



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



Mohammadhossein Ravanbakhsh<sup>a,c,\*</sup>, Abdol-Majid Ronaghi<sup>a</sup>, Seyed Mohsen Taghavi<sup>b</sup>, Alexandre Jousset<sup>c</sup>

<sup>a</sup> Department of Soil Science, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

<sup>b</sup> Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

<sup>c</sup> Utrecht University, Ecology and biodiversity, Padualaan 8, 3584CH Utrecht, The Netherlands

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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  $\mu\text{g/g}$  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  $\mu\text{g/g}$  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.

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## 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

\* Corresponding author at: Department of Soil Science, College of Agriculture, Shiraz University, Shiraz 71441-65186, Iran.

E-mail addresses: [m.ravanbakhsh@uu.nl](mailto:m.ravanbakhsh@uu.nl) (M. Ravanbakhsh), [A.L.C.Jousset@uu.nl](mailto:A.L.C.Jousset@uu.nl) (A. Jousset).

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 altitude and 640426 and 3158230 with the 982 m altitude. 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<sub>2</sub>-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<sup>-1</sup> Pb or 0, 50, 100,

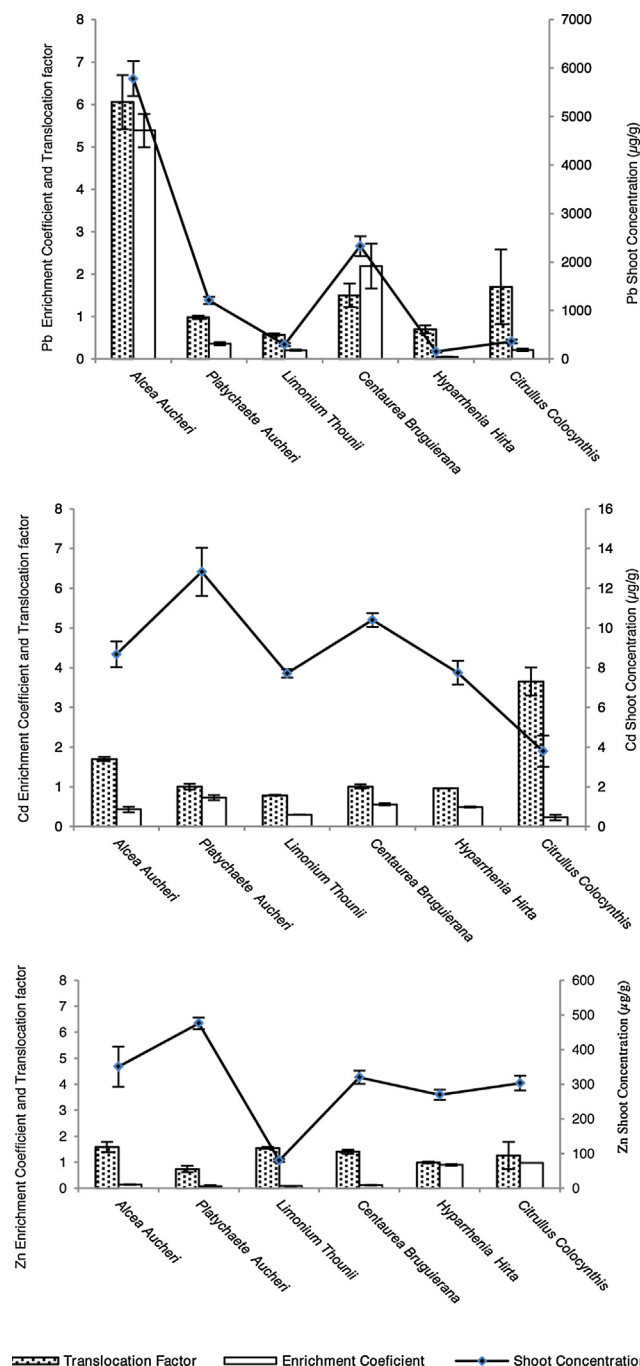


Fig. 1. Pb, Zn and Cd translocation factors, enrichment coefficient and shoot concentration in different plant in field survey. Line show Pb, Zn and Cd shoot concentration in field study. E.C; Enrichment Coefficient. T.F; Translocation Factor.

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