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# Screening for the next generation heavy metal hyperaccumulators for dryland decontamination

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ABSTRACT

Heavy metal removal by plants bears a great potential to decontaminate soils. A major challenge remains to find plant species that accumulate heavy metal, harbor a sufficient biomass and grow in the desired environmental conditions. Here we present candidate plants for phytoremediation in arid climates. We sampled sixteen dominant plants from mining area naturally polluted with high Pb-Zn and Cd concentration. Plants were assessed for their ability to accumulate Zn, Pb and Cd and six species were selected on the base of their heavy metal concentration in shoots and leaves, enrichment coefficient and translocation factor. Out of all the tested species in field study, *Alcea aucheri* was the most promising one which accumulated over than 460 and 4089 μg/g Pb in the roots and shoots, respectively. We confirmed this ability with a greenhouse experiment on soil spiked with different Pb and Cd concentrations. Concentration of Pb and Cd in aerial parts of *A. aucheri* were more than 1700 and 345 μg/g in 2400 and 200 mg/kg Pb and Cd soil treatment respectively. We propose that *A. aucheri* as model hyperaccumulator able to live in adverse condition, producing high biomass, and supersede heavy metal accumulation reported to other plants, making of this species one of the best Pb hyperaccumulator reported to date.


## 1. Introduction

Contamination of air, water and soil with heavy metals is a major environmental concern in many parts of the world. In absence of better alternatives, polluted zones are often treated by common method such as removal and burial of the contaminated soil. These methods are still expensive and inefficient, making them hard to apply on large area [1]. Phytoremediation, the removal of pollutants by plants, has often been proposed as an alternative and more practicable and environmental friendly strategy to restore soils. However, attempts to find new promising remediation methods have to date shown unsatisfactory results. Its low costs and disturbance coupled to its applications to a wide range of pollutants, makes phytoremediation very attractive [1]. However, a couple of challenges must still be addressed if we are to use large scale phytoremediation strategies. The interest of phytoremediation depends on the ability to extract high amounts of heavy metals from soil by plants. This requires both a high uptake of heavy metals per unit of plant weight and a high aboveground biomass of candidate plants [2,3], which is limited especially in case of Pb, Cu and Cr [2].

So far, more than 400 hyperaccumulator species are reported [2], with new species added every year. A promising strategy to discover new species is to sample the vegetation of heavily polluted zones, such as those around mines [4,5]. Tolerant or accumulating may be at an advantage so that dominant species will likely cope well with heavy metals [6]. Discovery of new plants is particularly needed for dry areas, where plants must cope with climatic extremes in addition to the heavy metal stress.

Here we sampled contaminated area in Iran to discover new hyper-accumulating plants. Iran is climatically located on Afro-Asian desert belt and consequently faced with high evapotranspiration and low precipitation. Screening semi-desertic area in order to find wild metallophytes for phytoextraction of contaminated

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drylands is a promising way to discover species suitable for large scale phytoremediation in difficult field conditions [7].

We sampled plants growing in a mine- contaminated area in Iran. We identified the dominant plant species and assessed their ability to take up Pb, Cd and Zn in their aerial parts. We report the results of six of the most promising plants with a special emphasis on *Alcea aucheri*, a new species that appears to accumulate heavy metals far beyond any other reported dryland plants and is especially efficient to remove non-mobile elements such as Pb in calcareous soils. We then validated heavy metal uptake of *A. aucheri* in controlled conditions in a separate greenhouse experiment.

## 2. Method and materials

### 2.1. Sampling site description

We sampled plants in an area enriched in Pb, Zn and Cd close to an active Pb and Zn mine in the Fars province, Iran. This area is located on the Pb-Zn ore deposit of the Zagros folded belt between UTM coordinate of 640300 and 3158136 with the 603 m attitude and 640426 and 3158230 with the 982 m attitude. Lead and zinc in the form of carbonate and sulfate are the main ores. Total area of site was about 80 ha with an average annual temperature of 24 °C and annual rainfall of 210 mm.

### 2.2. Soil and plant sampling

Plant samples and related rhizosphere were collected from January to March 2014. We selected two different location, a 20 ha and a 60 ha area, near two main ores with Pb concentration more than 500 mg/kg. Plant species and their abundance were surveyed by systematic sampling method. The dominant plants were recorded using 2 (10 m × 10 m) plots per hectare and identified. We assessed the six most abundant plants in relation to their ability to take up heavy metals uptake in roots and shoots. We then compared the heavy metal concentration to the surrounding soils to retrieve the translocation factor (transfer of heavy metals from roots to shoots), the enrichment coefficient (heavy metal concentration in plant relative to the surrounding concentration in soil).

### 2.3. Plant and soil analysis

Heavy metal content was determined separately in shoots and roots using standard procedures [8] with a few modifications. Briefly, plants were washed with deionized water and surface-adsorbed heavy metals were removed by immersing roots in 20 mM $Na_2$–EDTA for 15 min. Plant samples were dried at 60 °C, ground and sieved at 2 mm. Concentration of Pb, Zn and Cd were measured by atomic absorption spectrophotometer (AA-670 Shimadzu, Japan) after dry digestion [8]. The total concentration of Zn, Pb and Cd in soil were determined by standard method [9].

### 2.4. Enrichment coefficient and translocation factor

Enrichment coefficient, the capability of plants for adsorbing heavy metals from soil and accumulate in their roots was defined for each single heavy metal as follows:

Enrichment coefficient = Heavy metal concentration in plant above ground/Total heavy metal concentration in soil

The translocation factor, the ability of plants to translocate heavy metals from roots to shoot was defined as follows

Translocation Factor = Heavy metal concentration in shoots/heavy metal concentration in roots

### 2.5. Greenhouse experiment

The ability of *A. aucheri* to take up Pb and Cd was validated in greenhouse experiment with five levels of soil heavy metal concentration (0, 300, 600, 1200 and 2400 mg $kg^{-1}$ Pb or 0, 50, 100,

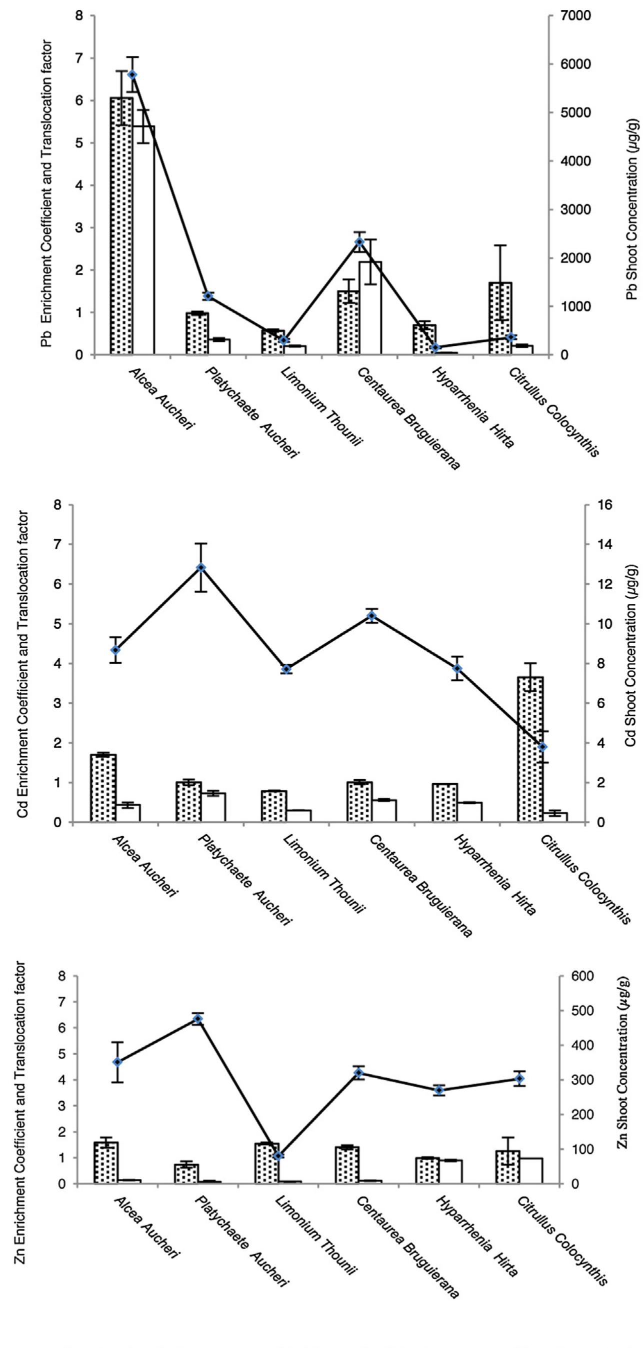


**Fig. 1.** Pb, Zn and Cd translocation factors, enrichment coefficient and shoot concentration in different plant in field survey. Line show Pb, Zn and Cd shoot concentration in field study. E.C; Enrichment Coefficient. T.F; Translocation Factor.

150 and 200 mg $kg^{-1}$ Cd, respectively). Metal level was replicated with or without EDTA (0.5 g $kg^{-1}$ soil), a chelating agent widely use to increase the bioavailability of micro nutrient and heavy metals in phytoremediation of Pb and Cd [10]. Two separate experiments were performed for Pb and Cd. Three replicates were set up per treatment. Plants were grown for 48 days in pots filled with 1 kg fine, mixed, mesic, fluventic calcixerepts soil at 28 ± 2 °C. Lead (Pb) and cadmium (Cd) were added in the form of $Pb(NO_3)_2$ and $Cd(NO_3)_2$. The amount of extra nitrate from metal treatment was calculated and added to control to avoid side effects of nitrate on treatment effects. After 48 days, plants were harvested. We measured shoot and root dry weight as well as Pb and Cd concentration in shoots, roots and soil.

### 2.6. Statistical analysis

#### 2.6.1. Field sampling

The effect of plant species on heavy metal uptake, translocation factors and enrichment coefficient was evaluated using separate way ANOVA tests. Individual means were compare by Duncan's multiple range test. Cluster analysis was applied to identify different plants group, based on similarity in shoot concentration, translocation factor and enrichment coefficient. This analysis was undertaken by Ward-algorithmic method.

#### 2.6.2. Greenhouse experiment

The interactive effects of soil heavy metal concentration (five levels) and EDTA (two levels) in the green house experiment was assessed with two way ANOVA tests. Separate analyses were performed for Pb and Cd.

## 3. Results and discussion

### 3.1. Dominant plant species in study area

In two different areas with average Pb concentration higher than 500 mg/kg, 16 different plant species were identified and recorded by systematic sampling method (Supplementary materials). Six dominant plants were selected and compared based on shoot concentration, translocation factors and enrichment coefficient.

### 3.2. Similarity of plants based on shoot concentration, translocation factor and enrichment coefficient

We selected six more frequent plant species in high Zn and Pb contaminated soils and classified them in two different clusters based on multivariate analysis of Zn, Pb and Cd Shoot Concentration, translocation Factors, Enrichment Coefficient, meaning plant in each cluster should have similar uptake, distribution characteristics in general for these three metals.

We observed two important clusters. The most promising one from a phytoremediation perspective comprised *Alcea Aucheri* and *Centaurea Bruguierana,* two plants showing a similarity level in their heavy metal accumulation patterns of more than 83%. This similarity was due to the high Zn, Pb and Cd shoot concentration, translocation factor, and enrichment coefficient in this cluster which makes this as a suitable candidate for phytoextraction. This cluster has this ability to uptake large amounts of metals with roots and translocates them efficiently to aerial part without limitation.

*Limonium Thounii*, *Citrullus Colocynthis*, *Hyparrhenia Hirta* and *Platychaete Aucheri* are belong to second cluster with similarity level more than 93% and difference with clusters 1 in terms of enrichment coefficient ($F_{1,69}$ = 15.7, $p < 0.001$) indicating significant difference between cluster one (M = 1.5, SE = 0.24) and cluster two (M = 0.3, SE = 0.17), translocation factors ($F_{1,70}$ = 7.1, $p < 0.001$) indicating significant difference between cluster one (M = 2.2, SE = 0.28) and cluster two (M = 1.2, SE = 0.20) by Duncan's Multiple Range Test. Shoot concentration in first cluster was 522.66 μg/g (SE = 98.71) compare to 335.43 μg/g (SE = 69.79) without statistical difference between two plants ($F_{1,70}$ = 2.4, $p$ = 0.13). Plants in cluster 2 mainly retrieved from soil with high level of Pb, Zn and Cd, and accumulate different and high concentration of heavy metals in roots (comparable with first cluster), but constant and relatively lower concentration in shoots, suggesting that exclusion was main

**Table 1**
Effects on soil Pb and Cd concentration on translocation factors, enrichment coefficient, and metal concentration in shoot and root in *Alcea aucheri* in the greenhouse experiment.

| | | Applied metal (mg/kg) | Shoot Concentration (μg/g) | Root Concentration (μg/g) | Soil Concentration (mg/kg)* | T.F | E.C |
|---|---|---|---|---|---|---|---|
| Pb | No-EDTA | 0 | 5.00 ± 3.3e | 7.53 ± 2.7c | 27.67 ± 8.4d | 0.65 ± 0.25b | 0.18 ± 0.09b |
| | | 300 | 43.61 ± 5.0d | 34.80 ± 3.5c | 143.50 ± 20.9 cd | 1.27 ± 0.23ab | 0.31 ± 0.07a |
| | | 600 | 94.22 ± 2.8c | 67.18 ± 6.7bc | 351.40 ± 23.9c | 1.41 ± 0.17ab | 0.27 ± 0.02ab |
| | | 1200 | 221.14 ± 21.4b | 123.55 ± 34.1b | 793.77 ± 67.4b | 1.93 ± 0.76a | 0.28 ± 0.02ab |
| | | 2400 | 375.31 ± 14.4a | 208.21 ± 61.4a | 1137.13 ± 267.9a | 1.89 ± 0.76a | 0.34 ± 0.10a |
| | EDTA | 0 | 4.392 ± 1.2d | 22.38 ± 8.9e | 23.66 ± 6.6e | 0.21 ± 0.06c | 0.20 ± 0.08c |
| | | 300 | 176.78 ± 10.9c | 75.77 ± 22.1d | 238.80 ± 43.9d | 2.48 ± 0.74a | 0.76 ± 0.13ab |
| | | 600 | 239.37 ± 4.0c | 296.27 ± 4.6c | 344.75 ± 31.9c | 0.81 ± 0.03bc | 0.70 ± 0.06b |
| | | 1200 | 579.65 ± 54.6b | 469.80 ± 13.8b | 881.90 ± 105.3b | 1.23 ± 0.12b | 0.66 ± 0.02b |
| | | 2400 | 1785.94 ± 153.6a | 1234.82 ± 24.3a | 2002.50 ± 45.4a | 1.45 ± 0.11b | 0.89 ± 0.08a |
| Cd | No-EDTA | 0 | 0.34 ± 0.1c | 0.20 ± 0.0e | 0.28 ± 0.02e | 1.73 ± 0.11a | 1.21 ± 0.10a |
| | | 25 | 27.58 ± 2.1b | 43.05 ± 4.0d | 17.98 ± 6.7d | 0.64 ± 0.01b | 1.68 ± 0.60a |
| | | 50 | 33.96 ± 10.1b | 218.06 ± 5.9c | 30.51 ± 7.6c | 0.18 ± 0.04c | 1.38 ± 0.68a |
| | | 100 | 38.50 ± 8.8ab | 336.87 ± 12.2b | 87.29 ± 2.0b | 0.10 ± 0.03c | 0.39 ± 0.09b |
| | | 150 | 46.19 ± 4.1a | 463.97 ± 7.2a | 164.61 ± 8.2a | 0.10 ± 0.01c | 0.28 ± 0.02b |
| | EDTA | 0 | 0.56 ± 0.1d | 0.28 ± 0.1e | 0.60 ± 0.1e | 2.10 ± 0.42a | 0.94 ± 0.16b |
| | | 25 | 41.10 ± 3.8 cd | 153.51 ± 25.4d | 45.81 ± 7.6d | 0.28 ± 0.07b | 0.91 ± 0.08b |
| | | 50 | 67.11 ± 2.5c | 322.95 ± 1.1c | 75.24 ± 6.2c | 0.21 ± 0.01b | 0.89 ± 0.04b |
| | | 100 | 132.25 ± 27.6b | 443.95 ± 34.4b | 144.84 ± 4.1b | 0.29 ± 0.08b | 0.91 ± 0.33b |
| | | 150 | 329.91 ± 15.6a | 612.66 ± 52.6a | 192.53 ± 4.9a | 0.54 ± 0.03b | 1.71 ± 0.07a |

a, b means sharing the same superscript are not significantly different from each other.
* Concentration of metal at the end of experiment.

survival mechanism of this cluster. Although this cluster may not be suitable for phytoextraction, it may prove interesting for other applications such as phytoremediation based on stabilization [5,11]. *Alcea aucheri* appears as the most suitable plant species for heavy metal phytoextraction due to high concentration of metals in roots and ability to translocate it to aerial parts compared to other evaluated species.

### 3.3. Criterion for hyperaccumulator plants

We classified plants as hyperaccumulator when they 1) accumulated Cd, Pb and Zn in the plant aerial parts more than 100, 1000 and 10000 mg/kg, respectively, 2) show an enrichment coefficient and translocation factors above 1, demonstrating an active heavy metal transfer from roots to aerial parts [5,6].

Mean concentrations of Zn, Pb and Cd in usual plants are 100, 5 and 1 mg/kg, respectively [5]. All studied plants (except *L. Thounii* for Zn) accumulated heavy metals more than usual plants. Concentration of Pb in all six plants was 30–1100 times more than non-accumulator plants. Among six evaluated plants, *A. aucheri* and *C. bruguierana* meet the criteria to be considered as Pb hyperaccumulator. They accumulate more than 5782.67 μg/gr and 2329.95 μg/gr Pb in aerial parts, respectively meaning 1156 and 93 times higher than non-accumulator plants. A typical Cd and Zn hyperaccumulator plants should has this ability to accumulate more than 100 and 10000 μg/g in the aerial parts. Although, based on field data, none of the studied plants could accumulate these

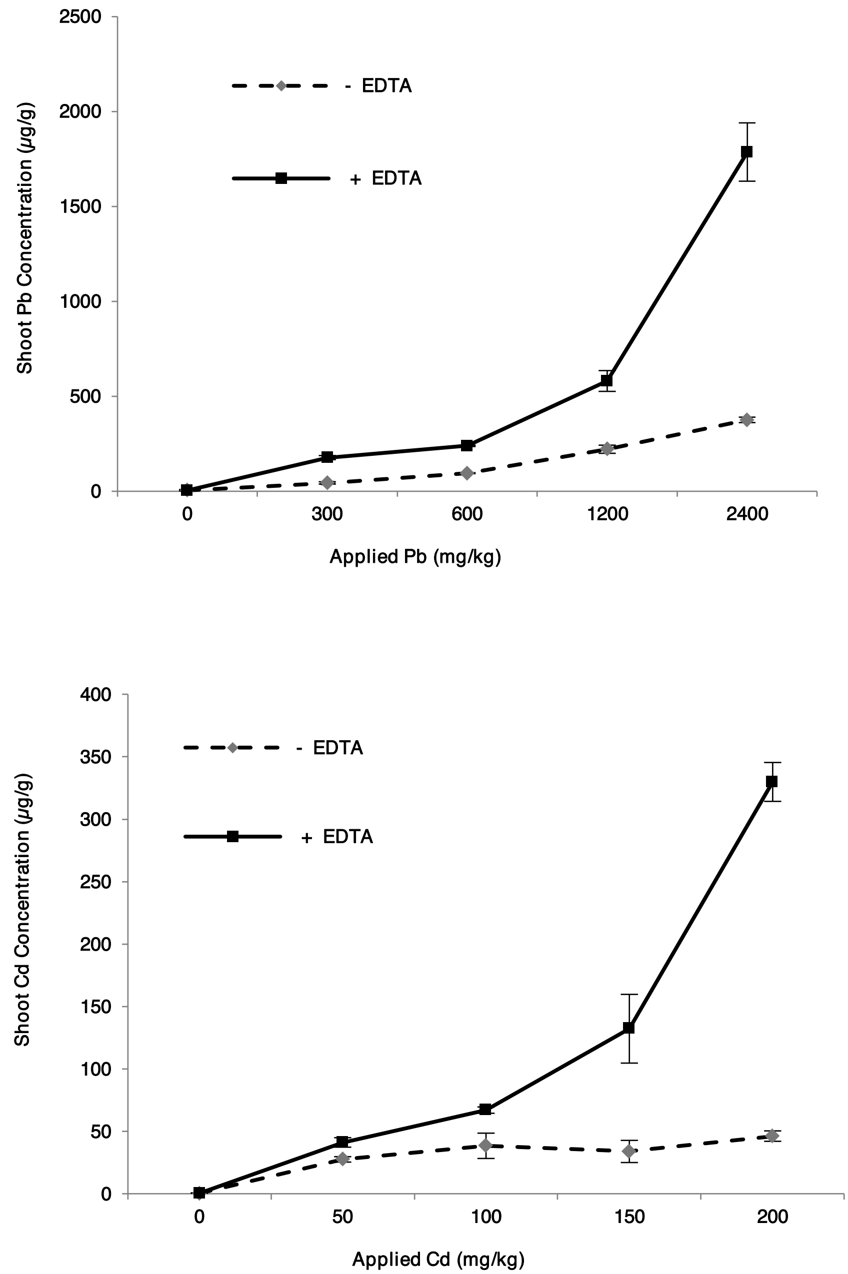


**Fig. 2.** Pb and Cd shoot concentration (±1SE) of *Alcea aucheri* after 48 days in greenhouse experiment at presence/absence of EDTA. Soils were spiked with different quantity of Pb (0, 300, 600, 1200 and 2400 mg/kg) and Cd (0, 50, 100, 150, and 200 mg/kg).

amounts of Zn and Cd (Fig. 1), we show that *A. aucheri* show this accumulation at greenhouse experiment and has this potential to evaluate as Cd accumulator.

Plants with translocation factor and enrichment coefficient more than one are suitable for phytoextraction process [12]. Lead translocation factor and enrichment coefficient in this two plants were 6.06, 1.50 and 5.38 and 2.19 respectively (Fig. 1). As *A. aucheri* shows a 30 times higher biomass than *C. bruguierana*, we selected it as main candidate for greenhouse experiment.

### *3.4. Dose-dependent Pb and Cd accumulation in* A. aucheri *under greenhouse conditions*

The concentrations of Pb in the shoot of *A. aucheri* increased significantly ($F_{4,20}$ = 408.3, $p < 0.001$) with increasing Pb concentration is soil with two ways ANOVA test from 4.69 μg/g in control to 1080.62 μg/g at higher applied Pb (2400 mg/kg) in the soil after 48 day exposure (Table 1).

These concentrations also increased significantly ($F_{1,20}$ = 458.2; $p < 0.001$) with application of EDTA from 147.85 to 557.23 μg/g (Fig. 2). The interaction effects of Pb level and EDTA application on Pb concentration in shoot was also significant ($F_{4,20}$ = 180.3, $p < 0.001$). There were no visual symptoms of toxicity in this plant even at highest level of Pb.

Cadmium concentrations in the shoot of *A. aucheri* increased significantly ($F_{4,20}$ = 112.5, $p < 0.001$) with increasing applied Cd in soil with two ways ANOVA test from 0.453 μg/g in control to 188.04 μg/g in higher applied Cd concentration (200 mg/kg) in the soil after 48 day exposure (Table 1). These concentrations also increased significantly ($p < 0.001$) in soil treated with EDTA from 29.31 to 114.18 μg/g ($F_{4,20}$ = 197.7, $p < 0.001$). The interaction effects of Cd level and EDTA application on Cd concentration in shoot was also significant ($F_{4,20}$ = 75.7, $p < 0.001$).

Concentration of Pb and Cd in shoot of hyperaccumolator plants correlated significantly with soil Pb and Cd, in line with previous study [13]. These uptake patterns confirm the field observation data and show that the *A. aucheri* could be a potential Pb-hyperaccumulator for phytoremediation process. The enrichment coefficient was less than 1 at greenhouse condition. Low availability of Pb in greenhouse calcareous soil may be possible explanation for low enrichment coefficient and translocation factor in greenhouse data. Enrichment coefficient increased significantly from 0.28 to 0.64 ($F_{1,28}$ = 11.0, $p < 0.001$) when EDTA applied to soil as chelator of Pb treatment. This index increased with increase soil Pb concentration. This pattern also reported by others [14]. The shoot concentration, enrichment coefficient and translocation factor of *A. aucheri* were increased with the increasing level of Pb in the soil indicating its ability to Pb adsorption and translocation in elevating Pb level in the soil. *A. aucheri* can accumulate more than 329 μg/g Cd in aerial parts in 48 days. It has noticeable enrichment coefficient, but relatively lower translocation factor compare to Pb.

Dry weight of *A. aucheri* was not affected by applied Pb ($F4,20$ = 1.1, $p$ = 0.39) or Cd concentration ($F4,20$ = 1.9, $p$ = 0.20), confirming the tolerance of this plant heavy metal contamination [15]. We did not observed visual symptoms of toxicity in any of the treatments. The mean dry weight of *A. Aucheri* was 5.3 g and 5.2 g at highest level of applied Pb and Cd after 48 days greenhouse experiment. The average amount of Pb and Cd concentration in aerial parts were 1081 μg/g and 196 μg/g in 2400 mg/kg Pb and 200 mg/kg Cd treatments. Considering a practicable sown density of 36 plants per square meter, we estimate that *A. aucheri* can reach a yield of 1.98 ton/ha after 48 days and remove 204.12 kg/ha Pb and 37.80 kg/ha Cd from soil in a 48 days period. Theoretically, *A. aucheri* would have the potential to remediate a 1000 mg/kg Pb contaminated area in 7 cultivation cycle, assuming 1400 tons surface soil per hectare. Total Cd uptake per hectare per year by *A. aucheri* is relatively higher than potential Cd uptake by the reference heavy metal accumulator *Thlaspi caerulescens* [16,17].

Application of EDTA to soil Pb and Cd further increased Pb and Cd shoot concentrations in *A. aucheri* for Pb ($F_{1,20}$ = 458.2, $p < 0.001$) and Cd ($F_{1,20}$ = 197.7, $p < 0.001$) respectively (Fig. 2). This increase is in line with previous reports showing that EDTA can help increase uptake of Pb and Cd [10,18,19].

### *3.5. Heavy metal distribution in* A. aucheri

In field conditions, concentration of Pb in *A. aucheri* exceeded 6800 μg/g in some old leaves, a value far above the root concentration of 400 μg/g in roots, a trend observed in hyperaccumulators. Concentration of metals in different organs of hyperaccumulator plants are in the order of: old leaves > young leaves > roots [20]. *A. aucheri* showed this trend for all three metals (Pb, Zn and Cd), and concentrations of Pb, Zn and Cd in its aerial parts were 5782.67 (SE = 824), 350.44 (SE = 93) and 8.67 (SE = 1.5) μg/g compared to 971.53 (SE = 210), 320.06 (SE = 54) and 5.085 (SE = 1.5) μg/g in their roots, respectively. Among these elements, Pb and Cd is regarded as none or slightly mobile element in plants [21], meaning that Pb level should decrease from root to shoot, leaves, fruit and seeds [22]. Low root to shoot translocation of Pb in plants has also been reported by others [18,21,22]. The high Pb translocation factor (above 8) is remarkable in *A. aucheri* and suggests that *A. aucheri* harbours a specialized mechanism for absorption, accumulation and translocation of Pb. This high transfer of non-mobile elements in aerial parts is a huge advantage for phytoremediation as it allows an easy harvesting of the biomass. This is clearly apparent in comparison with other candidate plants proposed for phytoremediation such as Indian mustard (*Brassica juncea*), which hyperaccumulates Pb but keeps it to more than 95%, complicating the extraction process [26].

High metal concentration in aerial parts and high biomass are two key factors for using plants in phytoremediation strategies [20] and reduce the contaminant metals to an acceptable level [21]. Even promising accumulators such as *Thlaspi rotundifolium* may not deliver sufficient results in field conditions due to their low biomass and growth rates [23]. *A. aucheri* had higher biomass compared to the other Hyperaccumulators assessed in this study and is adapted to dry conditions, allowing its use to decontaminate semi-desertic area.

We observed no visual symptom of chlorosis, necrosis and spot in the plants exposed to Pb and Cd even at highest level of heavy metal and also EDTA treatment. Furthermore the chlorophyll content in leaves did not show any significant difference at these levels of metal application (data not shown) confirming the ability of *A. aucheri* to accumulate Pb and Cd from highly contaminated soils without suffering from acute toxicity effects.

## 4. Conclusion

Wild plant species growing on heavy metal enriched soils bear a great potential for phytoremediation strategies. We propose *A. aucheri* as a candidate plant for large scale decontamination of heavy-metal contaminated drylands. It is especially efficient at taking up non-mobile elements such as Pb and to a lesser extent Cd. Its ability to remove high amounts of metals from the soil and translocating it to high concentrations in the aerial parts, coupled with a high biomass, allows removing high quantities of soil heavy metals in fewer growth cycles than alternative species. It is proposed that the loaded biomass could be incinerated under controlled conditions for retrieving energy from the plants and the ashes where the heavy metals would be concentrated could be extracted if economically viable or would undergo engineered landfill in accordance with the environmental regulations.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jece.2016.04.013.

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