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# Phytoremediation of stable Cs from solutions by *Calendula alata*, *Amaranthus chlorostachys* and *Chenopodium album*

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ABSTRACT

Uptake rate of $^{133}$Cs, at three different concentrations of CsCl, by *Calendula alata*, *Amaranthus chlorostachys* and *Chenopodium album* plants grown outdoors was studied. These plants grow abundantly in semi-arid regions and their varieties exist in many parts of the world. When exposed to lowest Cs concentration 68 percent Cs was remediated by *Chenopodium album*. $^{133}$Cs accumulation in shoots of *Amaranthus chlorostachys* reached its highest value of 2146.2 mg kg$^{-1}$ at a $^{133}$Cs supply level of 3.95 mg l$^{-1}$ of feed solution. The highest concentration ratio value was 4.89 for *Amaranthus chlorostachys*, whereas for the other tests it ranged from 0.74 to 3.33. Furthermore uptake of $^{133}$Cs by all three species increased with increasing metal concentrations. The results also indicated that hydroponically grown *Calendula alata*, *Amaranthus chlorostachys* and *Chenopodium album* could be used as potential candidate plants for phytoremediation of solutions contaminated with Cs.



## 1. Introduction

There has been significant interest in the use of plants for remediation of environmental contamination (Meyers et al., 2008). In recent years several preliminary cost effective analyses launch that phytoremediation should confine a significant portion of the market for waste management. A lot of plant species have been identified as hyperaccumulators, i.e., they have the ability to accumulate high concentrations of metals, without impact on their growth and development (Xiong, 1997). Many studies have examined the ability of plants to remediate a variety of elements from diverse media. The achievement of phytoremediation depends on plant growth rate and obtaining high metal concentrations in plant shoots (Alloway et al., 1990; Tanhan et al., 2007). Plants uptake system is defined as the system that involves in ions uptake. The main components of the species-specific uptake system are transporters and channels (Maestri et al., 2010). Depending on the plants uptake system, and following organ distribution of elements, their content and distribution is significantly diversified (Verkleij et al., 2009). Moreover plant concentrations of metals may be influenced by a variety of conditions. Not only pH but also other ions concentrations and environmental conditions may interact with uptake of elements and sometimes change the growth rate of plants (Massas et al., 2010). There has been an enduring interest in selecting native plants that are tolerant to pollutants and many studies have evaluated the phytoremediation potential of native plants under field conditions (McGrath and Zhao, 2003). Experimental real life studies are necessary and may have to include a range of contaminant concentrations, mixtures of various contaminants, and different experimental treatments. Plant selection is based on growth rate, contaminant translocation, accumulation potential and tolerance to contaminants. Singh et al. (2009) have found that plants belonging to Chenopodiaceae, Amaranthaceae and Asteraceae families are effective remediators of $^{137}$Cs. Interest in Cs distribution in plants and the movement of this element in ecosystems extends back to the 1950s by the development of nuclear technologies used for energy production (Cook et al., 2007). The radioisotopes of cesium ($^{134}$Cs and $^{137}$Cs) may be of special concern because of their similar behavior to the necessary element "K" in plants, their solubility in aquatic ecosystems, the volatilization, release and dispersal in major reactor accidents, and the great quantity and persistence of $^{137}$Cs in spent fuel and reprocessed wastes (Pipíska et al., 2004; Pinder III et al., 2006). According to studies carried out by Tsukada et al. (2002) and Vinichuk et al. (2010) strong correlations exist between distribution of $^{137}$Cs and stable Cs in plants. Moreover Soudek et al. (2006) did not find any differences between the uptake of radioactive and stable Cs isotopes by *Helianthus annuus* L. Stable Cs is phytotoxic in solution

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culture exceeding 200 μM (Borghei et al., 2011). The main purpose of this work has been largely to evaluate the potential of *Chenopodium album*, *Amaranthus chlorostachys* and *Calendula alata* (Borghei et al., 2011) to phytoremediate stable cesium as an analog of radioactive Cs. Moreover metal concentration in shoots of plants was compared with those in roots. *Chenopodium album*, *Amaranthus chlorostachys* and *Calendula alata* are fast growing plants that grow on wide geographical locations in arid and semi-arid regions of the world including Iran. There is no report on the use of these plants for phytoremediation of stable Cs from solutions as well as nuclear waste.

## 2. Materials and methods

Three plant species (*Amaranthus chlorostachys var. Chlorostachys*, *Calendula alata* Rech. F., Fl. Iranica and *Chenopodium album*) were used in this study with the aims to evaluate their potential for phytoremediation of Cs solutions and their tolerance to Cs.

### 2.1. Plant material and hydroponics culture

Healthy seeds of *Amaranthus chlorostachys*, *Calendula alata* and *Chenopodium album* were surface sterilized by one percent sodium hypochlorite for 20 min. *Calendula alata* seeds were sown in a substrate containing perlite and vermiculite 3:1 (v/v) moistened with distilled water for four weeks until seedlings with two leaf pairs were established. *Amaranthus chlororstachys* and *Chenopodium album* seeds were germinated in sand. Then one-month-old plantlets were transplanted in plastic trays containing 10 L nutrient solutions. The composition of macro elements per 100 L solution was as follows: 100 ml $NH_4H_2PO_4$ (115 g $l^{-1}$); 600 ml $KNO_3$ (107 g $l^{-1}$); 400 ml Ca $(NO_3)_2 \cdot 4H_2O$ (236 g $l^{-1}$); 200 ml $MgSO_4 \cdot 7H_2O$ (246 g $l^{-1}$); 150 ml Fe-EDTA (5 g $l^{-1}$). The composition of micro elements (100 ml of solution all together) also was: $H_3BO_3$ (0.38 g $l^{-1}$); $ZnSO_4 \cdot 7H_2O$ (0.22 g $l^{-1}$); $MnSO_4 \cdot 4H_2O$ (1.02 g $l^{-1}$); $CuSO_4 \cdot 5H_2O$ (0.08 g $l^{-1}$); $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.02 g $l^{-1}$). Solution pH was adjusted to 5.5 through 5.8 with 0.1 M NaOH or 0.1 M $HNO_3$ and continuously aerated with a pump (Islam et al., 2007). Nutrient solutions renewed at every 10th day. Level of the solutions in trays was made up with nutrient solution when required. Each tray contained 24 plants. Plants were grown outdoors with temperature ranging from 31 °C through 40 °C (maximum daily temperature) and 17 °C through 28 °C (minimum daily temperature) with natural light during the experiment. After four weeks plantlets with uniform size were selected and transferred to 1 L flasks (Singh et al., 2009).

### 2.2. Experiments using hydroponically grown plants

#### 2.2.1. Remediation of Cs solutions contaminated with Cs

The roots of cultured plants were washed thoroughly with distilled water and plants were incubated with roots immersed in 1 L solution with three different Cs concentrations. The treatment samples included: (1) control sample free of Cs, samples 2, 3 and 4 containing 0.5, 2 and 5 mg $l^{-1}$ of CsCl, respectively. Consequently the concentration of $Cs^-$ ions in the solutions was 0.47, 1.58 and 3.95 mg$l^{-1}$. The experiment was arranged with each treatment in triplicate samples. The treatment group was exposed to CsCl solution for a period of 15 days in 1500 ml flasks (Singh et al., 2009). pH of the solution was adjusted to 5.5. The average root lengths were 300 mm (*Calendula alata*), 350 mm (*Amaranthus chlorostachys*) and 200 mm (*Chenopodium album*). Those for plants shoots were 350 mm (*Calendula alata*), 400 mm (*Amaranthus chlorostachys*) and 300 mm (*Chenopodium album*). *Calendula alata* and *Amaranthus chlorostachys* plants had a massive root system. Each flask contained three plants, which represented one replicate. Plants grown in water served as control samples. Distilled water was used for solution preparation and for make-up of lost water. After treatment period samples of solutions were drawn out from the solutions and analyzed for Cs concentrations. In all experiments Cs contents of solutions were determined using atomic absorption spectrophotometry (Variance Spectra AA-55B). The percentage metal uptake was calculated from

$$\%\ \text{uptake} = [(C_0 - C_1)/C_0] \times 100$$

where $C_0$ and $C_1$ are initial and remaining concentrations of metal, respectively, in solution (mg $l^{-1}$) (Abdel-Halim et al., 2003; Tanhan et al., 2007).

#### 2.2.2. Distribution of Cs in Calendula alata, Amaranthus chlorostachys and Chenopodium album

At the end of the experiment, plants were thoroughly washed with distilled water, separated into root and shoot and dried in an oven at 60 °C for 48 h. The dried samples were digested in $HNO_3$:$HClO_4$ (5:1, V/V) and analyzed for Cs by flame atomic absorption spectrophotometry. The concentrations of elements in the samples are reported on a dry matter basis.

#### 2.2.3. Concentration ratio

The concentration ratio (CR), defined as the ratio of metal concentrations in plant shoots to those in the roots (Gonzaga et al., 2006; Bidar et al., 2007) was calculated to check the effectiveness of plants in translocating metals to their aerial parts (Dahmani-Muller, et al., 2000; Zabłudowska et al., 2009).

#### 2.2.4. Statistical analysis

The experiments were performed in triplicate and the statistical analysis was performed using Statistical Analysis System (SAS) software package. To confirm the variability of results, all the data were subjected to analysis of variance to consider the significance differences. Moreover means comparison between data was obtain using Duncan test.

## 3. Result

### 3.1. Cs remediation from solutions using hydroponically grown plants

In the present study, the plants were found to be efficient in remediating solutions contaminated with Cs (Table 1). As it is presented in Fig. 1 when these plants were exposed to lowest Cs concentration 68 percent Cs was remediated by *Chenopodium album* that was the highest remediation percentage found in this study.

### 3.2. Cs concentration in plants

As it is presented in Fig. 2, increased amounts of Cs in solutions led to significantly higher Cs concentrations in the shoots of *Calendula alata*, *Chenopodium album* and *Amaranthus chlorostachys*. *Amaranthus chlorostachys* showed a significant accumulation of Cs. This plant accumulated significantly more Cs in shoots than the other plants in all three treatments, and in treatment 1 (0.50 $mg^{-1}$ CsCl) *Calendula alata* roots showed the lowest Cs concentration. In this study all treated plants continued to produce new organs.

### 3.3. Cs concentration ratios in plants

Both the highest level of Cs accumulation and the highest ability to translocate it from roots to shoots were observed in *Amaranthus chlorostachys*. As shown in Table 2, the CR (concentration ratio) value was 4.89 for *Amaranthus chlorostachys*, whereas for the other tests it ranged from 0.74 to 3.33.

## 4. Discussion

In this study, the percentage uptake of Cs was highest for *Chenopodium album* at a Cs supply levels of 0.47 mg $l^{-1}$. In general, the metal accumulation in *Calendula alata*, *Chenopodium album* and *Amaranthus chlorostachys* increased with the increase in metal

**Table 1**
Cs concentrations. All the values are means of three replicates ± SD. $p < 0.05$, data differences are significant.

| Plant | In initial solution (mg $l^{-1}$) | After 15 days (mg $l^{-1}$) | Remediation (%) |
|---|---|---|---|
| *Calendula alata* | 0.47 | 0.25 ± 0.01 | 46 ± 2.12 |
| *Calendula alata* | 1.58 | 0.92 ± 0.02 | 41 ± 1.59 |
| *Calendula alata* | 3.95 | 1.89 ± 0.04 | 52 ± 1.02 |
| *Chenopodium album* | 0.47 | 0.15 ± 0.01 | 68 ± 2.12 |
| *Chenopodium album* | 1.58 | 0.95 ± 0.05 | 39 ± 3.48 |
| *Chenopodium album* | 3.95 | 1.85 ± 0.29 | 52 ± 7.57 |
| *Amaranthus chlorostachys* | 0.47 | 0.25 ± 0.04 | 45 ± 8.59 |
| *Amaranthus chlorostachys* | 1.58 | 0.54 ± 0.06 | 65 ± 4.11 |
| *Amaranthus chlorostachys* | 3.95 | 2.30 ± 0.15 | 41 ± 3.92 |