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Study the capability of *Ecliptaalba* plant to remove of lead from the polluted water by phytoremediation technique

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Abstract

One of the major environmental problems is the pollution of water and soilsby toxic heavy metals.The aim of current study was estimatation the ability of *Eclipta alba* plant for the removal of (Pb) from the polluted water by phytoremedation technique and study the effect of a number of parameters(Pb concentration, contact time and pHvalues). The plant showed higher removal efficiency (99.2%) after 7 days of treatment at 50ppm.The best removal efficiency of Pb 98.95% at 100ppm was occurred at pH7. The root ability to accumulate Pb was more than the shoot ability, after 7 days treatment of water and pH=7, they were 2852.5 mg/kg, 2497.5 mg/kg and 502.5 mg/kg in roots, stems and leaves respectively. These results showed that*Ecliptaalba* can be used in biological treatment of polluted water.

Keywords: *Ecliptaalba*, phytoremediation, lead, industrial waste water.

دراسة قدرة نبات الفريش لازالة عنصر الرصاص من المياه الملوثة بوساطة تقنية المعالجة بالنبات

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الخلاصة

تعد مشكلة تلوث المياه والتربة بالعناصر الثقيلة السامة من اكبر المشاكل البيئية. هدفت الدراسة الحالية الى تقدير قابلية نبات الفريش في ازالة عنصر الرصاص من المياه الملوثة بواسطة تقنية المعالجة النباتية مع دراسة تأثير عدد من العوامل المتغيرة(تركيز الرصاص، زمن التلامس و الاس الهيدروجيني). اظهر النبات كفاءه ازالة عالية (٩٩.٢%) بعد مرور سبعة ايام من المعالجة عند التركيز ٥٠ جزء بالمليون. ان افضل كفاءة ازالة لعنصر الرصاص(٩٨.٩٥%) عند التركيز ١٠٠ جزء بالمليون كانت عند الرقم الهيدروجيني ٧. كما اظهرت الدراسة ان جذور النبات تمتلك قابلية اكبر لتراكم عنصر الرصاص مقارنة بالسيقان والاوراق، بعد مرور ٧ ايام من المعالجة وعند الرقم الهيدروجيني ٧،اذ بلغ ٢٨٥٢.٥ ملغم/كغم و ٢٤٩٧.٥ ملغم /كغم و

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٥٠٢.٥ ملغم /كغم على التوالي. من خلال هذه النتائج يمكن استخدام نبات الفريش في المعالجة البايولوجية للمياه الملوثة.

Introduction

Several methods are already being used to clean up the environment from those kinds of contaminants, but most of them are costly and far away from their optimum performance. For at least 300 years, the ability of plants to remove contaminants from the environment has been recognized and taken advantage of in applications such as land farming of waste. Over time, this use of plants has evolved to the construction of treatment wetlands or even the planting of trees to counteract air, soil and water pollution [1]. Presently, phytoremediation has become an effective and inexpensive technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil and waterThis technology is environmental friendly and potentially cost effective. Phytoremediation takes the advantage of the unique and selective uptake capabilities of plant root systems, together with the translocation, bioaccumulation, and contaminant degradation abilities of the entire plant body [2].

Many species of plants have been successful in absorbing contaminants such as lead, cadmium, chromium, arsenic, and various radionuclides from soils and water. Among the existing pollutants, lead (Pb) is the major contaminant of the soil and water [3] which has significant environmental problems [4], including the risk of poisoning for humans and especially children [5].

Plants of the families Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Scrophulariaceae have been identified as for the extraction of lead from the soil [1]. Boonyapookana*et al.* [6] studied the hyperaccumulation potential for Pb of sunflower, tobacco and vetiver, and concluded that all three accumulated Pb in their leaves and stems, but the first one was the most efficient accumulator. Thus, this work is aimed to study the ability of *Ecliptaalba* plant for lead removal from the polluted water by phytoremediation technique.

Materials and Methods

Collection and growing of plant samples

The plant (*Ecliptaalba*) from the family (Asteraceae) (figure-1), was collected locally from different places near the channel around Baghdad University during October 2013 to May 2014.The collected plants were washed with clean tap water to remove dirt on the herbs .Cuttings with leaves of about 15cm were placed in distilled water and kept for rooting in water and then transfered to beakers filled with Hoagland’s hydroponics solution (containing macro and micro elements necessary for the plant growth and rooting) which prepared as described by [7].These propagules were used in the experiments.

Figure 1-*Ecliptaalba*grown in Baghdad University gardens

Estimation of lead concentration in the plant tissues

The concentration of lead ion (Pb^{+2}) in the plant samples (*E.alba*) was estimated before and after the treatment with industrial waste water.

Preparation of standard aqueous solutions

Lead standard aqueous solution was prepared depending on the atomic and molecular weight of Pb [8] as following: A standard solution of (1000 ppm) of lead Pb was prepared by dissolving 1.56g of lead acetate($Pb(C_2H_3O_2)_2$) in 1000ml of distilled water .This solution was passed through 0.45Mm membrane filter and served as a stock solution for the preparation of the required concentrations for the experiment.

Study the efficiency of *E.alba*for Lead removal

1-The effect of lead concentration and contact time

Five concentrations of lead (40, 50, 65, 85,100ppm) were selected and prepared according to the concentration of lead in the industrial waste water.Plastic containers of (40x30x15) cm was used and filled with 4liter of each concentration.Ten well rooted propagules were put in each container and three replicates were used for each treatment. Collection of water samples from all replicate containers every day from 1 to 11 days to estimate the residual quantity of lead by Atomic absorption spectrophotometer after treatment.

2-The effect of pH

The effect of pH on the uptake of lead (Pb) by *E. alba* was done by growing the plant in a range of pH values (4- 9), acidic and alkaline pHs were adjusted by adding dilute HCl and KOH respectively.

Plant analysis for estimation of pb concentration in plant tissues after phytoremediation process

For metal analysis, 0.5 g of roots, stems and leaves samples were taken. 5 ml of nitric acid (65%) and 1 ml of perchloric acid (70-72%) was added [9]. The digestion was allowed to proceed in microwave digester at 80 °C for 15 minutes. It was cooled and then the digests were filled up to 50 ml with distilled water [10]. Finally, 20ml of each sample solution was taken and used to determine Pb concentration using flame atomic absorption spectrophotometer.

Calculation of Removal efficiency (R.E)

The removal efficiency was calculated according to the following equation[11].

$$\text{Removal Efficiency (Re)\%} = \frac{C_0 - C_f}{C_0} \times 100$$

❖ *Where:*

C_0 = *the initial metal concentration (mg/l).*

C_f = *the final concentration (mg/l).*

Statistical analysis

The data were analyzed statistically by using statistical program, Anova test.

Results and discussion

The concentration of lead ion (Pb) in *E. alba* tissues before phytoremediation process

The analysis to different tissues of *E. alba*parts (roots, stems and leaves) for determination of Pb concentration inthe tissues before using in treatment of polluted waste water (control) showed that the concentration of lead was 0 mg/Kg. This means that the plant is free of pb ion in its tissue before phytoremediation process.

The removal efficiency of lead by *E. alba* from aqueous solutions

The effect of Pb ion concentration and time

E. alba plant shows higher efficiency for the removal of Pb ions from aqueous solution. The highest removal efficiency (RE) was 99.2% at 50ppm after 7 days of treatment while the lowest RE was 4.88% at 100ppm after one day of treatment (figure- 2).

The removal efficiency (RE) was ranged from 98.28% to 4.88% (mean 67.41%) after one day of treatment. It was noted that the RE was increased with increasing of the incubation time; they reached maximum 99.2% (mean 84.65%) after seven daysof incubation for all treatments.Further increase in incubation time, the RE was slowly decreased in all treatments (table 1).

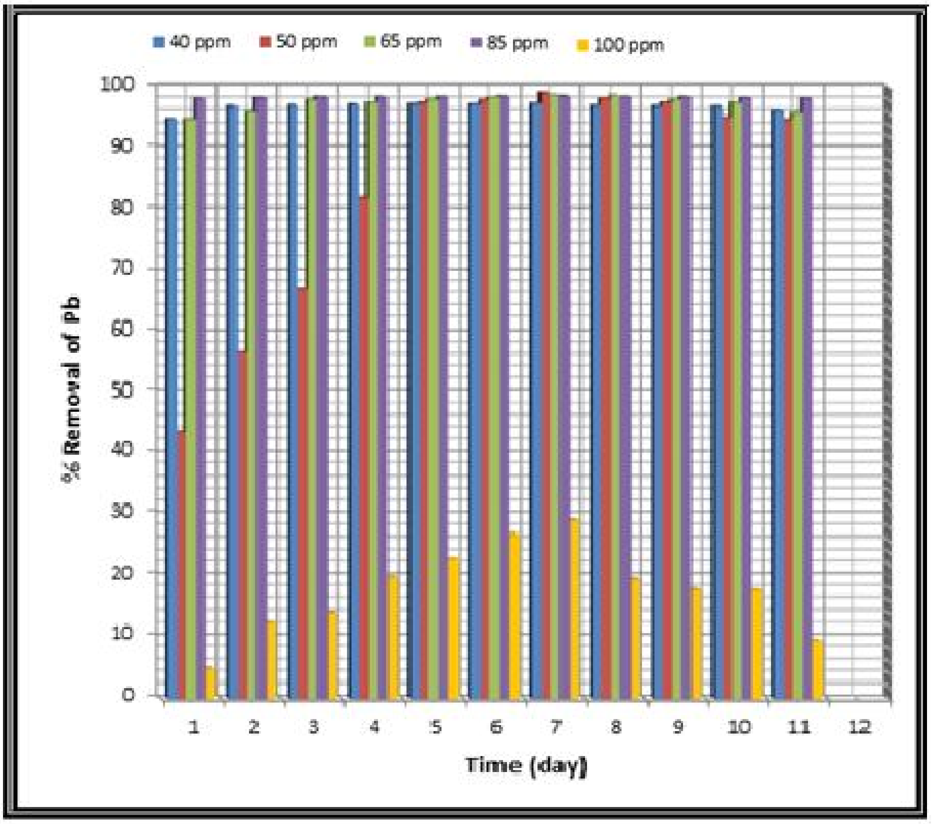

Figure 2- Effect of concentration and time on removal efficiency by *E. alba*

Table 1- Removal efficiency of lead at 5 different concentrations

day	R.E% 40ppm	R.E% 50ppm	R.E% 65ppm	R.E% 85ppm	R.E% 100ppm	Mean
1st	95.19	43.6	95.13	98.28	4.88	67.41
2nd	96.93	56.74	96.21	98.43	12.5	72.16
3rd	97.12	67	98.01	98.51	14.2	74.96
4th	97.27	81.99	97.56	98.53	19.9	79.05
5th	97.29	97.52	98.06	98.58	22.82	82.85
6th	97.28	97.99	98.46	98.6	26.86	83.83
7th	97.36	99.2	98.85	98.66	29.2	84.65
8th	97.02	98.2	98.78	98.58	19.6	82.43
9th	96.97	97.54	98.07	98.51	17.8	81.77
10th	96.87	95.4	97.56	98.35	17.7	81.17
11th	96.37	95	96	98.29	9.5	79.03
Mean	96.87	84.56	97.51	98.48	22.58	

The results of Anova test for the Pb remaining in the solution (table2) at probability level ($P \leq 0.05$) showed significant differences at the 2nd day for the concentrations (40, 50, 65, 85 ppm) while the concentration 100ppm showed not significant differences with the other concentrations of the experiment.

The 3rd, 5th, 6th, 7th and 8th days revealed significant differences for all the concentrations (40, 50, 65, 85 and 100ppm). At the 4th, 9th, 10th and 11th days, the concentration 85 ppm showed not significant differences with the other concentrations (40, 50, 65, and 100ppm) which they showed significant differences at $P \leq 0.05$.

Anova test was done for each concentration of Pb with the days of the experiment and there were found that the concentrations 40, 50 and 65ppm showed significant differences at $P \leq 0.05$ between all the 11 day of the experiment .

The concentration of 85 ppm showed not significant differences at the 4th, 9th, 10th and 11th days while showed significant differences at the 2nd, 3rd, 5th, 6th, 7th and 8th day of the experiment. On the other hand, the concentration 100ppm showed not significant differences between the 2nd and the 3rd day while showed significant differences for all the other days of the experiment.

The metal uptake mechanism is particularly dependent on the heavy metal concentration. In the present study*E.alba*showed higher removal efficiency for Pb ion ranged from 95.19% to 98.28% at the concentrations (40, 50, 65, 85 ppm); while the lowest RE was (4.88%) at 100ppm after the first day of treatment. In our study, the optimum Pb concentration was 50ppm because it gives the highest removal efficiency (99.2%) after 7 days of treatment (figure-2).

It is clear from Figure-2 that the removal efficiency of Pb ion increases at lower and medium concentrations while decreased at higher concentrations .These observations can be explained by the fact that at low concentrations of metal ion (Pb),the ratio of sorptive surface area to the total metal ions available is high and thus, there is a greater chance for Pb removal capacity. When Pb ion concentrations are increased, binding sites become more quickly saturated, therefore, more Pb ions were left unabsorbed in solution at higher concentration levels.

Contact time is one of the impact parameter on the successful of phytoremedation process. It is evident from figure -2 that the removal of metal ion increases as the contact time increases
Initially, the rate of uptake is fast. However, equilibrium was attained in 7 days. Further increase in contact time to 11 days, the percentage adsorption decreases very slowly. Therefore, the contact time was optimal at 7days. Similar data was recorded by [12] when he used Duckweed (*Lemna minor*) in the removal of some heavy metals. Gallardo *et al.*[13] found that after one week of exposure of*Hydrilaverticillata* to concentrated lead solution, maximum uptake (98%) of Pb was observed. Al-Bayati[14] found that the RE of Pb by *Eichhorniacrassipes*reached to (100%, 100%, 99.6%, 99.6%, 99.4%, 99.4%, 98.9%, and 96.33%) for the concentrations (1, 3, 6, 10, 12, 15, 20 and 30 ppm) respectively after 10 days of treatment.

Table 2-The residual lead remaining after treatment by *E. alba*at different concentrations of Pb

Day	Residual Pb at 40ppm	Residual Pb at 50ppm	Residual Pb at 65ppm	Residual Pb at 85ppm	Residual Pb at 100ppm
1st	1.92	28.2	3.16	1.45	95.12
2nd	1.22	21.63	2.46	1.33	87.5
3rd	1.15	16.5	1.28	1.26	85.8
4th	1.08	9.002	1.58	1.24	80.1
5th	1.08	1.24	1.25	1.20	77.18
6th	1.08	1.004	0.99	1.17	73.14
7th	1.05	0.4	0.74	1.13	70.8
8th	1.19	0.9	0.79	1.20	80.4
9th	1.21	1.23	1.25	1.26	82.2
10th	1.25	2.3	1.58	1.40	82.3
11th	1.45	2.5	2.6	1.45	90.5

The effect of pH on the removal of lead

The pH value of the solution is an important controlling parameter in the removal of heavy metals. It seems clearly that increasing the pH values have significant effects up on the ability of *E .alba* on removal of Pb ions.

The results were presented in table 3, showed that the removal efficiency (RE) of Pb ions from aqueous solution at (100ppm) was affected by the pH. The higher RE reached 100% was observed at pH 9 and after 7days of treatment, while the minimum RE was 0% at pH 4 after one day of treatment. It was noticed that as the pH increases, the RE also increases and reaches to the optimum values at pH above 6.

The results also showed that with pH values 8and 9(above 7), Pb ions are precipitated as lead hydroxide[15] and this case could give wrong initial concentration. Therefore, the optimum pH for removal of Pb ions fixed as pH 7 because it gives the highest RE (98.95%) after 7days of treatment figure- 3.

In addition, it was interesting to note that there was fluctuation of pH values for Pb solution after application of*E.alba*. Both acidic and alkaline pH of the solution shifted slightly towards neutral pH with increase in the number of days. This change in pH may be due to release of some root exudates in response to the stress so as to adapt itself to the existing environment. Thus, the result of present study shows that the plant *E. alba*has the pH neutralizing capacity.

Table 3- The removal efficiency of Pb by *E. alba*after using 6 different pH values

Day	R.E% pH=4	R.E% pH=5	R.E% pH=6	R.E% pH=7	R.E% pH=8	R.E% pH=9
1st	0	12.4	4.88	49.6	72.6	91.5
2nd	3.7	12.8	12.5	63.2	76.6	93.1
3rd	4.5	18.5	14.2	95.1	97.2	99.15
4th	5.1	20.7	19.9	95.2	97.48	99.85
5th	6.5	21.2	22.82	98.28	97.93	99.61
6th	7.8	51.6	26.86	98.91	98.21	99.89
7th	9.5	52	29.2	98.95	98.73	100
8th	6.2	32.3	19.6	98.88	99.11	100
9th	1.7	26.4	17.8	98.32	98.2	100
10th	0	22.7	17.7	97.61	98.21	100
11th	0	18.84	9.5	97.19	98.25	100

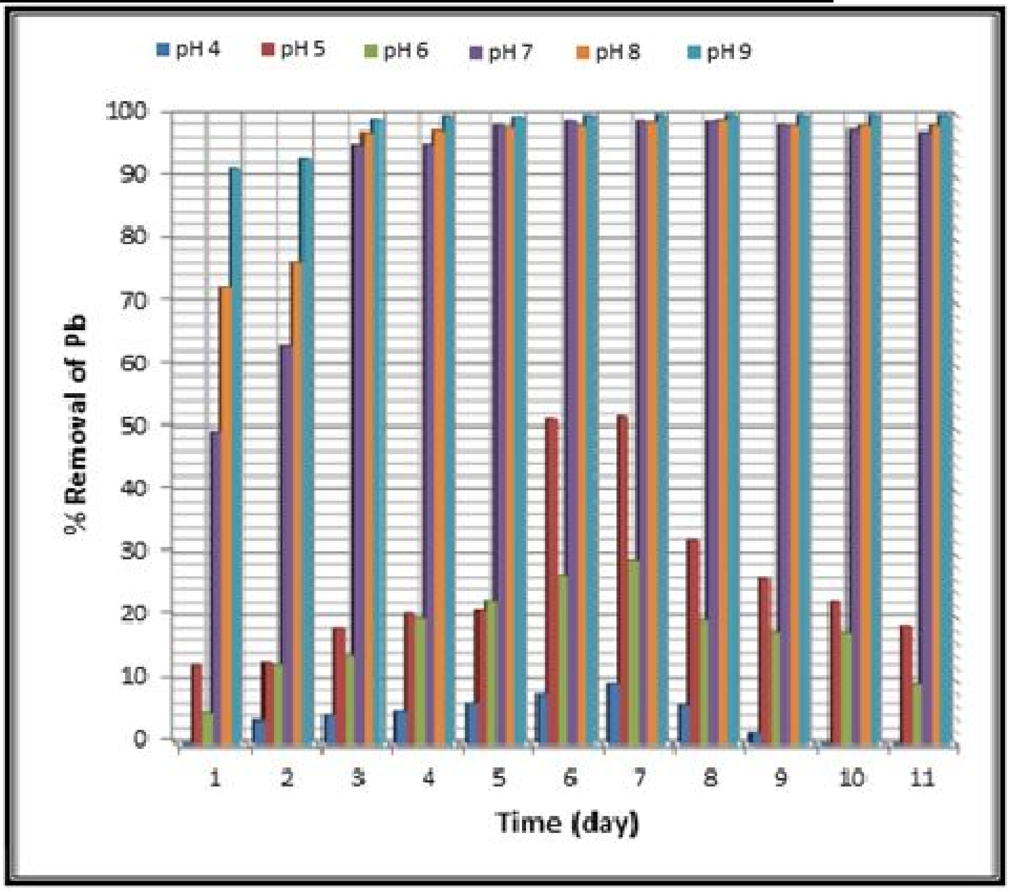

Figure 3- The removal efficiency of Pb at 6 different pH values

Anova test for the remaining of Pb ions in the solution (table 4) revealed significant effects of increasing pH values at levels (P≤ 0.05), also this metal was significantly affected by these pH levels at the same probability.

It was noticed that the ability of removing lead ions depends on the pH values of the solution and this depends on the ion state and nature of material. At low pH value, binding sites are generally protonated or positively charged (by the hydronium ions). Thus, repulsion occurs between the metal cation and the adsorbent. As the pH value increase, the binding sites start deprotonating and makes different functional groups available for metal binding. In general, cation binding increases as pH increases [16, 17].

The result obtained from current study was in accordance with the results of other authors.
Goswami*et al*. [18] found that *Eichhorniacrassipes*has the maximum removal efficiency of Pb at neutral pH.

Baharudin and Mohd [19] found that the constructed wetland containing 15-plants recorded the highest removal with 99.28% for lead at neutral condition (pH 7) compared to the base and acidic conditions.

However other studies obtained different results either less or more than of our study (pH 7). The amount of lead absorbed by plants is affected by the pH and to reduce lead uptake by plants, the pH of the soil or water is adjusted to a level of 6.5 to 7 [20].

While, Uysal and Taner[21] examined the ability of the *L. minor* to remove soluble lead under different pH values (4.5-8.0) and temperature (15-35°C) in presence of different Pb concentrations 0.1-10.0 mg/L for 7 days. They notice Pb accumulation at pH 4.5 and then it decreased to pH 6, but it did not change at pH range 6-8.

Table 4- The residual Pb ion remaining in the solution after treatment by *E. alba*at different pH values.

Day	pH=4	pH=5	pH=6	pH=7	pH=8	pH=9
1st	100	87.6	95.12	50.4	27.4	8.5
2nd	96.3	87.2	87.5	36.8	23.4	6.9
3rd	95.5	81.5	85.8	4.9	2.8	0.85
4th	94.9	79.3	80.1	4.8	2.52	0.15
5th	93.5	78.8	77.18	1.72	2.07	0.39
6th	92.2	48.4	73.14	1.09	1.79	0.11
7th	90.5	48.03	70.8	1.05	1.27	0
8th	93.8	67.7	80.4	1.12	0.89	0
9th	98.3	73.6	82.2	1.68	1.80	0
10th	100	77.3	82.3	2.39	1.79	0
11th	100	81.16	90.5	2.81	1.75	0

The accumulated lead (pb) ion in *E. alba* tissues after phytoremediation process

According to plant analysis with dry weight, the concentration of pb in *E. alba* tissues after 7 days at pH 7 was estimated. The average values 2852.5, 2497.5 and 502.5 mg/kg were observed in roots, stems and leaves respectively (figure- 4 and figure-5).

The results showed that *E. alba* had the ability to accumulate large quantities of lead, especially in roots comparing to stems and leaves. Ashokkumar*et al.*[22]reported that *E. alba*has the ability to uptake and accumulate heavy metals including Pb from polluted soils.

Metal concentrations in plants vary with plant species [23, 24, 25 and 26]. The high metal uptake may be attributed to highly efficient intracellular compartmentalization. Effective parameters of metal uptake by plants were reviewed to be genetic architecture of metal accumulation as well as binding ligands such as organic acids, amino acids, peptides and proteins including citrate, malate, histidine, nicotineamine, glutathione, phytochelatins, metallothioneins[27].

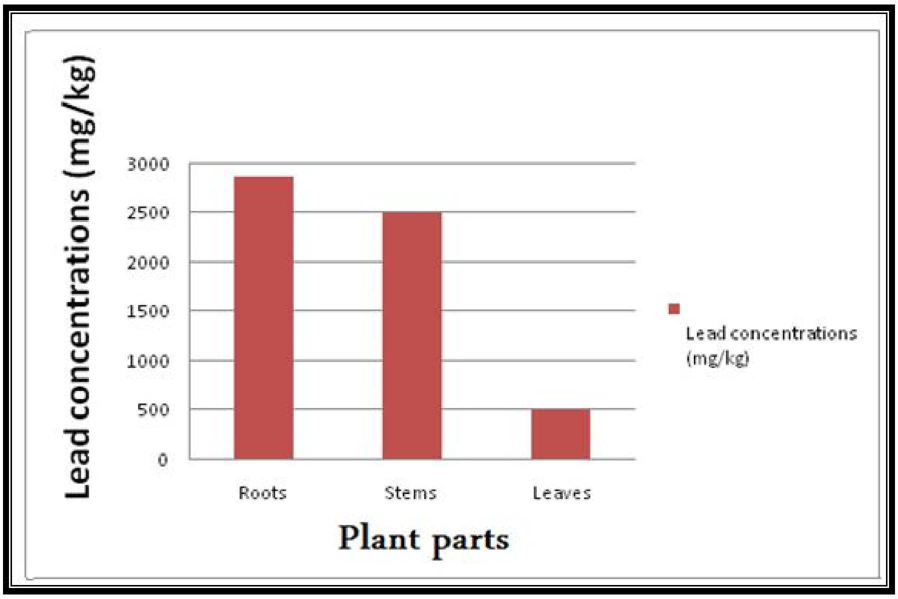

Figure 4- Lead concentrations (mg/kg) in *E. alba* parts after phytoremediation process

Figure 5-*E. alba* plant in the polluted water after 7 days of incubation at pH 713

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