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# Evaluation of sunflower (*Helianthus annuus* L.) for phytoremediation of lead contaminated soil

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**Abstract**

Lead is a toxic metal that affects plant growth and the environment. Phytoremediation utilizes plants to uptake contaminants and can potentially be used to remediate metal-contaminated sites. The aim of this study was to determine the effects of heavy metal (Pb) on plant growth and examine their uptake by different organs of sunflower ,and evaluated the lead (Pb) bioaccumulation potential of sunflower (*Helianthus annuus* L.). A pot experiment was conducted in a Completely Randomized Block Design (CRBD), with six treatments and three replications, Seeds were grown in plastic pots filled with 10 kg of soil. Lead was applied at the rates of 0; 50; 100; 150, 200 and 250mgPb.kg$^{-1}$ soil. The total concentration of these metals was measured in the roots, stems, leaves, whole plant, and the corresponding soil. The levels of photosynthetic pigments i.e. chlorophyll a and b, carotenoids, root, shoot and whole plant dry weight not affected significantly as the Pb concentrations increased, compared with control. Whilst at higher concentrations of Pb, there was a significant decrease in RWC and plant height. The Pb contents in the plants increased linearly with the Pb exposure concentration with the highest Pb content in the roots .The concentrations of Pb in the three parts of Pb-treated plants were in the order: roots > stem > leaves. . In addition, the determination of a bioconcentration factor, a translocation factor and tolerance index showed that sunflower was suitable for phytoextraction of Pb in all treatments**.** It is concluded from this study that sunflower plant can absorb and accumulate quantities of lead without affecting the production of biomass, indicating the possibility of using it as hyper accumulator successfully.

**Keywords:** Sunflower, phytoremediation, lead

## INTRODUCTION

The presence of heavy metals may vary from site to site, depending upon the source of individual pollutant (Adejube *et al*., 2017). At high soil concentrations, occur changes in macronutrient contents in plants grown under Pb stress(Lamhamdi *et al*., 2013; Augusto *et al*., 2014) and in organic solutes in plants grown in the presence of Pb (Lamhamdi *et al*., 2013). Once lead has entered into the root system, it may accumulate there or may be translocated to aerial plant parts (Shahid *et al*., 2011). Most of the lead uptake was accumulated in the roots, then in the stems and leaves, but when re-calculated per plant dry weight, the uptake of the metal did not depend on the lead dose applied **(**Wińska-Krysiak *et al*., 2015). While Sewalem *et al*. (2014) observed high amount of the total absorbed Pb was tranlocated to shoots of sunflower seedlings, Pb allocation between roots and shoots at the yield stage.

A toxic pollutant causes number of lamellar organization in the chloroplast, cell division, seed germination, chlorosis, decrease the process of photosynthesis and the number of mitochondrial cristae (Paliwal *et al*., 2014;Wińska-Krysiak *et al*., 2015), plant tolerance index (TI) of sunflower **(**Atta *et al*., 2014**).** reduce total protein content, modification in gene expression (Kovalchuk *et al*., 2005), reduces plant cell wall plasticity, increased ribonuclease activity (Gopal and Rizvi, 2008), However, certain amino acids, like proline, increase under lead stress (Qureshi *et al*., 2007), such proteins play a major role in the tolerance of the morphological and biochemical variations in the plants ( Kanwal *et al*., 2014). However, the extent of these effects varies and depends on (a) the lead concentration tested,(b) the duration of exposure, (c)the intensity of plant stress, (d)the stage of plant development, (e)and the particular organs studied (Pourrut *et al*., 2012 ).

Several methods are already being used to clean up the environment from heavy metals, physical and chemical methods are generally considered as destructive, expensive, labor-intensive and causing secondary problems ( Wu *et al*., 2010). Whereas, phytoremediation is a novel, less expensive, efficient, environment- and eco-friendly remediation strategy with good public acceptance (Revathi *et al*., 2011). Phytoremediation, involves the use of green plants to decontaminate soils, water, sediments and air. There are different categories of bioremediation which include phytoremediation, phytoextraction, phytofiltration, phytostabilization, phytovolatization and phytodegradation, depending on the mechanisms of remediation (Adejube *et al*., 2017).

Sunflower ( *Helianthus nnuus* L.) possesses important agronomic traits, such as tolerance to high and low temperatures and adaptation to different soil and climate conditions , is one of the most environmental crops that is being used in diverse situations for environmental clean-up since it grows very fast, have large biomass and it can hyper accumulate heavy metals(Paniego *et al*., 2007).The accumulation of metals was recorded highest in roots of the plant followed by shoots(Fulekar ,2016).The results of Adejube *et al*.( 2017).showed that the removal of heavy metals in the polluted soil varied based on the duration of sunflower plant (30days< 60days< 90days). The present study was designed to evaluate the phytoremediating activity of sunflower on soil polluted with lead (Pb).

## MATERIALS &METHODS

### Plant Materials

The experiment was carried out on sunflower plants (*Helianthus annuus* L., cv. Aqmar ) obtained from Field Crop Department, College of Agriculture, University of Baghdad**.**

**Experimental layout**

The study was carried out in the woody shade, Department of Horticulture and Gardens, College of Agriculture - University of Baghdad. Seeds were grown in plastic pots filled with 10 kg of soil. Surface soil were gathered at depths ranging from 0 - 30 cm, the soil was air-dried and then passed through 2 mm sieve, and large stones and plant root debris were removed. Physical and chemical proprieties of the soil samples were measured and the results were presented in table 1. The experiment was laid out in Completely Randomized Block Design with five levels 50, 100, 150, 200 and 250 $mg.kg^{-1}$ soil of lead acetate [$Pb(CH3COO)_2$] ,in addition to control treatment (tap water). Ten seeds were sown at equal distance and one inch deep in soil of corresponding pots, thinning to one plant after 10 days from sowing (five pots in each replicate for each treatment). The treatments were replicated three times (n=90 pots). Lead treatments were added to the soil at one month seedling age .Each treatment was dissolved in 0.250 litre of water for the purpose of distributing it evenly to pot soil; the pot bottom was sealed off to prevent lead leaching. Phosphate fertilizer 80 kg / ha (calcium superphosphate 45% $P_2O_5$) was added (65g per pot) pre sowing, Nitrogen fertilizer 160 kg / ha (urea 46% N) (130g per pot) was added on two equal amounts: the first at sowing and the second after one month of sowing .The experiment lasted for 100 days (from 15 March to 10 July 2018).

**Table 1.** Physical and chemical characteristic of soil before sowing.

| Characteristics | value |
|---|---|
| EC ($ds.m^{-1}$) | 3.80 |
| pH | 7.10 |
| Dissolved ions ($meq.L^{-1}$): | |
| Ca | 19.14 |
| Mg | 11.27 |
| Na | 6.83 |
| K | 1.28 |
| Cl | 33.10 |
| $HCO_3$ | 2.64 |
| $CO_2$ | Nil |
| $SO_4$ | 2.86 |
| Available ions : | |
| N ($mg.kg^{-1}$) | 21.10 |
| P ($mg.kg^{-1}$) | 6.55 |
| K ($mg.kg^{-1}$) | 120.41 |
| Pb ($mg.kg^{-1}$) | 3.882 |
| CaCO3 (%) | 29.71 |
| Organic matter (O.M) (%) | 0.69 |
| Soil texture | Silty loam |
| Sand (%) | 24.61 |
| Loam (%) | 53.29 |
| Clay (%) | 22.10 |
| Values are mean (n= 3) | |

**Sampling and measurements**

**Physiological parameters**

Relative water content(RWC) was determined at flowering stage using 1 cm2 segments of leaf tissue, which were weighed to record fresh weight (FW), floated in distilled water for 24 h to determine turgid weight (TW), then oven-dried at 70°C for 48 h to measure dry weight (DW). RWC was calculated using the following equation (Turner, 1988):

RWC= (FW – DW)/ (TW – DW)*100

For pigment analysis 1 g of fresh leaf tissue was measured and the leaves were cut into small pieces (about 1 mm wide). The pigments were extracted by grinding in a mortar and pestle for 5 minutes. Afterwards the extract was filtrated and transferred to 100 ml acetone, and filter with Whatman No. 1 filter paper. The extract obtained was diluted to a final volume of 100mL. Immediately after the solutions were prepared, measurement of pigments was performed in a spectrophotometer (Beckman®, model DU-640) at the following wavelengths: chlorophyll-a in 663.2 nm, chlorophyll-b in 646.8 nm and carotenoids (carotene[c] + xanthophyll [x]) in 470 nm. The chlorophyll (chl) and carotenoids (car) contents were calculated as described by Lichtenthaler (1987):

$$\text{Chl a} = 12.25 \times A_{663.2} - 2.79 \times A_{646.8}$$
$$\text{Chl b} = 21.50 \times A_{646.8} - 5.10 \times A_{663.2}$$
$$\text{Car c+x} = (1000\, A_{470} - 1.82\, \text{Chla} - 85.02\, \text{Chlb})/198$$

Pigment concentration was expressed in milligrams of pigment per gram of tissue fresh weight ($mg.\ g^{-1}$ FW).

**Growth Traits**

Plant length was measured at flowering stage in control and treated plants. After the plants were removed from the soil, they were washed with tap water to remove any residual soil or dust. Oven dried at 70°C for 48 h (to a constant weight), then the plants were cooled in a dry environment, and the plants were weighed for dry weight., then, separated into roots and shoots, dry weight of roots and shoots was recorded.

**Chemical analysis of the plant samples**

Plant samples were taken from different pots after harvesting and Lead accumulation was estimated from whole plant, root, stem, and leaves. Samples were washed with distilled water and HCl (0.01N), then sectioned and dried at 70°C during 48 h. They were ground and stored in glass containers until analysis. Each sample was separately analyzed for Pb concentration. Each sample was weighed (0.5g) and digested in 10 mL $H_2SO_4$-$H_2O_2$ for 24 .After digestion, samples were filtered and the volume of each sample was adjusted to 50 ml using deionized water. Total Pb of samples was analyzed by atomic absorption (Feng *et al*., 2010).

**Chemical analysis of Soil samples after Harvesting**

Soil samples from each pot were homogenized and airs dried in an oven at 30°C overnight to a constant weight and were then passed through a 2 mm sieve (Uba *et al.,* 2009) before analysis. Approximately 0.5 g of soil sample was digested with 6 mL of $H_2SO_4$:15 mL $H_2O_2$. The samples were then filtered and diluted with deionized water to 50 mL. The total concentrations of Pb were determined by atomic absorption.

**Bioconcentration (BCF)**

Bioconcentration factor (BCF) indicates the efficiency of a plant in up-taking heavy metals from soil and accumulating them into its tissues. It is the ratio of the heavy metal concentration in the plant tissue (root, stem or leaves) to that in soil. It is calculated using the equation (Zhuang *et al*., 2007):

$BCF = (C_{harvested\ tissue} / C_{soil})$

where $C_{harvested}$ tissue is concentration of the target metal in the plant harvested tissue (roots, stem or leaves) and $C_{soil}$ is concentration of the same metal in soil.

**Translocation Factor (TF)**

Translocation factor (TF) indicates the efficiency of the plant in translocating the accumulated heavy metals from roots to shoots. It is the ratio of the concentration of the heavy metal in shoots (stem or leaves) to that in its roots. It is calculated following the equation (Adesodun *et al*., 2010):

$TF = [C_{shoot\ (stem\ or\ leaves)} / C_{roots}]$

Where $C_{shoot\ (stem\ or\ leaves}$ is concentration of the target metal in the plant harvested tissue ( stem or leaves) and $C_{roots}$ is concentration of the same metal in the roots.

**Tolerance Index (TI)**

The tolerance index which is a measure of the plant tolerance to heavy metals was determined by comparing the dry biomass of plants subjected to metal treatment with the control using the relationship outlined by Wilkins (1978):

TI= (Biomass of treated plants / Biomass of control plant) * 100

**Statistical Analysis**

Results were analysed using Image J program (Schneider *et al*., 2012). Least significant difference – LSD-Test was used to significant compare between means in this study.

## RESULTS & DISCUSSION

**Results**

**Physiological parameters**

The lead levels applied and its accumulation in plants produced negative effects and produced significant ($p \geq 0.05$) effect on RWC response of the plants which decreased to 51% by increasing the level of Pb in the soil to 250 $mg.kg^{-1}$ soil compared to 85% at Pb 0. Photosynthetic pigments were compared in sunflower plants treated with lead polluted soil. The levels of photosynthetic pigments i.e. chlorophyll a and b and carotenoids not affected significantly as the Pb concentrations increased, compared with control. (Table 2).

**Growth Traits**

At 100 mg $.kg^{-1}$ soil of Pb, there was a significant decrease in plant height to76 cm, which started to drop more sharply to 75.67 and 75, respectively at Pb concentrations of 200 and 250$mg.kg^{-1}$soil compared with control which gave maximum height of 80.67 cm(Table 3). Generally, the data depicted in table 3 clearly shows that there were no significant differences between the plants for root dry weight, shoot dry weight and whole plant dry weight when they were treated with lead at concentrations of 0, 50,100,150, 200 or 250 $mg.kg^{-1}$ soil after 30 days from sowing.

**Lead Accumulation in Plant Tissues**

The main characteristics of the primary soil and heavy metals (before treating) are shown in table 1. The soil in the pots had a silty loamy texture, with an average EC of approximately 3.80 $ds.m^{-1}$, and they were slightly alkaline (pH=7.10), which means the pH conditions were suitable for plant growth (Table 1). After the treatment with the heavy metals and the removal of the plants, the total concentrations of Pb in leaves, stem, root and whole plants were measured separately. The results in figures 1 – 4 shows that the total concentrations for Pb heavy metal in plant parts were significantly different between the treated and control soils at some of the tested levels in Aqmar variety.

**Table 2.** Effect of lead concentrations on some physiological traits of sunflower plant.

| Treatment | Dose Pb $mg.kg^{-1}$ | | | | | | Mean | LSD 0.05 |
|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | | |
| (%) RWC | 85 | 79 | 76 | 63 | 61 | 51 | 69 | 29.44 |
| Chl a ($mg.g^{-1}$ fresh weight ) | 5.62 | 5.51 | 4.81 | 5.03 | 4.61 | 4.24 | 4.97 | N.S |
| Chl b($mg.g^{-1}$ fresh weight ) | 3.17 | 3.16 | 3.35 | 2.72 | 2.86 | 3.04 | 3.05 | N.S |
| Carotenoids ($mg.g^{-1}$ fresh weight ) | 2.59 | 2.90 | 2.34 | 2.31 | 1.99 | 2.10 | 2.37 | N.S |

Table 3.Plant height and Dry weight of organs and whole plant of sunflower.

| Organs | Dose Pb $mg.kg^{-1}$ | | | | | | Mean | LSD 0.05 |
|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | | |
| Plant height(cm) | | | | | | | | |
| | 80.67 | 78.33 | 76.00 | 77.67 | 75.67 | 75.00 | 77.22 | 4.55 |
| Dry weight (g) | | | | | | | | |
| Roots | 8.20 | 9.08 | 7.18 | 5.52 | 7.08 | 6.12 | 7.20 | N.S |
| Shoots | 73.43 | 73.58 | 70.12 | 71.97 | 68.70 | 61.01 | 69.80 | N.S |
| Whole plant | 81.63 | 82.66 | 77.30 | 77.49 | 75.78 | 67.13 | 77.00 | N.S |

At harvesting an overall increase of Pb in plant tissues was observed with increasing of Pb in soil. In particular, Pb increased of about 2.5-fold in leaves (from 4.07 of $Pb_0$ to 10.98 mg.kg-1 of Pbg.kg-1 siol) (Figure 1) ,and of about 3-fold in stems (from 8.58 of $Pb_0$ to 21.19 mg.kg-1 of $Pb_{250}$ treatment) (Figure 2), and of about 8-fold in roots (from 22.69 of $Pb_0$ to 183.59 mg.kg-1 of $Pb_{250}$ treatment) (Figure 3), and of about 6-fold in whole plant (from 35.34 of $Pb_0$ to 215.75 mg.kg-1 of $Pb_{250}$ treatment) (Figure 4). The concentrations of Pb in the three parts of Pb-treated plants were in the order: roots > stem > leaves.

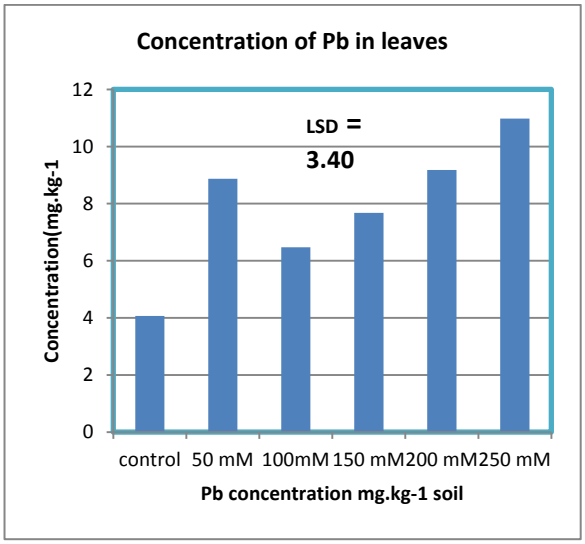


**Figure 1**.accumulation of Pb in leaves of sunflower.

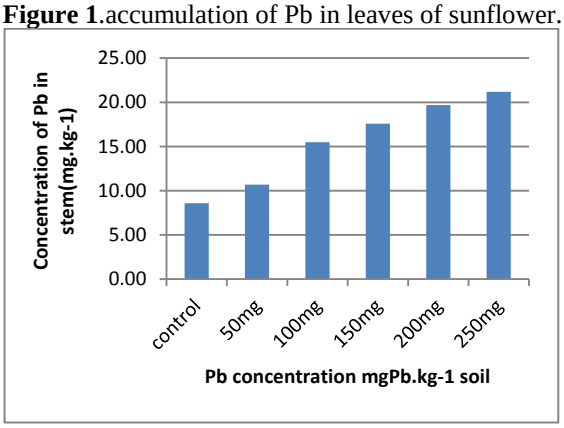


**Figure 2**.accumulation of Pb in stems of sunflower.

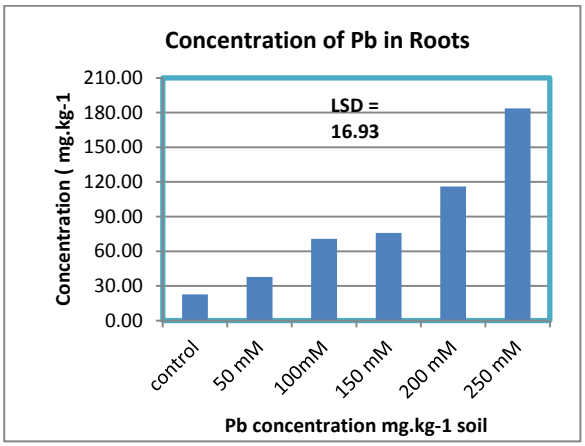


**Figure 3**.accumulation of Pb in roots of sun flower.

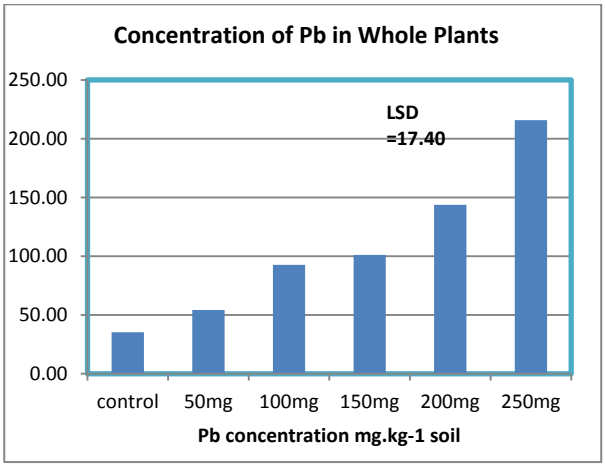


**Figure 4**.accumulation of Pb in whole plant of sunflower.

**Lead Accumulation in the Soil**

Lead accumulation in the soil after the harvesting increased linearly with the increasing the amounts of lead added to the soil. As can be seen in table 4, Pb accumulation in soil increased significantly in Pb treatments (p p ≥0.05) compared to control treatment. The table shows that at the concentrations 150,200 and 250 $mg.kg^{-1}$ soil the effect of Pb was higher and gave 154.84, 193.91 and 291.31 $mg.kg^{-1}$ than at 50 and 100 $mgPb.kg^{-1}$ soil which gave 26.03 and 51.74 $mg.kg^{-1}$.

**Table 5.** Bioconcentration factors of sunflower plant for lead concentrations.

| Pb conc. (mg. $kg^{-1}$) | Pb concentration in soil (mg. $kg^{-1}$) | | | Bioconcentration factor of harvested tissues | | |
|---|---|---|---|---|---|---|
| | Background Conc. | Con. added to soil | Total Conc. | BCF root | BCFstem | BCF leaves |
| 50 | 3.882 | 50 | 53.882 | 0.699 | 0.198 | 0.109 |
| 100 | 3.882 | 100 | 103.882 | 0.681 | 0.149 | 0.062 |
| 150 | 3.882 | 150 | 153.882 | 0.493 | 0.114 | 0.050 |
| 200 | 3.882 | 200 | 203.882 | 0.569 | 0.091 | 0.045 |
| 250 | 3.882 | 250 | 253.882 | 0.723 | 0.084 | 0.043 |
| LSD0.05 | | | | 0.09 | 0.04 | 0.03 |

**Table 6.** Translocation factors of sunflower plant for lead concentrations.

| Pb conc. (mg. $kg^{-1}$) | Pb concentration in soil (mg. $kg^{-1}$) | | | Translocation factor | |
|---|---|---|---|---|---|
| | Background Conc. | Con. added to soil | Total Conc. | TFstem | TFleaves |
| 50 | 3.882 | 50 | 53.882 | 0.251 | 0.130 |
| 100 | 3.882 | 100 | 103.882 | 0.218 | 0.092 |
| 150 | 3.882 | 150 | 153.882 | 0.231 | 0.101 |
| 200 | 3.882 | 200 | 203.882 | 0.161 | 0.079 |
| 250 | 3.882 | 250 | 253.882 | 0.116 | 0.060 |
| LSD0.05 | | | | 0.09 | 0.09 |

**Table 4.**Lead concentrations of tested soil before and after treatment with lead.

| Test soil | Dose Pb $mg.kg^{-1}$ | | | | | | Mean | LSD 0.05 |
|---|---|---|---|---|---|---|---|---|
| | 0 | 50 | 100 | 150 | 200 | 250 | | |
| Before treatment | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | 3.88 | — |
| After treatment | 2.80 | 26.03 | 51.74 | 154.84 | 193.91 | 2.19.31 | 108.11 | 58.13 |

**Bioconcentration factor (BCF)**

The bioconcentration factor (BCF) of Pb regarding leaf, stem, and root is given in table 5. Results are based on the total soil concentrations of lead (background concentrations plus concentrations added to soil). Values of background concentrations lead metal in the used soil were determined before sowing. There were significant differences between Pb concentrations for BCF of all plant parts. The maximum value was found in roots (0.723) at 250 Pb concentration followed by stem (0.198) and leaf (0.109) at 50 Pb concentration**.** The results show that sunflower plant at the different levels had BCFs<1 for Pb.

**Translocation factor (TF)**

The values of translocation factor (TF) of sunflower plant parts (stem and leaves) for the lead metal are given in Table 6. The effect of Pb concentration on shoot (stem and leaves)/root Pb concentration ratio (translocation factor) was significant, ($P \geq 0.05$) compared to control. Translocation factor decreased with increasing Pb concentration. TF values follow the order 0.251, 0.218, 0.231, 0.161 and 0.116 at 0,50, 100, 150, 200, 250 $mgPb.kg^{-1}$ soil for stem. Whereas for leaves TF values follow the order 0.130, 0.092, 0.101, 0.079 and 0.60 at 0, 50, 100, 150, 200, 250 $mgPb.kg^{-1}$ soil. The results show that sunflower plant at the different levels (treatments and control) had TFs<1for Pb; therefore, it was suitable for phytostabilization of this metal.

**Tolerance index (TI)**

Tolerance index of roots, stem, leaves and whole plant were affected by the polluted soil treatments .Tolerance index varied along increasing Pb metal concentration. Stem, leaves and whole plant at 200 and 250 $mgPb.kg^{-1}$ soil revealed reduced tolerance index to 0.161 and 0.116 for stem, 0.079 and 0.060 for leaves, and 0.93 and 0.82 for whole plant , respectively, while root exhibited reduced TI to 0.67 and 0.75 at 150 and 250 $mgPb.kg^{-1}$ soil (Table 7).

**Table 7.** Translocation index of sunflower plant in response to lead concentrations.

| Pb concentration (mg. $kg^{-1}$) | Translocation Index | | | |
|---|---|---|---|---|
| | TI root | TI stem | TI leaves | TI whole plant |
| 50 | 1.11 | 0.251 | 0.130 | 1.01 |
| 100 | 0.88 | 0.218 | 0.092 | 0.95 |
| 150 | 0.67 | 0.231 | 0.101 | 0.95 |
| 200 | 0.86 | 0.161 | 0.079 | 0.93 |
| 250 | 0.75 | 0.116 | 0.060 | 0.82 |
| Mean | 0.854 | 0.195 | 0.092 | 0.932 |
| LSD0.05 | 0.14 | 0.09 | 0.09 | 0.14 |

## DISCUSSION

Plants exposed to heavy metals reduced transpiration and increases stomatal resistance (Stancheva *et al*., 2014), especially Pb (Bharwana *et al*. 2013). A transpiration decrease leads to a reduction of water uptake intensity, and thence limits the uptake of metal ions from the substrate, which is a known stress avoidance mechanism (Wińska-Krysiak *et al*., 2015). In the present study, the exposure to Pb ions decreased the relative water content. In the control plants a very high RWC was observed accompanied by very good cell turgor. Plants treated $250mgPb.kg^{-1}$ soil showed significant decrease in RWC by 67% at $250mgPb.kg^{-1}$ soil (Table 2). Pb can alter the water relations by disturbing water balance throughout the effects on stomatal conductance, water transport and cell wall elasticity, and thereby influences the cell turgor pressure (Elzbieta and Miroslawa, 2005).

Decreased chlorophyll content that associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis Gangwar *et al*., 2011;Huang *et al*., 2013) or direct oxidative damage to the pigments (Oláh *et al*., 2010). SaiKachout *et al*. (2015) proposed for the reduction state of photosynthetic parameters to be a useful tool in bioassay toxicity testing of metal polluted soil. Carotenoids serve as antioxidants against free radicals and photochemical damage (Sengar *et*

*al*., 2008). Results of current study reveal no significant change in chlorophll a, and b and carotinoids (Table 2), indicated that sunflower plant may has a high ability to tolerate Pb stress. The deterioration of chlorophyll synthesis due to lead toxicity resulting in chlorotic leaves, changed ratios of chlorophyll a and b (Viehweger and Geipel, 2010) and photosynthetic activity (Küpper *et al*., 2007).

Many studies had reported an inhibitory effect of various heavy metals, including Cd and Pb, on plant growth (Sharma and Dubey 2005; Mishra *et al*., 2006). In agreement with these reports, results of this study showed that the growth of sunflower plants is reduced by Pb. The higher concentrations 200 and 250mgPb.kg$^{-1}$ soil reduced plant height by 6.6 and 7.6%, respectively (Table 3). The higher concentration of heavy metal has been reported to retard the cell division and differentiation, reduce their elongation and effect plant growth and development (Soares *et al*., 2001). Growth of sunflower was affected variably by the stress of Pb. Application of Pb produced no significant reduction in plant biomass parameters compared to control. The weak effects on physiological parameters were may be reflected on plant growth response and dry matter production. Moreover, application of 50mgPb.kg$^{-1}$ soil showed increase in root, shoot and whole plant dry weight compared with other Pb concentrations and control indicating low toxicity of Pb in the range of concentration. Increased Pb concentrations above 50mg Pb caused decreases in dry matter of root, shoot and whole plant. When analysing the dry weight of dissected organs, the weight of the shoot was significantly higher than that of the roots (Table 3). Other authors (Zhivotovsky *et al*., 2011; Hamadouche *et al*., 2012; Gupta *et al*., 2013; Hamvumba *et al*., 2014; Alves *et al*.,2016 ) found reductions of dry matter production in many plant species as a function of the application of increasing doses of Pb in experiments with soil and nutrient solution.

Based on the results presented in figures 1-3, it is clear that sunflower can uptake heavy metals to the plant tissue as previously reported by Spirochova *et al*.( 2003). From the experimental data it was clear that the largest portion of lead taken up by the plant was stored in the roots rather than in stem and leaves. This is because heavy metals are absorbed by roots from soil solution and later on translocated to leaves (through xylem vessels) where they are deposited in vacuoles (de Abreu *et al.,* 2017). Lead accumulation in roots is considered to be a factor that increases plant tolerance to Pb toxicity, because it prevents the metal translocation to leaves (Azad *et al*., 2011). Based on the experimental data it is clear that as the concentration of Pb in the soil increases the amount of metal taken up by the plant also increases. From figure 4 it is clear that the Pb concentration in the whole plant is less slightly than the metal concentration in the soil after harvesting. Based on lead accumulation in roots more than other parts of the plant, it could be concluded that roots play a very significant role in extra lead storage. The high Pb concentration, found mainly in roots, suggested that sunflower tend to avoid toxicity in the physiologically most active portions of the plants by reducing Pb translocation to the epigeous portion, and by promoting the re-translocation of toxic metals from shoots to the roots (Batista *et al*., 2017). The findings of Fulekar (2016) have proved the potential of sunflower plants for remediation of metals from contaminated soil-water environment. After harvesting, Pb concentrations in the soil increased linearly ($p \geq 0.05$) with increase of Pb doses added to the soil, the higher rate was (219.31mg.kg$^{-1}$) at 250mgPb.kg$^{-1}$ soil (Table 4). Heavy metal pollution can result in adverse effects on the composition of soil microbial community, adversely affecting soil characteristics (Xie *et al*., 2016). Alaboudi *et al. (*2018) obtained increase of Pb in the soil treated with 200mgPb .kg$^{-1}$ soil after harvesting of sunflower.

Bioconcentration (BCF), Translocation Factor (TF) factors are a key values that are needed to estimate a plant's potential for phytoextraction and phytostabilization. Table 5 depicted the significant ($p \geq 0.05$) reductions of BCF and TF in the roots, stems and leaves at high concentrations of Pb. The maximum BCF of Pb was found in roots followed by stem and leaf of sunflower. BCF was found approximately 9 times more in roots as compared to stems and 18 as in leaves at 250 mg concentration. BCF was found 2 times more in stems than leaves at the same concentration (Table 5). Plants exhibiting a shoot BCF >1 are suitable for phytoextraction, and plants with a root BCF >1 and TF<1 have the ability for phytostabilization (Zucchini *et al*., 2008; Sarawet and Rao, 2009). The results of TF revealed significant ($p \geq 0.05$) decrease at 200 and 250 mgPb for stems and at 250mgPb for leaves. All evaluated Pb concentrations showed TF < 1, which confirms the low ability to translocate Pb from roots to shoots (Table 6), a desirable factor for phytostabilization species. Translocation of the accumulated Pb metal from roots to shoots was limited as seen from TF values for this metal, which are less than 1 in all treatments. This is one of the limitations usually encountered in phytoremediation of toxic heavy metals. However, this limitation can be overcome by considering uprooting the plants in which case the accumulated heavy metals in the roots are removed from the soil(Ali *et al*., 2012). Tolerance index of roots, stems, leaves and whole plant were affected by the polluted soil treatments (Table 7). The extent of toxicity increased with increasing concentration of the metals. High polluted soil concentration had more severe inhibiting effect on leaves than on roots and stems. Results of this study are not agreement with the results of SaiKachout *et al*. (2015) who found that tolerance index of roots was more affected by the polluted soil with Pb treatments than on shoots. Also Atta *et al*. (2014) reported that Plant tolerance index (TI) reflected negative impacts of heavy metals on plant vigor.

Considering the results presented in Figures 1-3 so that sunflower plants accumulate the bulk of the absorbed Pb in their roots, it may be concluded that sunflower plants phytostabilize Pb. Cunningham *et al*. (1997) suggest that good hyperaccumulators can accumulate 1-3% Pb ions in leaves and stems. Data of this study showed the capability of sunflower to accumulate and tolerate significant quantities of Pb and thus its suitability for

phytoremediation. With the except for hyperaccumulator species, the tendency of low translocation and mobility of Pb, with intensive accumulation in the roots, has been largely documented in the literature for many plant species (Hamvumba *et al*., 2014; Kacálková *et al*., 2014; Selamat *et al*., 2014). Current data reveals the higher retention of Pb ions in the roots and the low translocation to the shoots (Figures 1, 2). However, it should be indicate that the data refer to the absorption in pots and the results must be confirmed with cultivation in soils.

## CONCLUSIONS

The present study suggest that sunflower with high biomass has efficiently removed lead demonstrate a suitable potential plant for cleaning of Pb metal polluted environment.

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