Research Article

Evaluation of lead phytoremediation potential of *Rumex dentatus*: a greenhouse experiment

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Citation
Muhammad Anwar Sajad, Muhammad Saleem Khan, Muhammad Anwar Saleem Khan and Zaib-Un-Nisa. Evaluation of lead phytoremediation potential of *Rumex dentatus*: a greenhouse experiment. Pure and Applied Biology. Vol. 8, Issue 2, pp.1499-1504. http://dx.doi.org/10.19045/bspab.2019.80090

Received: 08/02/2019 Revised: 04/05/2019 Accepted: 13/05/2019 Online First: 17/05/2019

Abstract
Phytoremediation is a recent approach that employs the use of plants for the removal of pollutants from the environment. This approach is cost effective and environment friendly in comparison of other conventional remediation techniques. A greenhouse experiment was conducted in pots to evaluate the lead phytoremediation potential of *Rumex dentatus*. Pots were divided into six groups and seeds of the plant were grown in them. Plant parts were analysed for the concentration of Pb. The highest concentration of Pb (423.33 mg/kg) was found in the root of group D plants (grown in 250 ppm Pb-contaminated soil) while the minimum concentration of Pb (296 mg/kg) was found in the root of group C plants (grown in 100 ppm Pb-contaminated soil). The maximum concentration of Pb (467.33 mg/kg) in the shoot of the plants was found in group D plants while its minimum concentration (85.33 mg/kg) was found in the shoot of group A plants. Bioconcentration factor (BCF) of the plants for Pb was found higher (18.39) in control group while minimum (0.61) in the plants of group E (0.61). The calculated translocation factor (TF) value of the plants for Pb was found maximum (1.10) grown in group D pots while its minimum value (0.25) was found in the plants grown in group A soil. The maximum bioaccumulation coefficient (BAC) was 6.74 for the Pb in the plants of control group while its minimum value (0.50) was found in the plants grown in group E soil. Only the plant of the *R. dentatus* grown in 270.77 ppm Pb-contaminated soil showed BCF, BAC and TF values > 1 for Pb. Based on BCF, BAC and TF values, *R. dentatus* was found feasible for the phytoextraction of Pb from contaminated soil having Pb concentration up to 270.77 mg/kg.

Keywords: Phytoremediation; Phytoextraction, *Rumex dentatus*, Pb-Contaminated soil

Introduction
Heavy metals occurrence in the soil, air, streams and food is an issue due to their detrimental effects on the health of human even at low concentration. Such contaminants in waste water is one of the problems facing humans [1]. Heavy metals are toxic to the life [2]. When the metal bearing waste water is not sufficiently treated over discharge, they enters into the environment and subsequently to the food chain. According to the world health
organization, one such hazardous metal is lead [1]. It is primarily of interest due to their toxic effects that may cause sub-acute or acute health effects in human beings [3]. Lead is among the common toxic heavy metals disturbing the environment [4]. It's poisoning results from the ingestion of Pb-containing ingredients like paint or water which has stood in lead pipes. Toxicity may also happen from the inhalation of fumes from burning storage batteries. There is no uncertainty that it is extremely toxic to humans. Most of the absorbed Pb is stored in the brain, blood and bones. Lead colic is characterized by abdominal pain. Due to it impairment of the brain may occur in children and is identified to cause mental retardation, convulsions and even death. It is also documented that Pb is destructive to the kidney and cause permanent neurological injury [5]. Many health disorders like a rise in blood pressure, disorder of the biosynthesis of haemoglobin and anaemia, behavioural conflicts of children; impulsive behaviour, aggression, hyperactivity and declined fertility of men through sperm damage are due to Pb pollutants. Through the placenta of the mother it can enters to the foetus and cause severe impairment of the nervous system and to the brains of unborn children. This metal has no known biological role in animals or plants and is extremely toxic to aquatic life and mammals [1]. Lead is a particularly hazardous chemical, as it may accumulate in individual organisms and also in food chains. Phytoremediation is an effective way for the remediation of metal contaminated soil [2]. Thus, lead pollution is a worldwide issue. Current research work was conducted to assess the Pb phytoremediation Potential of R. dentatus grown in Pb contaminated soil. BCF, BAC and TF of the plants grown in pots were calculated and based on these values the phytoremediation potential of R. dentatus for Pb metal was evaluated.

Materials and methods
Experimental design
A greenhouse experiment was conducted in the botanical Garden of the Department of Botany, University of Malakand. Eighteen pots were used in the experiment. About 6 kg soil was added into each pot. Pots were divided into 6 group’s (Control, A, B, C, D and group E). One was control group (three pots) to which no Pb was added. In the rest of the soil of the five groups different concentration of Pb (50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm) was added. Three pots were used for each concentration of Pb in order to significantly assess the uptake potential of R. dentatus in control and Pb-contaminated soil. After the addition of the concentration of Pb to the pots of five groups, seeds of the plants were sowed in them as well as in control pots. The same water was used for the germination and growth of the plant up to maturity in the green house. Upon maturity the plants were uprooted, labelled and dried for a week in shade. Plant parts (root and shoot) were separated and grinded into powder form through grinder for the preparation of samples for the analysis of Pb.

Lead analysis in the soil
The background concentration of the Pb was analysed in the soil, which was used in the experiment. Metal in the soil was determined using standard procedure [6]. Accordingly, 5 gram of soil was taken in a beaker of 100 mL and H$_2$O$_2$ (3 mL of 30%) was transferred to it. The soil with hydrogen peroxide was then left as such in the beaker for one hour up to the vigorous reaction stopped. HCl (0.5 M) of 75 mL was added into the soil and used hot plate to heat it for about 2 hours. It was cooled and filtered through filter paper. The filtrate obtained was diluted up to 50 ml using distill water and used as sample for the purpose of Pb determination in them. It was determined in the sample using atomic absorption spectrometry (AAS) in the laboratory of soil sciences, the University of Agriculture. This step was conducted in
triplicate. Results were shown as mean ± SD.

**Lead analysis in Rumex dentatus**

The plant parts (root and shoot) were initially washed thoroughly with tape H₂O and then with distilled H₂O in order to remove soil and dust particles. Oven at 105°C was used to dry roots and shoots for 24 hours. They were grinded with the help of electrical grinder. The powder was digested using the standard protocol [7]. Plant part in powder form (0.5 g) was taken into a beaker of 100 mL and 65% concentrated HNO₃ (5 mL) as well as HClO₄ (2 mL) were added to it. It was heated using hot plate till the digest became clear. The mixture was cooled and filtered through filter paper. The filtrate was collected in a volumetric flask (50 mL). It was diluted with distilled H₂O up to 50 ml. The filtrate was used as sample for the determination of Pb by AAS in the laboratory of soil sciences, the University of Agriculture. As previously mentioned, each step was conducted in triplicate and results were shown as mean ± SD.

**Evaluation of the lead uptake potential of Rumex dentatus**

The uptake potential of the *R. dentatus* for Pb was evaluated by the calculation of BCF, TF and BAC. BCF was calculated according to Zhuang *et al.* [8] while TF using the method of Adesodun *et al.*, [9], and Padmavathiamma and Li, [10]. BAC was determined according to the formula used by Cui *et al.*, [11] and Malik *et al.*, [12]. Feasibility of the phytoremediation potential of *R. dentatus* for Pb was assessed based on BCF, TF and BAC values.

**Statistical analysis**

Excel and Graph pad prism 6 was used for statistical analysis.

**Results and discussion**

**Concentration of lead in the root and shoot of Rumex dentatus**

The plant of *R. dentatus* was grown in control soil and in different concentration of Pb contaminated soil (25 ppm, 50 ppm, 100 ppm, 250 ppm and 500ppm). The Concentration of Pb was analysed in the plant parts (Roots and Shoots) grown in different concentration of Pb-contaminated and control soil. The permissible limit of Pb in soil is 32 mg/Kg [13]. The concentration of Pb in the soil used in the experiment was higher than this allowable limit except soil of the control pots. The concentration of lead in mg/kg in the roots of the analysed plants was found in the order: Control root (382) > root of group A plants (grown in 25 ppm lead contaminated soil) (336.67) < root of group B plants (grown in 50 ppm lead contaminated soil) (378) > root of group C plants (grown in 100 ppm lead contaminated soil) (296) < root of group D plants (grown in 250 ppm lead contaminated soil) (423.33) > root of group E plants (grown in 500 ppm lead contaminated soil) (318.67). The concentration of Pb in shoot of the plants in mg/kg was found in the order; Control shoot (140) > group A shoot (85.33) < group B shoot (104) < group C shoot (260) < group D shoot (467.33) > group E shoot (262) (Figure 1). The permissible limit of Pb in plants recommended by WHO is 2 mg/Kg [14]. Results shows that the concentration of Pb in the roots and shoots of the plants grown in control and Pb-contaminated soil is higher than this permissible limit.

**Lead phytoremediation potential of Rumex dentatus**

Bioconcentration factor (BCF), translocation factor (TF) and bioaccumulation coefficient (BAC) of the *R. dentatus* grown in control and different concentration of Pb-contaminated soil was calculated. The calculated BCF, TF and BAC values of the plant grown in control and Pb-contaminated soil was used for the evaluation of the plants for the phytoremediation; phytoextraction of Pb metal. BCF of the plants for Pb metal was found in the order; Control (18.39) > group A (7.36) > group B (5.34) > group C (2.45) > group D (1.56) > group E (0.61). Sheoran *et al.*, [15] stated that the plants are not feasible for the phytoextraction of metal if BCF is less than one. Fitz and Wenzel [16]
demonstrated that plants exhibiting BCF value less than one are unsuitable for the phytoextraction of metals. BCF of the *R. dentatus* for Pb was found greater than one except for those grown in 520.77 ppm Pb-contaminated soil. The TF values of the same plants for lead were found in the order; Control (0.37) > group A (0.25) < group B (0.28) < group C (0.88) < group D (1.10) > group E (0.82). Both BCF and TF are significant in screening hyperaccumulators for the phytoextraction of metals. The selection and evaluation of plants for the phytoremediation purposes exclusively depend on BCF and TF values [17]. TF value > 1 indicates the translocation of metals from root to aerial part [18]. According to Yoon et al., [19] only plant species with both TF and BCF > 1 have the potential to be used for the phytoextraction of metals. TF value of the experimental plant was found < 1 except for those grown in 270.77 ppm Pb-contaminated soil. BAC of the analysed plant for the Pb metal was found in the order; Control (6.74) > group A (1.87) > group B (1.47) < group C (2.15) > group D (1.72) > group E (0.50). Only plant species with BCF, BAC and TF > 1 have the potential for the remediation process [20]. Only the Plant of the *R. dentatus* grown in 270.77 ppm Pb-contaminated soil showed BCF, BAC and TF values greater than one for Pb metal. The plant *R. dentatus* was found feasible for the phytoextraction of Pb metal from Pb-contaminated soil which have Pb concentration up to 270.77 mg/kg. Increase in the concentration of Pb in the soil was found to decreases the absorption capacity of root of the plant to absorb Pb from the soil as shown in results. Our findings show similarity to Van der Ent et al., who [21] stated that high metal concentrations in soil could result in a BCF < 1 (Table 1).
Table 1. Bioconcentration factor, translocation factor and bioaccumulation coefficient of *Remex dentatus* for lead metal

| Group | Concentration of Lead in Soil (mg/kg) | Bioconcentration Factor, Translocation Factor and Bioaccumulation coefficient |
|-------|---------------------------------------|-----------------------------------------------------------------------------|
|       | Background concentration               | Concentration added to the soil | Total concentration | BCF  | TF   | BAC     |
| Control | 20.77 | 0 | 20.77 | 18.39* | 0.37 | 6.74* |
| A      |       | 25 | 45.77 | 7.36  | 0.25 | 1.87  |
| B      |       | 50 | 70.77 | 5.34  | 0.28 | 1.47  |
| C      |       | 100 | 120.77 | 2.45  | 0.88 | 2.15  |
| D      |       | 250 | 270.77 | 1.56** | 1.10** | 1.72** |
| E      |       | 500 | 520.77* | 0.61  | 0.82 | 0.50  |

*Represent maximum value in respective column, ** Represent feasibility of plant for the phytoextraction of Pb

**Conclusion**

It is concluded from the experimental work that the highest concentration of Pb (423.33 mg/kg) among the roots of the analysed plants was found in group D while its minimum concentration (296 mg/kg) was found in the root of group C plants. The maximum concentration of Pb (467.33 mg/kg) was found in the shoot of group D plants while its minimum concentration (85.33 mg/kg) was found in the shoot of group A plants. The concentration of Pb in the roots and shoots of the plants grown in control and Pb-contaminated soil was found higher than the permissible limit. BCF of the plants for Pb metal was found higher (18.39) in control group while minimum (0.61) in the plants of group E (0.61). BCF of the *R. dentatus* for Pb was found greater than one in all cases except for those grown in 520.77 ppm Pb-contaminated soil. The calculated TF value of the plants for Pb was found maximum (1.10) grown in group D pots while its minimum value (0.25) was found in the plants grown in group A soil. TF value of the experimental plant was found < 1 in all cases except for those grown in 270.77 ppm Pb-contaminated soil. The maximum BAC (6.74) for the Pb metal was found in the plants of control group while the minimum BAC value (0.50) of the same metal was found for the plants grown in group E soil. Only the Plant of the *R. dentatus* grown in 270.77 ppm Pb-contaminated soil showed BCF, BAC and TF values greater than one for Pb metal. An increase in the concentration of Pb in the soil was found to decrease the absorption capacity of root of the plant to absorb Pb from the soil. Based on BCF, BAC and TF values the plant *R. dentatus* was found feasible for the phytoextraction of Pb metal from Pb-contaminated soil which have Pb concentration up to 270.77 mg/kg.

**Authors’ contributions**

Conceived and designed the experiments: MS Khan & MA Sajad, Performed the experiments: MA Sajad, Analyzed the data: MS Khan & MA Sajad, Contributed materials/ analysis/ tools: MS Khan & MA Sajad, Wrote the paper: MA Sajad, MAS Khan & ZU Nisa.

**Acknowledgment**

Authors are thankful to the chairman, Department of Soil Sciences, the University of Agriculture, Peshawar Pakistan for providing AAS (Atomic Absorption spectrophotometry) facility in their laboratory.

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