Tolerance and accumulation of lead by fenugreek

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Abstract

In the present study, the response of fenugreek (*Trigonella foenumgraceum* L.) along with remediation potential was tested against lead (Pb). A pot trial was set up in order to evaluate remediation efficiency of *T. foenumgraceum*. Growth parameters and remediation potential of fenugreek against different concentrations of Pb was compared. Different physical parameters were studied. Biochemical parameters like chlorophyll content, soluble protein, total soluble sugar and proline were analyzed. The results showed that fenugreek accumulated Pb and translocated it in the harvestable parts of the plants. Metal accumulation increased consistently with increasing concentration of Pb in the treatments. Dry matter yield of plant increased with decreasing Pb concentration in the treatment. In conclusion, fenugreek can be used for remediation of Pb contaminated soil.

Key Words: Fenugreek, tolerance, accumulation, lead, remediation potential

Introduction

Pollution generated by heavy metals has become a foremost global problem causing diseases and deaths to human. Toxic metals pollution in the biosphere has speeded up remarkably since the commencement of the Industrial Revolution (Nagajyoti et al. 2010). The huge industrial wastes, sewages and application of different chemical fertilizers and pesticides have resulted in the contamination of soils. Heavy metal contaminations due to industrial effluents discharge may pose a severe risk to human health. At present, ion exchange columns, alkaline precipitation, filtration, electrochemical removal, and membrane technologies are being used for heavy metal

removal. These technologies may produce adverse impacts on environment and are not economical. Heavy metal phytoremediation is considered as an option as it is a cost-effective, clean and green technology based on the use of specially selected metal accumulating plants to remove toxic metals from soils and water. Due to plants exclusive genetic, biochemical and physiological features they are supreme mediator for soil and water remediation (Lone et al. 2008).

Lead is one of the most common and toxic heavy metal. So forth, the present study concentrates on lead phytoremediation using fenugreek plant. Fenugreek (*Trigonella foenumgraecum* L.) is a herb of leguminosae family. Tolerance and accumulation of heavy metals by fenugreek have been studied by various authors (Parida et al. 2003; Dheri et al. 2007; Sinha et al. 2007). In this study, the objective was the assessment of morphological and biochemical response of fenugreek (*Trigonella foenumgraecum* L.) to lead bioaccumulation.

Materials and Methods

Plant Cultivation: A pot trial was set up in order to evaluate remediation efficiency of *T. foenumgraecum* L. Two kilograms of soil per treatment in earthen pots was treated with 100, 200, 400 and 800 mg/l of lead (Pb) as lead nitrate. Fenugreek seeds were grown in these treatments. Harvesting was done at 3 stages i.e., pre-flowering, flowering and post-flowering stages. Plants were watered daily using tap water to maintain optimal growth conditions. At the end of the experiment the plants were harvested and the aerial part was divided from the roots to determine the metal content. Dry weight, survival percentage and biochemical parameters of plants were estimated.

Biochemical analysis: Biochemical parameters such as chlorophyll (Arnon method), soluble protein (Bradford method), total soluble sugar (Anthrone method) and proline (Ninhydrin method) were analyzed (Thimmaiah 1999). The total chlorophyll contents were analyzed by the arnon method (Arnon 1979). Leaf samples were homogenized in 80% acetone and optical densities were measured at 645 nm, 663 nm and 652 nm with Spectrophotometer. Proline was estimated by the ninhydrin method (Bates et al. 1973). Proteins were precipitated as a protein-sulphosalicylic acid complex during extraction of tissue with sulphosalicylic acid. The extracted proline was made to react with ninhydrin under acidic conditions to form a red colour which was measured at 520 nm spectrophotometrically. Total soluble sugars were estimated by the anthrone method (Yem & Willis 1954). Carbohydrates were dehydrated by concentrated H_2SO_4 to form furfural. Furfural condensed with anthrone to form a blue-green coloured complex which was measured at 630 nm. The soluble protein content in plants was determined by the method of Bradford (1976). Absorbance was measured at 595 nm by spectrophotometer.

Metal analysis: Plant samples were washed with tap water and dried at 70°C for 48 hours. The dried material was digested with aqua regia ($1HNO_3:3HCl$). All determinations were performed

in triplicate. The concentrations of Pb in plant samples were determined by ICP-OES (Varian Vista-MPX CCD Simultaneous ICP-OES, Varian Australia Pty. Ltd).

Translocation factor and Bioaccumulation factor: Phytoextraction potential of *T. foenumgraceum* L. were evaluated by translocation factor (TF) which is a ratio of the metal concentration in aerial parts to metal concentration in roots. It signifies the metal translocation ability of plant from the roots to the aerial parts of the plant (Marchiol et al. 2004). Translocation factor (TF) = Metal concentration in aerial parts/Metal concentration in roots. Bioaccumulation Factor (BAF) is used to estimate toxic element accumulation efficiency in plants by comparing the concentration in biota and an external medium e.g. soil (Baker 1981, Ma et al. 2001). It is represented as follows: Bioaccumulation factor (BAF) = Metal concentration in aerial parts/Metal concentration in soil

Statistical Analysis: Statistical analysis of the data was done following analysis of variance (ANOVA) in MINITAB version 15 software. The mean values were compared by applying Tukey tests at 5% probability level.

Results and Discussion

Effect of lead on plant survival and dry weight: Effect of lead on plant survival and dry weight are shown in Figure 1 and 2 respectively. The maximum survival of fenugreek was found in 100 mg/l of Pb treatment (99.3 ± 2.5 %) while the minimum plant survival was noted at 800 mg/l of Pb treatment (87.6 ± 1.5 %). The maximum dry weight of fenugreek was 1.94 ± 0.16 g (100 mg/l Pb) whereas the minimum dry weight of fenugreek was 1.36 ± 0.07 g (800 mg/l Pb). Dry matter yield of plant increased with decreasing Pb concentration in the treatment. Similarly, declination in dry weight proportionately with increasing concentrations of Pb was also obtained by Sengar et al. (2008) and Bhardwaj et al. (2009). Azmat et al. (2009) showed that Pb physically block the uptake of water and water stress led to substantial losses in dry weight.

Fig. 1. Effect of Pb treatments on survival of fenugreek

Fig. 2. Effect of Pb treatments on dry weight of fenugreek

Effect of lead (Pb) on biochemical parameters: Effects of different lead concentrations on chlorophyll, total soluble sugar, soluble protein and proline contents of fenugreek are shown in Figure 3, 4, 5 and 6 respectively. The highest total chlorophyll content was 2.66 ± 0.11 mg/g FW

(100 mg/l Pb) and the lowest total chlorophyll content was 2.07 ± 0.04 mg/g FW (400 mg/l Pb). The application of Pb at concentrations of 100, 200, 400 and 800 mg/l caused a significant decrease in the total chlorophyll contents of fenugreek plants. Abiotic stress decreases the chlorophyll content in plants (Ahmad et al. 2007, John et al. 2008) because of change in pigment content that can be evidenced by plant illness and photosynthesis (Pandey et al. 2011). The chlorophyll degradation by metals in higher plants has been reported in several research works (John et al. 2008). Overall, fenugreek plants exposed to Pb stress showed a decrease in chlorophyll content which is supposed to be due to either inhibition of enzymes such as δ -aminolevulinic acid dehydratase (ALA dehydratase) and protochlorophyllide reductase associated with chlorophyll biosynthesis (Ma et al. 2001, John et al. 2008, Lone et al. 2008) or peroxidation of chloroplast, pigments and membrane lipids by oxygen radicals as a result of oxidative stress (Romero Puertas et al. 2004, Zengin & Kirbag 2007). Moreover, lead can directly destroy the structure and function of chloroplast by binding with -SH group of enzymes (Rausser 1995). Besides, heavy metals ions prevent uptake and transportation of Mn, Zn and Fe by diverging effects and therefore the capacity of synthesis of chlorophyll is lost by leaves (Gardea-Torresdey et al. 2004, Zengin & Kirbag 2007). These results are supported by Siedlecka & Krupa (1996), Pandey et al. (2011) and Zengin & Kirbag (2007) who also found a reduction in chlorophyll content with heavy metal stress in *Zea mays*, *Shorea robusta* and *Phaseolus vulgaris* L.

Effects of lead treatments on total soluble sugar content of *T. juncea* are depicted in Figure 4. The highest content of total soluble sugar was found in 100 mg/l of Pb treatment (230.37 ± 22 %mg) and the lowest content of total soluble sugar was found in 400 mg/l of Pb treatment (83.38 ± 7 %mg). Our findings pertaining to effect of lead on soluble sugar are corroborated with the results obtained by Bhardwaj et al. (2009). Soluble sugars are involved in various metabolic events and act as molecule signals regulating different genes, especially those involved in photosynthesis, sucrose metabolism and osmolyte synthesis (Rosa et al. 2009). The highest content of soluble protein was found in 100 mg/l of Pb treated plants (178 ± 17 mg/g) and the lowest content of soluble protein was found in 800 mg/l of Pb treated plants (128 ± 10 mg/g) (Figure 5). The decrease in protein content as observed at higher concentrations of Pb may be because of enhanced protein degradation process as a result of increased protease activity (Palma et al. 2002) which is found to increase under stress conditions. Figure 6 shows the influence of Pb on proline content. The maximum proline content was 0.156 ± 0.04 micromoles/g (100 mg/l Pb) and the minimum was found in 800 mg/l of Pb treatment (0.073 ± 0.02 micromoles/g). The results show that lead induced an increase of proline concentration in fenugreek leaves which may be due to the changed water status of the plants. Proline has been suggested to act as an appropriate osmolyte (Verbruggen & Hermans 2008). Proline accumulation in plants following water stress is a well-established fact (Yamada et al. 2005, Handique & Handique 2009). Water deficit conditions change the regulation of pyrroline-5-carboxylate (an enzyme involved in proline synthesis) resulted in high proline accumulation (Ali et al. 2001, Nikolic et al. 2008).

Proline increases the stress tolerance of plants through providing stability of enzymes to denaturation and improving of mRNA translation (Shafi Tantrey & Agnihotri 2010, Pandey et al. 2011). Heavy metal induced proline accumulation has been reported in cabbage (Pandey & Sharma 2002), lemongrass (Handique & Handique 2009) and gram (Shafi Tantrey & Agnihotri 2010).

Fig. 3. Effect of Pb treatments on chlorophyll content of fenugreek

Fig. 4. Effect of Pb treatments on total soluble sugar of fenugreek

Fig. 5. Effect of Pb treatments on soluble protein of fenugreek

Fig.6. Effect of Pb treatments on proline content of fenugreek

Lead content in root and shoot: Lead accumulations in root and shoot of fenugreek plant after 120 days of treatments are shown in Figure 7. The maximum Pb accumulation was 43256 ± 526 mg/kg in roots of 800 mg/l Pb treated fenugreek plant and the minimum Pb accumulation was

1879±105 mg/kg in shoots of 100 mg/l Pb treated fenugreek plant. Fenugreek accumulated high content of lead at higher concentrations. In our studies the maximum lead content was observed in the root than the shoot, which showed low transport of lead towards aerial parts of plants. Uptake in root and transportation in shoot may be based with low translocation factor which indicated great potential for phytostabilization of heavy metals in root. Other works confirmed more accumulation of heavy metal in root than the shoot in radish and spinach (Vajpayee et al. 2001, Ramos et al. 2002, Wojcik et al. 2005, Pandey et al. 2011). Furthermore, the root cell walls are first target for metals ion in soil solution. The integration of the metal ions in to the cell wall has been noted in several papers reviewed by Ernst et al. (1992). Root cells uptake only a portion of metal ions out of the total amount of ions connected with the root. This metal ion fraction is attached to the negatively charged sites (COO^-) of the root cell walls. The cell wall bound fraction cannot be translocated to the shoots and likely exhibit a limited capacity for phytoextraction (Ernst et al. 1992). Metals can also be separated in cellular organelles (e.g. vacuole) becoming inaccessible for translocation to the shoot (Lasat et al. 1998). Moreover, some plants, named excluders, have specific mechanisms to reduce metal uptake into roots. However, the idea of metal exclusion is not well known (Peterson 1983).

Fig. 7. Pb accumulation in roots and shoots of fenugreek

Translocation factor and Bioaccumulation factor: The Translocation Factor (TF) of various lead concentrations treatments are represented in Figure 8. The highest translocation factor was noted as 0.407 ± 1.09 (800 mg/l Pb) and the lowest TF was 0.182 ± 0.65 (100 mg/l Pb). Our result showed a $\text{TF} < 1$ suggesting that lead could not be effectively translocated from the roots to the shoots. Translocation factor (TF) can be used to estimate a plants potential for phytoremediation purpose. However, plants with a high translocation factor have the potential for phytoextraction (Ghosh & Singh 2005, Yoon et al. 2006). Plants grouped to three type i.e. accumulator, excluder

and indicator. Translocation factors for these three category plants are >1 , $<<1$ and near 1, respectively (Baker 1981). Thus, *Trigonella foenumgraceum* L. in this study was an excluder for Pb. They mainly restrict lead in their roots. The plant may change its cell membrane permeability, alter metal binding capacity of cell walls, or exclude more chelating substances (Lasat et al. 2000). High accumulation of lead in roots and low translocation in shoots may indicate appropriateness of a plant species for phytostabilization (Shu et al. 2000, Archer & Caiwell 2004, Malik et al. 2010).

Bioaccumulation factors (BAF) of lead are presented in Figure 9. The highest bioaccumulation factor was 25 ± 0.61 (400 mg/l Pb) and the lowest BAF was 18.8 ± 1.05 (100 mg/l Pb). Plant species under investigation had $BAF > 1$, that shows fenugreek can be considered lead accumulator. The BAF represents the contaminant concentration in plants comparing with the environment concentration for example in soil (Scragg 2005). BAF values are mostly based on metals particularity, environmental efficacy, disposal route and species-specific features. The BAF have to be higher than 1 for selection of a plant to the phytoremediation of contaminated soil (Luoma & Rainbow 2005).

Fig. 8. Translocation factor of fenugreek for different lead treatments

Fig. 9. Bioaccumulation factor of fenugreek for different lead treatments

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