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Comparative analysis of tissue compartmentalized heavy metal uptake by common forage crop: A field experiment

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ABSTRACT

Heavy metal contamination of agricultural soils is a severe cause of concern globally. The heavy metals can enter crops through roots and can result in biomagnification in the different plant tissues such as roots, stems and leaves. From plants these metals are transferred into animal and human systems resulting in serious health problems. Various physical and chemical methods are available for heavy metal removal from soil but phytoremediation is considered as one of the most sustainable and cost-friendly method. Although many studies have been carried throughout the world to assess phytoremediation potential of plants in controlled conditions, few studies are available on the metal uptake capabilities of plants growing in natural conditions. Therefore, the present study was conducted to assess the phytoremediation potential of *Trifolium alexandrinum* (Berseem), an important forage crop growing in intensively cultivated agricultural soils of Punjab, India with main focus on the accumulation and mobility of metals (Cr, Cd, Cu, Co, Fe, Mn, Pb and Zn) in various plant tissues like roots, stems and leaves. The maximum contents of Cd, Co, Fe and Pb were observed in roots whereas for Cr, Cu, Mn and Zn maximum content was observed in leaves of Berseem. Overall among the metals studied Fe content was highest in all tissues of Berseem, which could be due to the higher content of Fe in soil. Metal Bioaccumulation Factor (BAF) and Translocation Factor (TF) calculated for assessing metal uptake and transport by plant tissues were found to be above 1 for the studied metals (except Co and Fe), which indicated Berseem to be a suitable accumulator of these metals in natural conditions.

1. Introduction

Heavy metal contamination of soils is a very serious issue affecting plant, animal and human health throughout the globe. Indiscriminate anthropogenic activities such as industrialization, urbanization, excessive agrochemical application have contributed significantly to heavy metal contamination of soils. The soil physico-chemical characteristics such as pH, soil organic matter (SOM), texture, soil nutrients are very important in determining the retention and mobility of heavy metals in soils (Kavianpoor et al., 2012). Several conventional physical, chemical and biological approaches such as in situ vitrification, soil incineration, excavation and landfill have been employed for decontamination of soil. But these methods have limited applicability due to their high costs, intensive labor requirements, irreversible changes to soil structure and disturbance of soil microflora (Ali et al., 2013; Ma et al., 2016). Hence, there is a strong requirement for cost effective and environment friendly methods for cleanup of metal contaminated soils. Phytoremediation is one such approach, which employs plants for removal of metals from soil.

For phytoremediation, plants having high metal uptake capabilities are grown on metal contaminated soils. These plants either absorb and retain the metals in their roots (known as phytostabalization) or transport the metals to above ground tissues (known as phytoextraction). Usually plants having high metal extraction properties, high biomass, rapid growth and ability to accumulate > 1000 mg/kg of a metal in various tissues are considered ideal for phytoremediation (Baker and Brooks, 1989; Vamerali et al., 2010; Malik et al., 2010). *Trifolium alexandrinum* (Berseem) is one such plant. It is a leguminous crop belonging to family Fabaceae and having fast growth, high biomass and high metal extraction properties (Prasad, 2007; Ali et al., 2012). It flourishes best in neutral to alkaline soils and is usually grown in winter season where it has frost tolerance to temperatures as low as −6 °C (Muhammad et al., 2014). Although many studies have been conducted to assess phytoremediation potential of plants (including

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Berseem) in controlled conditions with metal spiked soils (Ali et al., 2012; Ma et al., 2016), these studies do not represent the actual metal accumulation capabilities of plants in open field natural conditions in the different plant tissues such as roots, stems, leaves and inflorescence. Thus, plants growing in natural habitats must be studied for assessing their phytoremediation potential (van der Ent et al., 2013).

Punjab is an important agrarian state situated in north-western part of India. Berseem is one of the main fodder crops grown in Punjab. The main sources of irrigation are river water and groundwater. However recent studies have indicated that the water of two main rivers of Punjab i.e. Beas and Sutlej are polluted due to industrial and urban activities (Singh et al., 2013; Kaur et al., 2014; Shrivastava, 2014; Kumar et al., 2016), which are further polluting the groundwater (Bhatti et al., 2015, 2016). Therefore, these polluted irrigation sources and intensive agricultural practices using agrochemicals are causing heavy metal contamination of soils of Punjab. In our earlier studies (Bhatti et al., 2015, 2016), physico-chemical characteristics and heavy metal contents in soils and crops (food and fodder) were analyzed in areas around Beas and Sutlej rivers. In these studies, main focus was overall contamination of the shoots of plants (including Berseem) without concentrating on the metal contents in different plant tissues. However, there is a strong need to assess the metal accumulation in various tissues of plants such as Berseem to understand their phytoremediation potential completely in order to provide a green solution for metals removal from soils. Hence, a detailed study was conducted in intensively cultivated areas around Harike wetland to assess the phytoremediation potential of Berseem grown in these areas by analyzing the metal uptake and accumulation in different tissues (roots, stem and leaves) of Berseem.

2. Material and methods

2.1. Study area

Punjab (Lat. 29°30 to 32°32′N and Long. 73°55 to 76°50′E) is a state located in the north-western part of India bordering Pakistan. The climate of Punjab is continental, semiarid to sub-humid with 435.6 mm annual rainfall. Punjab has two main crop seasons Kharif (fall) and Rabi (spring). Beas and Sutlej are the two main rivers of Punjab, and Harike wetland is the confluence point of these two rivers. The main sources of irrigation in areas around Harike wetland are the river water and groundwater. The sampling was done from the area surrounding Harike wetland, a Ramsar site where rivers Beas and Sutlej meet (shown in Fig. 1). The main occupation in the study area is agriculture, which involves extensive use of fertilizers, pesticides and weedicides, which are potent sources of heavy metals and contaminates the soil (Mortvedt, 1996; Milinović et al., 2008; Savci, 2012). There is also substantial industrial and urban activity (leather tanning, dyeing, electroplating, textile in cities such as Ludhiana, Jalandhar and Kapurthala. upstream of Harike wetland, which discharge sewage and industrial effluents in the rivers Beas and Sutlej, which contain several heavy metals such as Cr, Cd, Cu, Pb etc. (Kaur et al., 2017; Sharma and Walia, 2017). Thus, the irrigation sources i.e. river and ground water, are contaminated with heavy metals in this area, which further aggravates the problem of heavy metal contamination of soil (Singh et al., 2013; Kaur et al., 2014).

2.2. Sampling and preparation

Soil sampling was done during March–April 2015 (March 28, 2015, April 04–05, 2015). Six composite soil samples in triplicates were collected from fields under Berseem cultivation from the selected area which are shown in Fig. 1. At least five subsamples of soil were pooled to form a composite sample. Soil samples were taken from depths of 0–15 cm. Six composite samples of Berseem plants were collected in triplicates from corresponding soil sampling fields for heavy metal analysis. All soil and Berseem samples were stored in clean polythene bags and were brought to the laboratory. The soil samples were air-dried, ground and passed through 2 mm sieve for physicochemical and heavy metal analysis. The roots, stems and leaves of Berseem were separated in lab, washed with deionised water, oven dried at 70 °C and then ground to fine powder with pestle mortar (Bhatti et al., 2015).

2.3. Physico-chemical analysis of soil

Soil pH and conductivity were determined in 1:5 soil:water suspension. The mixture was shaken for 2 h and the supernatant was filtered and used for analysis of pH and conductivity using HM digital meter-COM-100 (New Delhi, India) and Equip-tronics EQ-614-A (Mumbai, India), respectively (Rodriguez Martin et al., 2013). Soil organic carbon content was determined by Walkley Black wet oxidation method (Nelson and Sommers, 1982). A factor of 1.72 was multiplied with organic carbon content to determine soil organic matter (SOM). Soil texture was determined by Hydrometer method (Jacob and Clarke, 2002), and an EDTA titration method was used for measuring Calcium (Ca) and Magnesium (Mg) (Lanyon and Heald, 1982). Total nitrogen (N) and available phosphorous (AP) were determined by the Kjeldahl method (Bremner and Mulvaney, 1982) and Olsen method (Olsen et al., 1954), respectively. The carbonates ($CaCO_3$) were analyzed using acid neutralization method (Hesse, 1971), and potassium (K) and sodium (Na) were measured by using a Systronics Flame Photometer-128, after digesting the samples in a diacid mixture ($HClO_4$/HNO_3 in a 4:1 ratio) (Bhat et al., 2014).

2.4. Heavy metal analysis

For heavy metal (Cd, Cr, Co, Cu, Fe, Pb, Mn and Zn) determination, one gram of soil was digested with 15 mL of aqua regia (HNO_3: HCl in 3:1 ratio) and 1 g each of ground root, stem and leaf samples of Berseem with 15 mL of triacid mixture (HNO_3:H_2SO_4:$HClO_4$ in 5:1:1 ratio) at 80 °C till a transparent solution was obtained (Allen et al., 1986). The digested samples were filtered and diluted with de-ionized water up to 50 mL and analyzed for different metals viz. Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn) and Zinc (Zn) by flame Atomic Absorption Spectrophotometer (FAAS) (Agilent 240 FS AA model). The limits of detection of the instrument are given in Table 2. Properly washed glassware, double distilled water and analytical grade reagents were used throughout the study. The standard solutions of selected heavy metals were procured from Agilent (1000 mg/L) and were used to make solutions of varying concentrations by dilution of the standards. For quality assurance, the standards were run after every ten sample readings, to assure the working of machine with 95% accuracy as done by Arora et al., 2008. The samples were also run in triplicates per sample to maintain accuracy of the results.

2.5. Metal Bioaccumulation Factor (BAF)

Heavy metal accumulation in soil and Berseem were calculated on the basis of dry weight. The metal Bioaccumulation Factor is a ratio of heavy metal concentration in crop tissue to soil (Ali et al., 2013) and was calculated as follows:

$$BAF = C_{\text{plant tissue}}/C_{\text{soil}} \quad (1)$$

where $C_{\text{plant tissue}}$ and C_{soil} are the concentrations of heavy metal in Berseem tissues (roots, stems and leaves) and soil, respectively, on a dry weight basis.

2.6. Metal Translocation Factor (TF)

Translocation Factor (TF) is the ability of a plant to move the accumulated heavy metal from roots to above ground tissues (stems,