td>
<td>35.33 ± 9.33 C</td>
</tr>
<tr>
<td>50</td>
<td>11219.00 ± 9.87 B**</td>
<td>498.67 ± 20.83 B**</td>
<td>300.33 ± 0.67 B**</td>
<td>42.33 ± 8.97 B</td>
</tr>
<tr>
<td>25</td>
<td>12219.67 ± 10.97 A**</td>
<td>579.67 ± 10.97 A**</td>
<td>442.67 ± 15.25 A**</td>
<td>48.33 ± 6.7 A</td>
</tr>
</tbody>
</table>

Note: Mean values labeled with different letters are significantly different (â = 0.05) in each metal. (Non-star – p > 0.05), (* – p ≤ 0.05), (** – p ≤ 0.01).

3.3.3. Effect of citric acid (Modification stage)

Adding citric acid as a chelating agent to the soil can form citrate complexes of heavy metals (Chen et al., 2003). These complexes can facilitate the transportation of heavy metals to aboveground plant tissues (Mihalík et al., 2010; Muhammad et al., 2009; Shakoor et al., 2014). In addition, citric acid can decrease the pH of the soil and as a result of this, heavy metal uptake and mobilization are increased (Chen et al., 2003). Also, the other mechanisms such as cation exchange can enhance heavy metal accumulation (Quartacci et al., 2005).

Fig. 5 illustrates the mean BCFs of zinc, lead, nickel, and cadmium in the A. retroflexus plants harvested from the pots that the low level (0.005 M) and high level (0.1 M) of citric acid were added to the soil. Adding low level of citric acid to the optimum conditions of two previous stages (25 wt% of the leached ZLR + 75 wt% of the soil), the BCFs of A. retroflexus for zinc, lead, nickel, and cadmium were obtained as 0.6838, 0.3042, 1.1027, and 2.3621, respectively. This result ascertained that citric acid is a practical chelating agent to increase the heavy metals bioavailability and the metal accumulation in the aerial parts of A. retroflexus (Sinchal et al., 2010). With higher BCFs for nickel and cadmium, in comparison with zinc and lead, can be attributed to the natural trait of A. retroflexus that is a hyper-accumulator for cadmium and a high tolerance plant for nickel (Osmolovskaya et al., 2018; Pietrini et al., 2015).

Using a high level of citric acid (0.1 M) illustrates a very low metals uptake by the plant. Based on literature, this observation is attributed to the soil compaction and the degradability of citric acid (Afshan et al., 2015; Sinhal et al., 2010), but we believe that it is necessary to concentrate deeper on the mechanism of phyto-extraction. The mechanism consists of the following stages: mobilization of heavy metals in soil, uptake of metal ions by plant roots, translocation of the accumulated metals from roots to aerial plant tissues, and sequestration of the metal ions in plant as well as metal tolerance (Ali et al., 2013). It is suggested that each plant intrinsically behaves similar to a resin, and it should be mentioned that metal separation by resins consists of two steps: sorption and elution. In the sorption step, pH is usually higher than the elution step (Jackson, 1986). The metal uptake step in the plant’s root can act similar to the sorption or elution step due to the pH variation. The specialized transporters (channel protein) or H+ coupled carrier proteins in plants that are available in the plasma membrane of the root uptake the heavy metal ions (Ali et al., 2013). In fact, the performance of these channel proteins is very similar to the functional groups in resins. There is a family of different proteins that are able to uptake various oxidation states of metals with different radii, i.e., for divalent, trivalent, and etc. Similar to resins, phyto-extraction suffers from lack of selectivity (Jackson, 1986). To overcome this shortage, organic acids are suggested as chelating agents for the heavy metal ions to increase selectivity (Ali et al., 2013). In this case, using low level of citric acid, the plant behaves like cationic resins in the sorption step. By increasing citric acid concentration, plants behave similar to the elution step as a result of pH reduction. Therefore, severe reduction in the BCFs observed in Fig. 5 can be related to the higher acid concentration that results in elution of the metal ions from the plant. In conclusion, the low level of citric acid (0.005 M) is an effective dose to enhance the accumulation of zinc, lead, nickel, and cadmium in the biomass of A. retroflexus and improve the plant growth. Hence, a citric acid induced phyto-extraction by A. retroflexus from ZLR can be an effective alternative to the other methods, which have the following advantages: it can be applied in situ, it has not any hazardous solid or liquid residue, it can provide a green landscape and
rehabilitate the contaminated lands, as well as it can recover valuable elements and remove contaminants. Table 8 shows the heavy metal concentrations in the dried shoots of plants in this stage.

3.4. Metal recovery

The efficiency of extraction is an important parameter in extractive metallurgy. Therefore, phyto metal extraction index (PME) is suggested that can be used to calculate metal recovery:

$$PME = \frac{\text{Metal concentration in dried shoot} \times (\frac{\text{MS}}{\text{WS}})}{\text{Metal concentration in ZLR} \times (\frac{\text{MS}}{\text{WS}})} \times 100 \quad (3)$$

Table 9 illustrates the PME for zinc, lead, nickel, and cadmium at the optimum conditions of the three abovementioned stages. As can be seen in Table 9, at the modification (last stage), the recoveries of heavy metals increased and reached to 17.09%, 7.60%, 27.57%, and 59.05% for zinc, lead, nickel, and cadmium, respectively. The separation factor (SF) is used to determine the selectivity of metal extraction. This factor is a measure of the ability of a plant to separate two metals. The SF is the ratio of the BCFs for two metals:

$$SF = \frac{BCF_A}{BCF_B} \quad (4)$$

where A and B are the two different metal ions uptaken by a plant. If the SF is greater than unity, it means that the tendency of the plant to extract A is greater than that of B. The results showed that if the SFs are calculated based on the BCF of lead—as the fraction denominator—the SF values are obtained 7.83, 3.6, 2.27, and 1 for cadmium, nickel, zinc, and lead, respectively, which shows the tendency of A. retroflexus for heavy metal extraction is in ascending order for lead, zinc, nickel, and cadmium, respectively.

4. Conclusions

In the present research, phytoextraction by Amaranthus retroflexus was used to extract zinc, lead, nickel, and cadmium from ZLR. The experiments were performed at three stages of screening, soil amendment, and modification to optimize the plant lifetime, biomass, and heavy metal BCFs. The results showed that:

- At the screening stage, in 25 wt% of ZLR +75 wt% of the soil, A. retroflexus survived for a longer time (4 weeks) and had a higher heavy metal BCFs (0.3452, 0.1797, 0.7268, and 1.7360 for zinc, lead, nickel, and cadmium, respectively).

- At the soil amendment stage, in order to further reduce salinity of the ZLR, two separate operations of draining and leaching of the ZLR were performed. The plants that were cultivated from the pots containing the leached ZLR (25 wt% leached ZLR +75 wt% the soil) lived longer (5 weeks) with more biomass and heavy metal uptake. The BCFs were 0.5870, 0.2552, 0.9474, and 2.0091 for zinc, lead, nickel, and cadmium, respectively.

- At the modification stage, two levels of citric acid (0.005 M and 0.1 M) were used as chelating agent. After 5 weeks the plants were harvested. By adding 0.005 M of citric acid in 25 wt% leached ZLR +75 wt% soil, heavy metal BCFs were reached to the highest amount among all the experimental pots. Zinc, lead, nickel, and cadmium BCFs were reached to 0.6838, 0.3042, 1.027, and 2.3621, respectively. Also, the plants had a greater biomass in comparison to the other experiments.

- It is suggested that each plant intrinsically acts similar to a cationic resin. Therefore, in lower level of citric acid (higher pH), the plants behaved as a resin in the sorption step and in higher level of citric acid (lower pH), the plants behaved as a resin in the elution step.

- Phyto metal extraction index (PME) was suggested as a parameter for indicating the efficiency of extraction. At the optimum condition (25 wt% leached ZLR +75 wt% soil), PME was 17.09%, 7.60%, 27.57%, and 59.05% for zinc, lead, nickel, and cadmium, respectively.

- The separation factor (SF) was calculated to determine the A. retroflexus’s tendency to extract heavy metals. The plant tendency to uptake the heavy metals is in ascending order for lead, zinc, nickel, and cadmium, respectively.

In conclusion, with all this taken into account, zinc, lead, nickel, and cadmium can be extracted from ZLR in zinc production plants using A. retroflexus. This is a safer and cleaner method in comparison with the common pyrometallurgical and hydrometallurgical methods since metal species will be removed and recovered without the use of any hazardous chemicals and with a lower energy consumption. Moreover, hazardous solid and liquid residues are not produced in this newly proposed process.

<table>
<thead>
<tr>
<th>Table 7</th>
<th>Mean metal concentrations in the dried shoot, leached, and drained.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals</td>
<td>Zn (mg/kg DW)</td>
</tr>
<tr>
<td>Leached</td>
<td>20602.33 ± 20.34 A**</td>
</tr>
<tr>
<td>Drained</td>
<td>18562.67 ± 16.38 B**</td>
</tr>
</tbody>
</table>

| Note: Mean values labeled with different letters are significantly different (α = 0.05) in each metal. (Non-star – p > 0.05), (* – p ≤ 0.05), (** – p ≤ 0.01). |

<table>
<thead>
<tr>
<th>Table 8</th>
<th>The mean heavy metal concentrations in the dried shoot, low and high levels of CA.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals</td>
<td>Zn (mg/kg DW)</td>
</tr>
<tr>
<td>Low level of CA</td>
<td>24000.00 ± 23.05 A**</td>
</tr>
<tr>
<td>High level of CA</td>
<td>963.00 ± 8.08 B**</td>
</tr>
</tbody>
</table>

| Note: Mean values labeled with different letters are significantly different (α = 0.05) in each metal. (Non-star – p > 0.05), (* – p ≤ 0.05), (** – p ≤ 0.01). |

Table 9 | PME of heavy metals at the optimum conditions of the three stages. |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Stage</td>
<td>Zn (%)</td>
</tr>
<tr>
<td>Screening</td>
<td>8.63</td>
</tr>
<tr>
<td>Soil amendment</td>
<td>14.67</td>
</tr>
<tr>
<td>Modification</td>
<td>17.09</td>
</tr>
</tbody>
</table>

Note: Mean values labeled with different letters are significantly different (α = 0.05) in each metal. (Non-star – p > 0.05), (* – p ≤ 0.05), (** – p ≤ 0.01).
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Melina Roshanfar: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing - original draft, Visualization.

Misagh Khanlarian: Conceptualization, Methodology, Software, Formal analysis, Writing - review & editing, Project administration.

Feresthett Rashchi: Conceptualization, Validation, Resources, Data curation, Writing - review & editing, Supervision, Funding acquisition.

Babak Motesharezadeh: Methodology, Validation, Resources, Data curation.

Glossary

Saturation capacity The maximum amount of water that soil can absorb.

Water-holding capacity The amount of water that soil can maintain for plant use.

Ultrastructural The architecture of a plant’s cells that is visible at higher magnifications than found on a standard optical light microscope.

Phytochelatin The most important peptides/proteins involved in metal accumulation and tolerance.

References


