PHYTOEXTRACTION OF LEAD FROM POLLUTED SOIL BY *Silybum marianum* L.

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ABSTRACT

The process of remediation soils polluted by heavy metals require huge efforts and economic costs, therefore phytoextraction of heavy metals from these soils is very important, because it is a cost effective and environmentally friend method. This study conducted to estimate lead phytoextraction ability of Milk thistle (*Silybum marianum* L.) from soil polluted with lead. A pot experiment was conducted on Milk thistle under plastic house conditions at the Department of Horticulture, College of Agriculture, University of Duhok. The plant grown in soil polluted artificially with different concentrations of lead (40, 80, 160, and 320 mg. kg^{-1} soil) and irrigated with tap water. The study lasted about five months from January 2015 to the end of May 2015. At the end of the experiment the biomass of roots and shoots determined. Concentration of lead in roots and shoots also determined. Bioconcentration factor (BCF), translocation factor (TF), bioaccumulation factor (BAF), and removed lead also measured. Plant's biomass decreases as lead concentration in soil increased and the lowest biomass recorded in plants sampled from pots received higher lead. Lead content in shoots and roots of *S. marianum* ranged from (26.06-49.56) and (26.74-44.70) mg.kg^{-1} d.wt respectively. Values of (BCF), (BAF), and (TF) ranged from (0.13-1.07), (0.14-1.04) and (0.92-1.20) respectively. The amount of removed lead by each plant ranged from (213.3-285.6 µg. plant^{-1}). Milk thistle plants showed a good ability for translocation of absorbed lead from roots to shoots therefore it can be considered a hyperaccumulator plant of lead because TF was more than one.

KEYWORDS: Phytoextraction, Pb, ***Silybum marianum L.,*** *bio-accumulation factor (BAF), bio-concentration factor, and Translocation factor.*

INTRODUCTION

The pollution of the environment with heavy metals has become a global problem, therefore cleaning the soil from these metals is necessary (Angelova et al., 2018). Remediation of these soils require removal of these toxic heavy metals (Henry, 2000). Although the cleaning of the soils contaminated with heavy metals by conventional methods is very efficient, but they are very expensive, sever labor demanding and cause bad disturbance to soil biota (Pulford and Watson, 2003).

As an alternative to these traditional methods of environment cleaning, phytoextraction strategies have been developed; which is the use of some plants in order to remove heavy metals from the environment and accumulate it in the above ground tissues then removed by harvesting (Meers et al., 2005). Phytoextraction is a promising low cost technique compared with traditional methods (Evangelou et al., 2007). It is in situ methods for removing heavy metals from the soil and water (Fulekar et al., 2009).

Lead it is one of the most important toxic heavy metals that are present naturally in the earth crust (Gabos et al., 2009). But over 200 years ago a considerable amount of these metals have been emitted significantly more than natural sources (Clemens, 2006). These due to many industrials and commercial uses of lead such as in ceramic, medicals, batteries, solders, alloys, and in insecticides also lead used as anti knock in gasoline as tetraethyl lead or as tetramethyl lead (Kumar et al., 1995). The main source of lead pollution in the Iraq are automobiles (Mohamed et al., 2003). Tetraethyl or tetramethyl lead converted to inorganic salts and exhaust out of the engine of automobiles as halides, oxides, sulfates and carbonates (National academy of Science, 1972). Lead halides are easy instant in soil solution and more toxic to plants (Zimdahl and Koeppe, 1979).

Lead produced from human activities released into soil, water and air (Fulekar et al., 2009). When soil polluted with lead it can very easily incorporated into food chains (Awofolu, 2005). It enters human body through inhalation and by absorption, lead and other heavy metals are non biodegradable and accumulate in organism's tissues (Ali et al., 2012). Emission of high concentrations of lead affects human health in different ways, such as mental declining and cognitive effects of human (Paz-Alberto et al., 2007). Because lead is continuously used; its accumulation in the environment have been increased dangerously. (Nas and Ali, 2018).

Silybum marianum L. is belong to the family Asteraceae, and it is a biannual or annual plant grown originally in Southern Europe, Asia, North Africa, America and Australia, is now distribute overall the world (Ghafor et al., 2014 and Wianowska and Wiśniewski, 2014). In Iraq and Kurdistan Region milk thistle is a biennial weed plant that completes its live cycle in winter and spring. It consumed fresh; peeled stems and young heads are eaten raw, or mixed with sour materials such as sumac and lemon, also used as animal feed. In some area consumed as dietary supplement because of its benefits for liver health, in cosmetic, production of biofuel (Tournas et al. 2012). In Europe and USA used as nutritional supplements in various forms such as seeds, fresh herb, powder of the herb, oil of seeds and capsules (Andrzejewska et al., 2015). Many studies have shown that Milk thistle is tolerant to heavy metals pollution, it considered a hyperaccumulator plant of lead, and accumulate a considerable amount of cadmium and zinc. The plant can be successfully used in the phytoremediation of heavy metal contaminated soils (Angelova et al., 2018).

The main aim of this study is to evaluate the potential of lead phytoextraction ability of *S. marianum* L. and to estimate the values of lead transported from substrate through roots to shoots.

MATERIALS AND METHODS

The study was conducted under plastic house conditions during the period January 2015- end of May 2015 at the Department of Horticulture, College of Agriculture, University of Duhok, Kurdistan Region, Iraq.

Preparation substrate

Surface soil was collected from a depth of (0-20) cm from the fields of college of Agriculture-University of Duhok. The collected soil was air dried, and clods were crushed, then sieved through a 4-mm sieve. Sieved soil mixed with loam in ratio of (3 loam:1 soil). The chemical and physical properties of substrate are shown in table (1):

Table (1): Some chemical and physical properties of substrate.

Soil parameter	Unit	Value
Clay	%	69.5
Sand	%	11.1
Silt	%	19.4
Soil texture	---	Clay
E C	$(ds.m)^{-1}$	0.462
pH	---	7.4
Moisture	%	3.87
Total $CaCO_3$	%	13.74
Organic matter	%	0.95
Total Pb	mg. kg^{-1}	25

Lead addition

10 kg of the mixture (loam+soil) were added to the pots. Four concentrations of lead (40, 80, 160, and 320 mg. kg^{-1} soil) was applied to pots by dissolving analytically grade $Pb(NO_3)2$. in two litters of distilled deionized water. Because field capacity of the mixture was 20 % it means that we need two litters of water to confirm that all the soil present in the pot had been saturated with the added solution. in addition to control (cont.) which itself contains 25 mg. kg^{-1} soil of lead, all treatments were replicated five times.

Pots were placed in the plastic house for a period of one month. During that period, the pots were watered with deionized distilled water. This process was taken to enable added $Pb(NO_3)2$ to reach a steadiest condition (Blaylock *et al.,* 1997).

At the end of the incubation period (beginning of December), the pots were distributed on five blocks, each block contained five experimental units (five treatments; control, 40, 80, 160, and 320 mg. kg^{-1} soil). Each experimental unit composed from five pots. Randomized Complete Block Design (RCBD) with five replicates was adapted for the implementation of the experiment.

Source of seeds

Ten seeds of *Sylibum marianum* L. were planted in each pot (seeds were collected from the same region in the year prior to experiment). Pots were watered with tap water (specifications of irrigation water are represented in table 2). after full germinations and emergence of real leaf, seedlings were thinned to three plants for each pot. Saucers of 30 cm diameter were placed under pots to prevent leaching of water from pots. During the period of the experiments plants were irrigated with tap water only. Any weeds or foreign plants were removed immediately after the germination. Also we didn't record any cases of disease or insect infection on plants.

Table (2): Some chemical properties of water used for irrigation

Water characteristics	Unit	Value
Turbidity	NTU	0.1
Color	----	Clear
pH	----	7.27
EC	ds. m^{-1}	0.792
Total dissolved solids	$mg.l^{-1}$	506.7
Total alkalinity	$mg.l^{-1}$	386.0
lead	$mg.l^{-1}$	0.0062
Calcium	$mg.l^{-1}$	79.9
Magnesium	$mg.l^{-1}$	60.0
Chloride	$mg.l^{-1}$	21.4

Plants uprooting

The plants were uprooted at the end of April and before flowering. Shoots were cut at soil level, substrate of pots was broken carefully and all roots were collected. Both of roots and shoots gently washed with tap water and then with distilled water, then air dried for two days followed by drying in an oven at 70°C for 48 hours. Dry weight of roots and shoots was recorded.

Dry samples of roots and shoot were grinded well and 0.5 g of each sample was taken and putted in a 100 ml conical flask. Samples were wet digested by a mixture of HNO_3 and $HClO_4$ at a ratio (4:2) (Tandon, 1999).

Lead concentration in samples determined by Atomic Absorption Spectrophotometer type (G B C) in the laboratory of researches center in College of Agriculture, University of Duhok.

Analysis of data

Randomized completely block design (R C B D) was applied (Al-Rawi and Khalaf, 2000). The program Microsoft (S A S 2002) was used for statistical analysis. The differences between means of treatment were tested with Duncan Multiple Range test at 5% level (Duncan, 1955).

RESULTS AND DISCUSSION

The dry weight (plant biomass) of *S. marianum* L. (table 3) of control plants differed non-significantly from plants treated with (40 mg Pb. kg^{-1}soil) but both of them differed significantly from plants sampled from pots treated with (80, 160, and 320 mg Pb. kg^{-1}soil), also there were no significant differences among last three treatments. Roots biomass decreased spontaneously with the increased lead concentration, but non-significantly.

The reduction of *S. marianum* L. biomass is due to the various negative effects of lead such as reduction of photosynthesis rate through inhibition of chloroplast formation (Habash et al, 1995); inhibition in transport chain of both photo system 1 and 2 (Sharma and Dubey, 2005). Lead inhibit root hairs formation and lower the permeability of membrane (Mukhopdhyay and Maiti, 2010). Also inactivation of different enzymes throughout binding wit SH- groups have been recirded by (Pinho and Ladeiro, 2012). Mukhopadhyay and Maiti (2010) found that lead bind to the ion-carriers thereby preventing absorption and translocation of other essential metals for plant growth such as P, Ca, Mg, Zn, Mn, and Fe.

Table (3): Shoots and roots dry weight (g. plant^{-1}) of *S. marianum* L.

Pb levels	Shoots	Roots
cont.	8.22a	4.71 a
40	8.34 a	4.63 a
80	6.58 b	4.50 a
160	6.42b	4.08 a
320	5.50 b	4.07 a
Mean	**7.01**	**4.40**

In each column values with same letter are not significantly different.

Lead content in roots and shoots of *S. marianum* L.

Discovery of heavy metals concentration in plant tissues is a major step in developing phytoremediation process of soils impacted by toxic metals (Angelova, 2013). Lead concentration in shoots and roots of *S. marianum* L. is summarized in table 4, it can be seen that the higher lead content (49.56 mg. kg^{-1} d.wt) was measured in plants grown in pots treated with (320 mg. kg^{-1}soil), which differed non-significantly with treatment (160 mg. kg^{-1}soil), but both of them differed non-significantly from treatments (40 and 80 mg. kg^{-1}soil) which themselves all different significantly with control but non-significantly from each other.

However the higher lead content (44.70 mg. kg^{-1} d.wt) was recorded in plant roots treated with (320 mg. kg^{-1}soil) of lead but no significant differences recorded among it and both of (40 and 80 mg. kg^{-1}soil) treatments. Lead content in roots of control plants different significantly from all other treatments but non-significantly with (40 mg. kg^{-1}soil) treatment. These results are more than (20.5 and 20.6 mg.kg^{-1} dwt.) of Pb respectively in roots and shoots of *Silybum marianum* found by (Angelova, 2013). Also our results were more than (2.34 mg.kg^{-1} dwt.) of Pb in stems of *S. marianum* recorded by (Ghafor et al., 2014). On other hand our results were less than the results of (Del Rio-Celestino, 2006); who found (6211.4 mg.kg^{-1} dwt.) of Pb in roots and (735.4 mg.kg^{-1} dwt.) in shoots of *S. marianum* grown in pots contained 500 mg.kg^{-1} dwt. Pb. Also Sajad et al., (2018) recorded more lead in roots and shoots of Milk thistle (148.0 and 49.33 mg.kg^{-1} dwt.) respectively.

Duplication of lead concentration in substrate didn't show same manner of lead concentration in Milk thistle tissues, this because lead is one of the heavy metals that dissolve very slowly in the soil (Gabos et al. 2009).

Table (4): Lead concentration (mg. kg^{-1} d.wt) in roots and shoots of *S. marianum* L.

Pb levels	Shoots	Roots
cont.	26.06 c	26.74 c
40	33.72 b	33.78 bc
80	34.52 b	37.56 ab
160	44.34 a	38.38 ab
320	49.56 a	44.7 a
Mean	**37.64**	**36.232**

In each column values with same letter are not significantly different.

Biocncentration (BCF), translocation (TF), and bioaccumulation (BAF) factors

The ability of plants to concentrate heavy metals from soil estimated by the biocncentration factor (BCF) ***[Pb in roots] / [Pb in soil]*** which is a ratio between metals concentration in roots and soil (Qu et al., 2011). The absorbed metal must be transported to the above ground parts in order to be easy for harvesting, plants ability for translocation of metals from roots to shoots is measured as translocation factor (TF) ***[Pb in shoot] / [Pb in roots]*** which is a ratio between metal concentration in shots and roots (Qu *et al.*, 2011). Another good indicator of phytoextraction is the bioaccumulation factor (BAF) ***[Pb in shoot] / [Pb in soil]***, which is the metal content in the plant shoots compared to metal concentration in the substrate (Goswami *et al.*, 2010)

In table (5) we can see significant differences in BCF among all treatments except between treatments (160 and 320 mg Pb. kg^{-1}soil) which was non-significant. The value of TF of all treatments shows insignificant differences. BAF of control plants and treatment (40 mg Pb. kg^{-1}soil) differed significantly from all other treatments.

It is clear that the BCF and BAF decreased as lead concentration in the soil increased, because of low lead content in roots and shoots, it can be explained by the fact that the higher lead concentration in the soil does not mean necessarily high lead content in plant tissues (shoots and roots) because of low lead solubility (Gabos et al, 2009).

According to Suchkovaa et al (2010) a hyperaccumulator plant are that plants that transport amount of metal from their roots to their shoots to achieve translocation factor more than 1; in our study the mean translocation factor of all treatments is slightly exceeded 1 therefore *S. marianum* can be considered a Pb hyperaccumulator plant.

Table (5): BCF, TF, and BAF of ***S. marianum*** L.

Pb levels	BCF	TF	BAF
Cont.	1.07 a	0.99 a	1.04 a
40	0.52 b	1.00 a	0.52 b
80	0.36 c	0.92 a	0.33 c
160	0.21 d	1.20 a	0.24 cd
320	0.13 d	1.12 a	0.14 d
Mean	0.46	1.05	0.45

In each column values with same letter are not significantly different.

Removal lead

Figure (1) shows removal lead by individual plants of *S. marianum* which calculated from the equation ***[Pb in shoots]* × *[plant weight]*** it can be seen that removal lead by plants in pots treated with (40 and 160 mg Pb. kg^{-1}soil) didn't different significantly from each other but significantly from treatments (control; 80 and 320 mg Pb. kg^{-1}soil). No significant differences appeared among plants of three treatments. The maximum removed lead (285.66 µg. $plant^{-1}$) obtained from plants grown in pots polluted with (160 mg Pb. kg^{-1}soil).

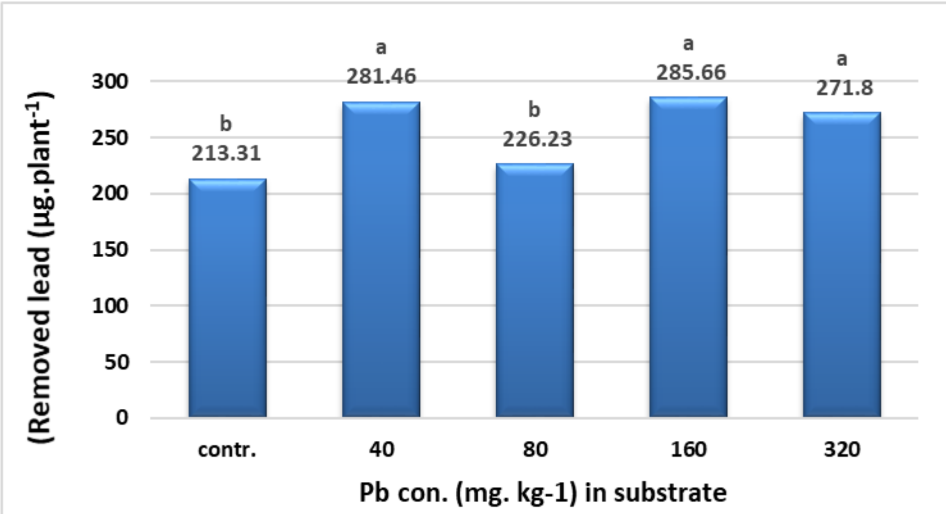

Fig. (1): Removed lead (µg. $plant^{-1}$) by shoots of *S. marianum* L.
Values with same letter are not significantly different.

CONCLUSIONS

In conclusion, it is evident that this study has shown that *S. marianum* L. can be considered a hyperaccumulator plant because it was able to transport same amount of lead from roots to shoots (TF was more than one), therefore it is useful for phytoextraction of lead from polluted soils.

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هەلمژاناندنا رووەكی بو قورقوشم ژ ئاخێت پیس ب رێكا قیڤارێ Si l ybummar i anumL.

پوخته

پروسێسا پاكرنا ئاخێ ژ توخمێن ب سەنگ پێدڤی كارەكێ مەزن و مەزاختنا دارایێ یە، ژ بەر ڤێ چەندێ هەلمژاندنا رووەكی بو توخمێن ب سەنگ ژ ڤان ئاخا گەلەك یا گرنگە ژ بەر كەلەك مفایێت وێ یێت هەین وەك مفایێت دارایی و هەر وەسا رێكەكا هەڤالە بو ژینگەهێ. ئەڤ ڤەكولینە هات ئەنجامدان لسەر رووەكێ قیڤارێ **Silybum marianum L**. ژ بو زانینا هەلمژاندنا رووەكی یا فی رووەكی بو توخمێ قورقوشەمی ژ ئاخێت پیسبوی ل بن بارودوخێت خانیێ پلاستیكی ل پشكا بیستان كاری ل كولیژا چاندنی-زانكویا دهوكێ. رووەك هات بی چاندنێ دناڤ قافكیت پلاستیكی دا و ئاخا ڤان قافكا هات بی پیسكرن ب چەند ئاستێن قورقوشمی ، وئەوژ (**40** ، **80** ، **160** ، **320** ملغ.كغ-**1**) وهاتبی ئاڤدانێ ب ئاڤا حەنەقییێ. ئەڤێ ڤەكولینێ بو ماوێ پێنج هەیڤا ڤەكێشا ژ كانوینا دووێ **2015** تا دوماهیكا گولانا **2015**. لدوماهیكا ڤەكولینێ بایوماسا رها وچەقا و بەلگا هات پیڤان. كومبینا قورقوشمی ل ناڤ رە و چەقا دا ژ هات وەرگرتن. فاكتەرێ كومبینا چراتیتا قورقوشمی (**BCF**) ، فاكتەرێ وەرگێرانا قورقوشمی ژ رها بو چەق و بەلگا (**TF**) ، فاكتەرێ كومبینا بایولوجی یێ قورقوشمی (**BAF**) و وچەندیا قورقوشمی هاتی راكرن ژ ئاخێ ب رێكا هەر رووەكەكێ ژ هات پیڤان. بایوماسا قیڤارێ كێمبی دگەل زیدەبینا رێژا قورقوشەمی دناڤ ئاخێ دا و كێمترین بایوماس یا وان رووەكا بی ئەوێت هاتین چاندنێ ل وان قافكیت پتر قورقوشم تێدا هەی. چراتییا قورقوشمی ل ناڤ چەق و رهێت قیڤارێ ب ڤی رەنگی بی (**26.06 – 49.56** ملغ.كغ-**1** كێشا. هشك) و (**26.74 – 44.70** ملغ.كغ-**1** كێشا. هشك) ل دویف ئێك. چەندیا (**BCF**) و (**BAF**) و (**TF**) ب ڤی رەنگی بی (**0.13 – 1.07**) و (**0.14 – 1.04**) و (**0.92 – 1.20**) ل دویف ئێك. و وچەندیا قورقوشمی هاتی راكرن ژ ئاخێ ب رێكا هەر رووەكەكێ (**213.30 -285.60** مایكروغرام.رووەك -**1**). قیڤارێ دیاركر كو شیانێت باش یێت هەین بو ڤەگوهاستنا قورقوشمی ژ رها بو چەقا و بەلگا، ژبەر ڤێ چەندێ ئەم دشین بێژن ڤی رووەكی هایپەراكیومیولیتر ژبەر فاكتەرێ وەرگێرانا قورقوشمی ژ رها بو چەق و بەلگا پتر بی ژ ئێكێ.

الاستخلاص الخضري للرصاص من الترب الملوثة بواسطة الكلغان *Silybum marianum* L.

الخلاصة

ان عملية أستصلاح الترب الملوثة بالعناصرالثقيلة تتطلب جهد كبير وتكاليف اقتصادية هائلة، لذلك فأن الاستخلاص الخضري للعناصر الثقيلة من الترب الملوثة ذات اهمية كبيرة وذلك لكونها عملية اقتصادية وصديق للبيئة. نفذت هذه التجربة لدراسة قابلية نبات الكلغان في الاستخلاص الخضري للرصاص من الترب الملوثة به. طبقت تجربة سنادين على نبات الكلغان تحت ظروف البيت البلاستيكي في قسم البستنة، كلية الزراعة، جامعة دهوك. زرعت النباتات في تربة ملوثة اصطناعياً بتراكيز مختلفة من الرصاص (40، 80، 160، 360 مغ.كغ $^{-1}$ تربة) وتمت عملية السقي بماء الحنفية. استغرقت فترة الدراسة خمسة اشهر من الكانون الثاني 2015 الى نهاية شهر مايس 2015. في نهاية التجربة تم قياس الكتلة الحيوية للجذور والمجموع الخضري. وتم ايضاً تقدير العناصر الثقيلة في الجذور و المجموع الخضري. كذلك تم تقدير معامل التركيز الحيوي (BCF) و معامل الانتقال (TF) و معامل التراكم الحيوي (BAF) و كمية الرصاص المزالة بواسطة النبات. الكتلة الحيوية انخفضت بأزدياد تركيز الرصاص في التربة وأقل كتلة حيوية كانت للنباتات المزروعة في التربة الحوية على اعلى تركيز للرصاص. تركيزالرصاص في اوراق و جذور الكلغان تراوح مابين (26.06-49.56 مغ.كغ $^{-1}$وزن جاف) و (26.74-44.70 مغ.كغ $^{-1}$وزن جاف) على التوالي. قيم ال (BCF) و (TF) و (BAF) تراوحت ما بين (0.13-1.07) و (0.14-1.04) و (0.92-1.20) على التوالي. وأن الكمية المزالة من الرصاص تراوحت ما بين (213.30-285.60 مايكروغرام. نبات $^{-1}$). اظهرت نباتات الكلغان قابلية جيدة لانتقال الرصاص الممتص من الجذور الى الاوراق، لذلك يمكن اعتبار هذا النبات ذات معامل تراكمي عالي للرصاص حيث كان كان معامل الانتقال للرصاص اكبر من واحد.