Lead Uptake, Accumulation and Effects on Plant Growth of common reed (Pharagmites Australis(Cav.) Trin. ex Steudel) plants in Hydroponic Culture

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***Abstract*--**Reed plants (Pharagmites australis) may reduce heavy metals pollution of water used for irrigation of crop plants by phytoremediation. However, the accumulation of high shoot concentrations of heavy metals might increase their distribution and may harm grazing animals. The aim of this study was to investigate the characteristics of root and shoot accumulation of lead as well as its accumulation level that occur in reed plant (Pharagmites australis) that naturally grow with wetlands in the Kingdom of Saudi Arabia using a hydroponic experiment. Shoot and root parts of *Pharagmites australis* were sampled and analyzed for lead (Pb). Differences in uptake and translocation characteristics of the plant samples were observed between naturally-grown reed plants and plants grown in the hydroponic experiment. These differences were explained by the differences in soil x root interactions. Reed plants were found to have restricted translocation of Pb to the shoot system and the highest concentration of the element was found in the root system. In conclusion, the results of this study indicated that the results hydroponic experimental studies must be confirmed by field studies in order to find suitable plant species for phytoremediation of polluted waters. In any case, reed plants represent a safe method to reduce Pb contamination.

***Keywords*:** Lead, lead uptake, lead accumulation, common reed, Pharagmites Australis, Al-Hassa oasis.

I. INTRODUCTION

Along with the fast economic and social development in the kingdom of Saudi Arabia (KSA) , the environmental condition in different parts of the country is worsening more and more especially in agricultural regions such Al-Hassa oasis. In Al-Hassa oasis, pollution by domestic wastewater, which is in most cases is not treated effectively, has significant negative effects on both agricultural production as well as environment quality and the quality of life for Saudis in these areas. The treatment processes of wastewater cannot be effectively monitored in rural areas in order to significantly reduce the impacts of pollution. This is due to the fact that advanced treatment technologies require high technical knowhow as well as the establishment of extremely expensive facilities. It is deem necessary to explore wastewater treatment technologies that are highly efficient and inexpensive to solve the problems of wastewater in rural Saudi Arabia.

Heavy metals e.g. lead (Pb) occur naturally in earth surface and are released during the weathering process. However, human activities such as disposal of industrial and domestic waste water, car emissions, Pb acid batteries wastes, paints and treated woods and the use of various organic and mineral fertilizers are the main sources of Pb contamination (Srivastava et al.,2015). Pb is one of the most toxic heavy metals in the environment to plants, animals, and humans (Tamura et al., 2005 and Zhou et al., 2014). Through the uptake of water contaminated with Pb, Pb enters the plants and accumulate in the plant tissues as it isn't metabolized (Pezzarossa et al.,2011).

There are different methods by which plants respond to the toxic impacts of Pb. These includes, selective uptake of the element, Pb binding to the root surface, and formation of antioxidants such as proline, glutathione, cysteine, ascorbic acid and antioxidant enzymes, such as guaiacol peroxidase , superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase (Gupta et al.,2009). Pb interacts with the cellular components and increases the cell wall thickness. Usually, the plant cell wall contains pectin and Pb can form a complex with the carboxyl group of pectin and this process is regarded as the corner stone of the plant cells resistance to Pb toxicity (Eun, et al.,2002) and it acts as a physical barrier and restricted the movement of Pb through the plasma membrane.

As the uptake of Pb from the soil increases, it can damage the ultrastructures of the organs, tissues, chloroplast, mitochondria, nucleus, cell wall, and cell membrane in the plants. This damage can cause a loss of organelle function, and can eventually affect the normal physiological functions that include photosynthesis, respiration, protein synthesis, cell division within the plant species (Salazar and Pignata, 2014).

Plants can tolerate Pb be either by external exclusion or internal tolerance. By the external exclusion, Pb ions are excluded from entering the plant cells and thus Pb cannot

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accumulate in the organelles and excess Pb ions are removed out of the plant cell (Sharma and Dubey 2005). The internal tolerance of Pb is mainly due to the synthesis of organic lead compounds (cysteine, glutathione, phytochelatin, etc and eventually the Pb ions are transformed in the cell into chemically bound structures with lower toxicity, alleviating the Pb toxic effect on the plants tissues (Pourrut et al., 2011).

Yang and Ye (2014) reported significant differences among plants in their Pb tolerance and uptake in the shoots and the roots. Superoxide dismutase and peroxidase activities in the leaves of most tested plants increased with the increase in the level of Pb in the growth media, while the catalase activity in the leaves declined when plants were subjected to Pb stress (Yang and Ye,2014). Pb accumulation by the wetland plant species screened in the study was strongly dependent on the plant species and Pb concentrations. However, Pb translocation from the root to the shoot was greatly low in all species(Yang and Ye,2014). Increases in the activities of superoxide dismutase and peroxidase indicate that antioxidants may have an important role in reducing Pb toxicity in plants.

Common reed (Phragmites australis (Cav.) Trin. ex Steudel, Poaceae) is one of the most widely distributed species in the world including KSA and it is a typical rhizomatous perennial plant, whose extensive rhizomes form a dense, network from which new plants originate (Bonanno and Giudice,2010). Common reed can tolerate extreme environmental stresses, including high concentrations of heavy metal(Fediuc and Erdei, 2002). Common reed plants have been widely used in the construction of wet sites for the treatment of industrial wastewaters polluted with different types of metals,chemicals and fertilizers from agricultural soils and toxic and biological waste from industries and health centres.There is a strong evidence that common reed has high tolerance of heavy metals such as Pb (Ye et al.,1998) and high ability to remove heavy metals from soils, and thus can be used effectively in the phytoremediation processes (Iannelli, 2002).

Nutrient leaching, industrial and domestic waste water discharge and eutrophication of canals are major problems is Al-Hassa and other part of KSA. Existing methods of filtration, adsorption, chemical precipitation and ion-exchange are not sufficient to remove even low heavy metal and toxic concentrations. The present study was conducted to investigated the growth characteristics, Pb uptake and accumulation by common reed (Phragmites australis (Cav.) Trin. ex Steudel) growing in hydroponic culture in the presence of Pb ions.

MATERIAL AND METHODS

Experimental setup

P. australis (common reed), were propagated from seeds at the greenhouse King Faisal University, Al-Hassa ,Kingdom of Saudi Arabia. After germination, individual seedlings were potted in 30 cm pots containing a mixture (50:50) of sand and peat moss. When the plants were 15 cm tall, the soil was carefully washed off the roots, and the plants were rinsed in distilled water. Three similarly sized plants were selected and distributed randomly in each treatment. The plants were initially incubated for 10 days in hydroponic solution without lead for acclimatization to the hydroponic growth conditions. After the 10-day acclimatization period, dead leaves were genteelly removed, then, the plants were weighed and the hydroponic solutions were replaced by the treatments. The treatments included (i) 0 ppm, (ii) 25 ppm,(iii) 50 ppm of Pb in the form $Pb(NO_3)$. The plants were mounted in the lids of 700-mL glass vessels using soft polyethylene foam, making sure that all roots were submerged in 500 mL of hydroponic solution, and were then placed in a growth chamber (Fig. 1).

Fig. 1 Main Experimental Set up

Temperature, light and humidity were as under the laboratory conditions et al. 1998), and maximum/minimum temperatures were around 34/26 °C (day/night) during the treatment period of 90 days from 1st June to 1^{st} of September, 2015.

Five randomly chosen seedlings from each treatment per replication were washed gently with tap water, washed gently three times with deionized water and then divided into shoot and root and their respective total length was assessed before being oven-dried at 80 °C to a constant weight, and then the dry weights were measured. The dried shoots and roots were ground into fine powder with (MM400,Retsch, Haan, Germany) ball mill for the determination of their Pb content.

DETERMINATION OF PB CONTENTS

The homogeneous reed power samples (0.5 g) were digested in a microwave oven (CEM Mars5; Analyx, Wellesley, MA, USA) with solutions of 5:1 HNO_3: HF and HNO_3:$HCLO_4$(v/v) for 120 min. The clear liquid was further digested in a digestion apparatus (DigiBlockED36; LabTech, Cary, NC, USA) until around 0.5 cm height at 180 °C. The cooled solutions were made up to 50 ml total volume. The Pb contents were measured using a graphite furnace atomic absorption spectrometer (Spectr A A Z220; Varian, Palo Alto, CA, USA). The detection limits for Pb are in the range 10–100 ppm.

STATISTICAL ANALYSIS

Data were checked for normality and homogeneity of variance before the ANOVA was conducted. Treatments means multiple comparisons were performed using LSD tests at a probability = 0.05 when ANOVA results were significant. All statistical analyses were performed with the SPSS version 13.0 (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The effects of different Pb levels on root and shoot length and dry weights are shown in Fig. 2 and 3. Pb treatments had remarkable impacts on the shoot and root length and dry weights of the seedlings that reflect a concentration-dependent reduction of these traits. Significant reductions ($P<0.05$) of these characteristics with the increase in Pb concentration in the growth solution were recorded. However, the response to Pb concentration differs among the root and shoot length and dry weights. The results demonstrated that the roots appears to be more sensitive to Pb as compared to the shoot. Similar results were recorded by Fargasova (1994) in Sinapis alba and by Nwosu et al.(1995) in both radish and lettuce. Patra et al.(1994) have indicated that low concentrations of Pb can promote plant growth, but concentrations greater than 0.5 μM can inhibit plant growth. In this study, the shoot and root lengths of common reed seedlings under all Pb treatments showed noticeable differences when compared with the control seedlings, but these differences were not significant except for root length of reed seedlings growing at Pb concentration of 50 ppm, in the 20 treatments the root length was suppressed, but not significantly (Fig. 2). The fact that the roots are more responsive to the impact of Pb in the environment can be explained by the idea that the roots are the specialized absorptive tissues and the nearest to the source of Pb contamination and thus they are affected earlier and subjected to the accumulation of high Pb than the stem, branches and leaves. This could be the reason for using root length as a measure for determining Pb tolerance ability of plant. The reduction of both shoot and root lengths was maximized when the higher concentration of Pb was used. A number of studies have reported plant growth inhibition across different plant species by Pb toxicity (Islam et al., 2008, Huang et al. 2012 and Kumar et al. 2012),). The unchecked shoot growth as well as root growth at the 25ppm treatment of common reed seedlings as affected by Pb conditions may indicate that the species is some tolerant to the increase in Pb concentrations in its growth environment.

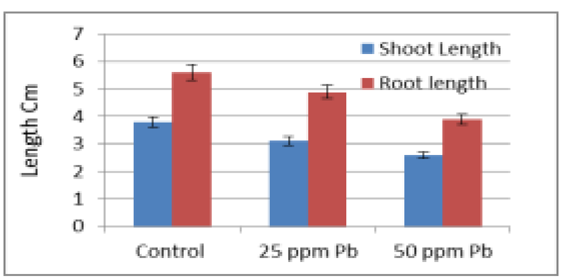

Fig.2.Shoot and root lengths of common reed seedlings as affected Pb concentration in the growth media bars represent mean ± S.E. of three replicates

The reed plant shoot and root dry weights as affected by various content of lead are presented in Fig3. Shoot and root dry weights of the common reed seedlings were not signifficantly different when the control and in 25 ppm Pb treatments were compared (Fig.3),however when these seedlings traits were compared with those grown in the 50 ppm Pb treatment signifficant differences were recorded. The effects of the two Pb concentrations (25 and 50 ppmPb) recorded in this study were not significantly different ($P>0.05$). The 50ppm Pb treatment caused noticeable declines in both shoot and root dry weight of common reed seedlings when compared to the control treatment (Fig. 3). The results of this indicate that common reed hass some degree of Pb tolerance. This degree of Pb tolerance depends markedly on the prevailing climatic and other environental conditions and ecotypes of this plant species (McKee and Richards, 1996).

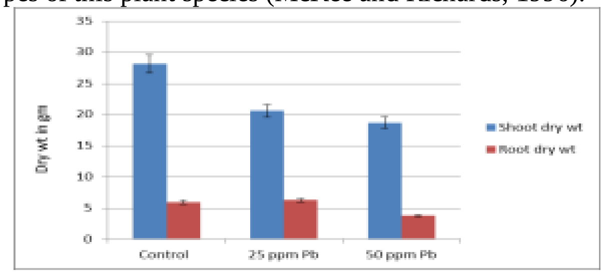

Fig.3.Shoot and root dry weights of common reed seedlings as affected Pb concentration in the growth media bars represent mean ± S.E. of three replicates

The Pb concentrations in the the shoots and roots of reed plants are presented in Fig 4. Accumulation of Pb by shoots of reed seedlings was not signicantly different when seedlings were grown in the control and 25 ppm Pb treatments.The Pb concentrations in the shoots and roots decreased in the order roots > shoots and in the roots were higher in the 50 ppm Pb treatment than in the 25 ppm Pb treatment (Fig. 4). So, the Pb concentration in the reed plant tissue is significantly affected by both the lead concentration supplied in the growth solution and by the reed plant tissue as well as by the interaction between the two factors. In all reed plant tissues, the Pb content increase without any exception with the increase of Pb concentration in the growth solution. Many studies indicated that heavy metal content in plant tissues is a function of heavy metal content in the growth media.The Pb contents in the roots significantly increased from 0 to 55.7and 82.6 ppm as the Pb concentration increased (Fig. 4),but Pb was extremely low in the stems. The roots of many tolerant plants appear to take up more of the heavy metal to which they are tolerant than the roots of susceptible plants (Baker and Walker, 1990). So, the Pb content in the roots is more closely related to that of the growth medium than in the shoots.

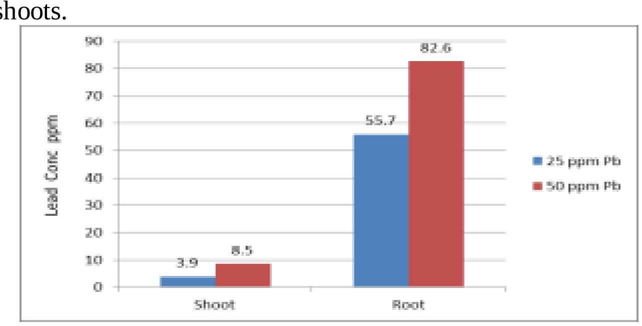

Fig.4.Accumulation of Pb by common reed seedlings as affected Pb concentration in the growth media

Higher Pb content in reed plant tissues can be expected as the Pb concentration in the growth solution is further increased.

The findings of this study show that common reed plant species in KSA can uptake Pb, hence it is recommended to use these plants for the removal of Pb from the water in Al-Hassa oasis.as seen in previous studies, reed plants can be utilized to extract other heavy metals both from waters and soils.The data obtained should help in future species selection for the use in designing wetlands in Pb-contaminated environments.

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