

## PHYTOREMEDIATION EFFECTS OF SOME TURFGRASS SPECIES IN DIFFERENT CONTAMINATED CONDITIONS

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### ABSTRACT

Remediation of toxic metals, including Pb and other pollutants has been an important research subject for environmental studies. In a complete controlled growth chamber we prepared different Pb treatments and pH values. Four turfgrass species were used in order to study the potential role of species that may play an important role for environmental remediation of contaminated soils, and possible mechanisms of Pb translocation from root to shoot system using kinetic studies.

Results revealed that, after 14 days most of Pb accumulated more in root than shoot. Increasing pH value for one unit particularly at high rate of Pb show that, mean values of the ratio root/shoot system reached to almost 12 times. Results also indicate that increasing Pb concentration, gradually increased Pb content in both root and Shoot. It was noticed that *Spartina Patens* (D) species, showed higher content of Pb as compared with other Species. Data showed that increasing Pb concentration up to 500 ppm led to increase the dry matter for both *Centipede grass* and *Buffalo grass*. The obtained results also show that rate constant values of  $\beta$  were affected by plant species and part of plants as well. Rate of the translocation of Pb from root to shoot parts Showed that increasing pH values from 4.5 to 6.5 led to increase the translocation of Pb from root to shoot.

Our results emphasize that turfgrass can accumulate substantial amounts of Pb and thus could be a major component of such a green liver particularly in urban and Sub urban regions.

### INTRODUCTION

In the time when population of the world is increasing drastically, the quality of agricultural land is deteriorating for many reasons. One of the main reasons is related to soil contamination with heavy metals such as Pb creating from industrial areas, mining activity, pesticides and application of high amounts of sewage sludge. Heavy metals are persistent unlike other contaminants because they are not degradable and their residence time in the soil is a matter of thousands years. Contaminated soils will become unsuitable for food production due to the restrictions for human and animal health, decreased plant growth and ground cover, and negative impact on soil microorganisms (McGrath *et al.*, 1998).

Remediation of toxic metals, including Pb, has been an important research subject for environmental studies. For example, metal remediation in USA cost multibillion-dollar to minimize industrial pollution, usually relying on engineering-based technologies, such as isolation and contamination, and decontamination by physical, chemical or biological treatments (Cunningham and Berti, 1997). Over the past decade Phytoextraction of heavy metals contaminated soils is a great challenge for the modern environmental technologies especially if these metals have ecotoxicological effect such as lead (Pb).

Today and in many cases, the soils are not actually cleaned, but simply removed from the site of contamination and deposited somewhere else, where they are supposed to cause no harm to humans or nature. This is an expensive method and, more importantly, it will not be sustainable in the long run. The development of economically and environmentally sustainable methods is therefore of utmost importance. One of the most recent techniques which have been developed during the last couple of decades, is phytoextraction, where plants are used to remove the contaminants from the soil. The identification of metal hyperaccumulator species demonstrates that plants possess the genetic potential to remove toxic metals from contaminated soil. Understanding the plant-based remedial mechanisms is important for several reasons. For example, the elucidation of these mechanisms may provide clues for optimizing the effectiveness of phytoremediation with appropriate agronomic practices. In addition, the identification and biochemical characterization of the remedial mechanisms are necessary preliminary steps to isolate plant genes responsible for the expression of the remediating phenotype. The identification and isolation of these genes may open the opportunity to use biotechnology to ameliorate plants for environmental cleanup. Phytoextraction takes advantage of this ability. The plants are grown on contaminated area, harvested and incinerated or composted to increase the concentration of pollutants. The plant material is then deposited, but in some cases of metal phytoextraction the concentrations are high enough to allow recycling of the metals. This of course is the ultimate goal of any remediation technique, but even if recycling is not possible the concentrations are increased as compared to the soil and therefore the volume, which has to be deposited, is reduced.

The aims of this research are to study the potential role of turfgrass species that may play relevant part particularly for environmental remediation of contaminated soils, and to study the possible mechanisms of the Pb uptake and translocation from root/shoot system through the kinetic studies.

## **MATERIALS AND METHODS**

### **1. Plant material and experimental conditions**

In completely controlled growth chamber in NRC four plant species:

(A) Centipede grass

(B) Buffalo grass

(C) Tall fescue

and (D) *Spartina patens*

were planted in sandy culture to start the root development process. At 15 cm plant height, the grown plants were collected, rinsed with deionized water several times to remove the sand particles attached. Plants were then cultivated hydroponically in Pb-containing solution. Each hydroponic unit consist of jar (10 cm diameter and 25 cm in height), containing 500 ml solution varying in their Pb concentration (0, 250 and 500 mg l<sup>-1</sup>). The pH of the water cultures were then adjusted at different pH values of 4.5, 5.5 and 6.5 using sulfuric acid. Plant roots of turfgrass species were then completely

immersed in the adjusted solutions, total volume of the solutions was kept constant by adding deionized water daily to compensate for water loss through evapotranspiration. The experiment was kept at 25°C temperature through the whole experiment. After 1, 3, 5, 7, 10 and 14 days plant samples were collected and rinsed, separated into shoot and root, and dried at 70°C. The dry matter of both shoot and root were analyzed for Pb content by flame atomic absorption spectrophotometry as described by Cottenie *et al.*, (1982).

## 2. Kinetic study

The Elovich equation is generally expressed as follows"

$$dq/dt = a \exp bqt$$

where  $qt$  is the sorption capacity at time  $t$  ( $\text{mg g}^{-1}$ ),  $a$  is the initial sorption rate ( $\text{mg .g}^{-1} \cdot \text{min}^{-1}$ ) and  $b$  is the desorption constant ( $\text{g .mg}^{-1}$ ) during any one experiment. To simplify the Elovich equation, Chien and Clayton (1996) assumed  $abt \gg 1$  and by applying the boundary conditions  $qt = 0$  at  $t = 0$  and  $qt$  at  $t = t$  the used equation becomes:

$$q_t = 1/\beta \ln \alpha\beta + 1/\beta \ln t$$

Where:

$q_t$  = the amount of Pb desorption in time  $t$

$\alpha$  = a constant related to the initial rate of heavy metals release in  $\text{mg.g}^{-1} \text{min}^{-1}$ .

$\beta$  = a constant in  $(\text{mg Pb}/100\text{g plant})^{-1}$ .

Thus, the constants can be obtained from the slope and the intercept of a straight line plot of  $qt$  against  $\ln(t)$  will be used to test the applicability of the Elovich equation to the kinetics of absorption.

The kinetic parameters  $\alpha$  and  $\beta$  of Elovich equation were calculated under different treatment conditions. Regression analysis using regression SAS software (SAS institute, 1985), was also applied to test the conformity of Pb uptake by turfgrass plants to the applied model and to test the significant differences in rate coefficients. In addition, averages and standard errors of the means were also calculated within each treatment group. Pair-wise comparisons were made between treatment group averages to reveal any significant differences on the 5% level, using the one-way ANOVA

## RESULTS

Fig 1 and 2 represent the effect of turfgrass species on Pb uptake as affected by Pb concentration and solution pH. Data showed that after 14 days most of Pb accumulated in root than in shoot regardless the different species of turfgrass used. At 500 ppm Pb and pH 4.5; data showed that the mean values of the ratio between root and shoot in their Pb concentration for different plants tested reached to almost 12 times. By increasing the pH to 6.5 the same ratio was decreased to reach only about 10 times, similarly such trend was also observed at the low concentration of Pb (250 ppm).

Data also showed that in root system increasing the Pb concentration in hydroponic system led to increase the Pb content in all plant species. In (D) plant, results showed that increasing Pb concentration from 0 to 250 to 500 ppm increased Pb content gradually from 0.4 to 21.48 and 31.82

mg.100g<sup>-1</sup>, respectively. Although the same trend was also observed in different plant species used, obtained results showed also that there is a variation between these species in Pb accumulation.

After 14 days particularly at 500 ppm Pb, the (D) plant type showed a higher Pb accumulation as compared with the other species. The values of Pb content were 19.6, 23.62, 26.53, and 31.82 mg. 100g<sup>-1</sup> grain in (A), (B), (C) and (D) plants respectively. Similarly this result was also observed at 250 ppm Pb concentration.

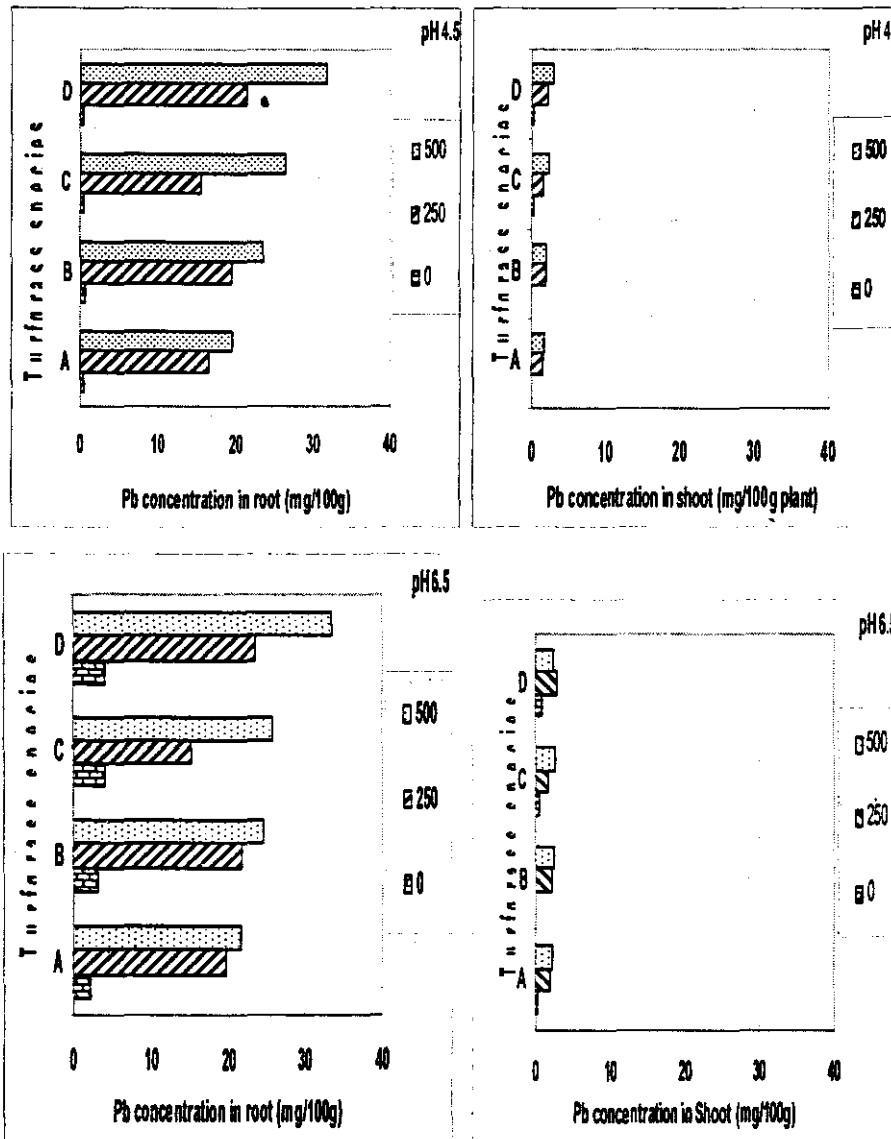
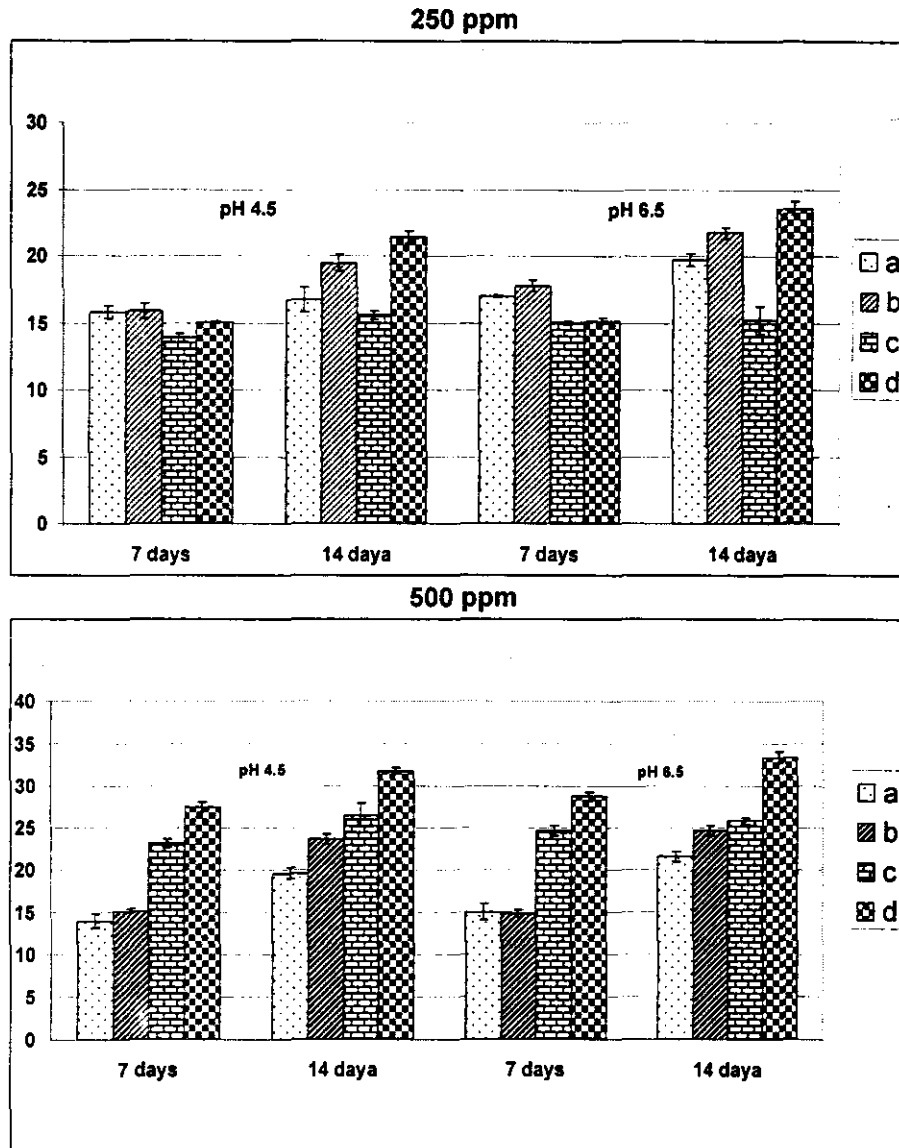


Fig (1): Pb concentration in Shoot and Root of different plant species grown in hydroponics system at different solution pH.



**Fig (2): Effect of growing time and solution pH in hydroponics system on Pb uptake by different plant species at different concentrations**

Fig. (3) represents the effect of the pH of hydroponic solutions on Pb accumulation in roots of different turfgrass studied. Results generally indicate that by increasing the pH values from 4.5 to 6.5 can stimulate the accumulation of Pb in root in different species in both concentrations used i.e. 250 and 500 ppm. At pH 4.5 and 250 ppm it was noticed that higher accumulation of Pb was observed in (A) species 17 % after 14 days of

plantation followed by (B) and (D) species, however, the (C) plant showed the lowest one..

### **1- Effect of Pb treatment and pH values on green yield biomass of the studied turfgrass species.**

The effect of different Pb concentrations and pH of solution on the green yield of the plant species are presented in Tables (1-3). It was observed that, an average decrease of dry matter of different species was ranged between 1.5 and 7% particularly at 250 ppm Pb and pH values 4.5.

Higher decreasing value was observed in ( C ) plant followed by (D) one, however, the (A) and (B) plants took the same trend in decreasing order, however, the minimum decreasing order of DM was observed in these two species. Increasing Pb concentration at 500 ppm led to increase the DM content in (A) and (B) species by the values of 6 and 42 % respectively, however, no change was observed in (C) and (D) species.

Concerning the pH values in these solutions, it was noticed that increasing of Pb concentration at 250 ppm was joined with decreasing the pH values to 4.71 and 4.83 in (A) and (B) plants and increasing the same parameter to 5.12 and 5.13 in (C) and (D) ones. Similar trend was also observed at higher rate of Pb (500 ppm).

Under pH 5.5 condition in different treatments, data showed that, the DM of different treatments were consistently increased regardless the concentration of the hydroponic solutions.

Higher values of increase in Pb concentration at pH 5.5 were observed in (A) and (B) species i.e. 19 and 16 % as compared with control in 250 ppm, and 26, 41% in 500 ppm of Pb respectively. The respective values were 16, 18 % and 37 and 17 % in (C) and (D), (Table 2).

Decreasing order of the pH values in most treatments as compared with control (i.e., Pb=0 conc.) becomes an important phenomena. The increasing of DM% under pH 6.5 of hydroponic solution was noted in most treatments and becomes the higher values as compared with other pH (4.5 and 5.5). The obtained results showed that, at 500 ppm hydroponic solution concentration, the percent of decrease in C plant was 7 % under control treatment and at pH 4.5 the same value was increased to 37% at pH 5.5, the DM was consistently increased to 48% as compared with control in pH 6.5.

### **2 Kinetic study**

Table (4) represents the  $\beta$  constants of Elovich equation as affected by pH and Pb concentration of hydroponic solution. In this study we used this important constant to represent the rate of Pb uptake or absorbed by plant root species used meanwhile, to represent the translocation of the studied element to shoot system

The obtained results showed that the rate constant values of B were influenced by the plant part and high values of accumulated Pb were observed in root parts than shoot under different conditions of pH and Pb concentration in the hydroponic solution.

The pH values of the hydroponic solutions considerably influenced the rate of Pb absorption by root. Results also indicated that increasing the pH values from 4.5 to 6.5 led to increase the Pb absorption from 3.78 to 4.19 mg 100g plant particularly in A plant grown in 500 ppm Pb.

**Table ( 1 ) Effect of Pb concentration in hydroponics system on biomass of the studied genotypes at pH 4.5**

Turfgrass species	0 ppm			250 ppm			500 ppm		
	DM	% of change	pH	DM	% of change	pH	DM	% of change	pH
<b>A</b>	0.73	100	5.01	1.71	98.84393	4.71	1.84	106.3584	4.59
<b>B</b>	1.74	100	4.93	1.72	98.85057	4.83	2.48	142.5287	4.68
<b>C</b>	2.1	100	5.1	1.96	93.33333	5.12	2.03	93.33333	5.09
<b>D</b>	2.22	100	4.89	2.09	94.14414	5.13	2.04	94.14414	5.14

**Table ( 2 ) Effect of Pb concentration in hydroponic system on biomass of the studied genotypes at pH 5.5**

Turfgrass species	0 ppm			250 ppm			500 ppm		
	DM	% of change	pH	DM	% of change	pH	DM	% of change	pH
<b>A</b>	1.2	100	5.42	1.43	119.2	5.13	1.51	125.8333	4.98
<b>B</b>	1.27	100	4.98	1.47	115.8	5.01	1.79	140.9449	5.11
<b>C</b>	1.41	100	4.92	1.64	116.3	4.89	1.94	137.5887	5.01
<b>D</b>	1.74	100	5.01	2.07	118.9	4.89	2.05	117.8161	4.93



**Table ( 3 ) Effect of Pb concentration in hydroponic system on biomass of the studied genotypes at pH 6.5**

Turfgrass species	0 ppm			250 ppm			500 ppm		
	DM	% of change	pH	DM	% of change	pH	DM	% of change	pH
<b>A</b>	1.76	100	6.59	2.28	129.5455	6.67	1.91	108.5227	6.62
<b>B</b>	1.64	100	6.58	2.15	131.0976	6.61	2.05	125	6.71
<b>C</b>	1.63	100	6.71	2.23	136.8098	6.68	2.40	147.2393	6.69
<b>D</b>	1.56	100	6.89	2.41	154.4872	6.78	2.53	162.1795	6.79

**Table (4)  $\beta$  constants of Elovich Equation representing Pb translocation of Pb to root and then to shoot as affected by pH, Pb concentrations of hydroponic solutions used**

Turfgrass species	Root						Shoot					
	pH 4.5			pH 6.5			pH 4.5			pH 6.5		
	0	250	500	0	250	500	0	250	500	0	250	500
	Pb, ppm			Pb, ppm			Pb, ppm			Pb, ppm		
<b>A</b>	0.10	3.42	3.78	0.66	4.21	4.19	0.05	0.07	0.27	0.08	0.40	0.35
<b>B</b>	0.14	3.64	4.52	0.94	4.05	4.90	0.05	0.33	0.31	0.05	0.41	0.45
<b>C</b>	0.11	3.25	3.89	1.22	3.02	3.40	0.10	0.32	0.29	0.08	0.28	0.25
<b>D</b>	0.11	5.63	4.56	1.21	6.29	4.95	0.06	0.54	0.47	0.24	0.57	0.58

Although the same trend was observed in other plant species used, the obtained data indicate that ( C ) plant took a reverse trend since the  $\beta$  values were decreased from 3.89 to 3.4 (mg 100g plant)<sup>-1</sup> by increasing the pH values from 4.5 to 6.5 of the growing solution.

Results also showed that the higher absorption of plants was observed in (D) species. Under pH value of 6.5 and at rate of 500 ppm Pb, data showed that Pb absorption reached to 4.56 (mg/100g)<sup>-1</sup> and decreased to 3.78 (mg/100g)<sup>-1</sup> plant and inbetween values for both B and C plant species. Although the same trend was observed at pH 4.5, a reverse trend was observed in ( C ) plant species at both pH treatments.

The rate of Pb translocation from the root to shoot parts showed that increasing pH from 4.5 to 6.5 led to increase the translocation of Pb from root to shoot system. Results revealed that at 500 ppm Pb concentration the  $\beta$  values were increased from 0.47 (mg 100g plant)<sup>-1</sup> to 0.58 (mg 100g plant)<sup>-1</sup> plant in (D) plant. Similar trend was also observed in (A) and (B) species. However, ( C ) species showed a reverse trend in decreasing the  $\beta$  value from 0.29 to 0.25 (mg 100g plant)<sup>-1</sup>.

## DISCUSSION

Turfgrasses are an important landscape plants used to beautify and protect our environment. Numerous studies reported that this plant species could be trusted to facilitate degradation of organic pollutants (Briggs *et al.* 1999; Hossler and drake, 1999; Fiorenza, *et al.*, 2000; qiu, *et al.*, 1997; Epuri and Sorensen, 1997; Fetterolf *et al.*, 1999). However a little number of researches has been performed to evaluate turfgrasses tolerance to another groups of pollutants, heavy metals and their capacity to absorb these metals.

Genetically engineered, turfgrass plants may further enhance their ability to perform this function (Rugh, *et al.* 1996). However, very little is known about the uptake and translocation of heavy metals in turfgrasses and the associated mechanisms or the mechanisms of turfgrasses degradation of toxic organics. If turfgrass that translocate substantial amounts of heavy metals to the shoots can be identified as in *Brassica juncea* (Nanda Kumar, *et al.*, 1995), clipping removal of turfgrass can be an efficient way to remove heavy metals from the polluted soils. Research in this point will help further understanding of the interaction between turfgrass and toxic pollutants and their effects in remediation of environmental pollutants.

In this study we characterized Pb accumulation in four turfgrass species Centipede (A), Buffalo (B), Tall fescus ( C ) and *Spartina patens* (D) and its effects on apparent plant health. To prove our hypothesis, we used hydroponic system to eliminate any side interaction between Pb and active soil particles under different conditions applied. We observed some turfgrass species, have the capacity to absorb and accumulate substantial amounts of Pb in their roots and that some turf species are more tolerant than others. In our experiment, D plant tolerated higher Pb concentrations and thus could be our better choice for planting in Pb contaminated soils.

Results referred that maximum absorption and accumulation of Pb in the roots of investigated plants were affected by the pH of the hydroponic solution and the concentration of different treatments of Pb used. Data showed that under pH 4.5 condition the maximum absorption was ranged between 3-4.5% at low Pb concentration. By increasing the Pb concentration up to 500 ppm the maximum absorption was ranged between 3-6.5% from the total concentration applied.

Increasing of pH of hydroponic solution to 6.5, increased the percent of maximum Pb absorption which ranged between 0.5 and 1 % in low and high concentrations used over the low pH one. A majority of Pb still in solution, the lack of aeration in our experiment may have inhibited Pb uptake to certain extent. Our results indicate that turfgrass can accumulate substantial amounts of heavy metals and thus could be a major component in our environment.

An important issue concerning other combinations of mechanisms which may exist and could have produced; a number of alternative combinations of mechanisms were considered that could have given rise to the reference mode behavior. For example, efflux of Pb was considered from either the roots or the shoots, or both. Such mechanism was rejected for the following reasons. It appears that once Pb is taken up into the root symplast, precipitation occurs rather rapidly (Malone *et al.*, 1974). Because of this, it appears that only relatively small amounts of Pb would be available to leak back out of the roots, and continuously balanced with the Pb being translocated to the shoots in the xylem for the reference mode steady state to be achieved. This phenomena could be considered unlikely and others gave support to the view that efflux from either roots or shoots was likely to be negligible if it occurred at all.

One of the other important mechanisms is precipitation, which can be took place at the root surface and throughout the plant. This mechanism was identified as having high uncertainty and high impact upon model behavior. There has been little research into this mechanism since Koeppel (1977) and Malone *et al.* (1974). Cunningham and Perti. (199.) have suggested that the dominant form of Pb-precipitate may be Pb-carbonates due to their greater intracellular availability. Though it would appear from model testing and validation that the assumptions made concerning Pb precipitation are reasonable. These results point out the need for more research concerning this mechanism. Concerning the effect of the concentration of different pH solutions, the results showed that higher DM content was observed at pH 6.5 followed by pH 5.5 and the lower ones was at pH 4.5. Increasing pH values reached in some cases to 82% over control treatment. The D plant gave the higher accumulation of Pb followed by C plant.

Hyperaccumulation of Pb seems to depend on constitutive overexpression of root cell plasma membrane transporter genes, such ZTP2 and ZTP3 that were recently found in *Thlaspi caerulescens*. In normal plants these genes are exclusively expressed under conditions of Zn deficiency. The molecular basis of the strongly increased root to shoot metal transport and extreme metal tolerance levels in hyperaccumulators remains elusive. ZTP1 from *T. caerulescens* is particularly overexpressed in leaves and seems to be

mainly involved in the foliar sequestration of Zn. As a conclusion the present investigation shows that many wetland plants can colonize heavily metal-polluted areas, and are found to absorb a wide range of soil metals (Pb, Zn, Cu and Cd). These species that do not appear to be affected by excessive metal contents may possess metal resistance capabilities, or higher tolerance than more sensitive species. As metal concentrations in shoots are usually maintained at low levels, metal tolerance in wetland plants may mainly depend on their metal exclusion ability. However, the higher-than-toxic level of metal concentrations in some species indicating that internal detoxification metal tolerance mechanisms might also exist. These plants are almost exclusively annual or perennial herbaceous species and produce relatively large biomass; therefore, their utility for phytoremediation is possible. Results within species revealed the factors influencing metal uptake in field. This implies that by controlling water level, soil nutrients and other factors, metal removal by wetland plants would be different and could be utilized for both phytostabilization and phytoextraction. It also suggests that full consideration of plant-soil interactions should be taken into account when choosing plant species and suitable conditions during wetland system construction and management.

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## تقييم المعالجة الحيوية بأستخدام بعض أنواع من النجيلية تحت ظروف مختلفة من التلوث

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القاهرة

تعتبر الدراسات العلمية في مجال معالجة الأراضي والمياه الملوثة بالعناصر الثقيلة من أهم الأهداف التي يتم الاستعانة بها من أجل الوصول إلى بيئة نظيفة خاصة إذا كانت هذه المعالجات تتم باستخدام طرق حيوية لا ضرر منها على البيئة ونمو النباتات المختلفة. وتهدف هذه الدراسة إلى إجراء تقييم لامكانية استخدام بعض أنواع النجيليات كوسيلة لامتناس عناصر الرصاص تحت ظروف مختلفة من التلوث كالتركيز ودرجة حموضة الوسط.

ولتحقيق هذا الهدف أجريت تجربة تم فيها انبات بذور أربعة أنواع من النجيلية

هي:

(A) Centipede grass (B) Buffalo grass (C) Tall fescue  
(D) Spartina patens

وذلك في بيئة رملية نظيفة وعند تمام الوصول إلى ارتفاع ١٥ سم للنباتات النامية تم نقل الأنواع المختلفة إلى بيئة نمو مائية ، تحتوي على تركيزات مختلفة من عنصر الرصاص (صفر - ٢٥٠ - ٥٠٠ جزء في المليون) تحت درجات حموضة مختلفة (٤,٥ ، ٥,٥ ، ٦,٥) .

أوضحت النتائج المتحصل عليها الآتي:

- ١- بعد ١٤ يوم أوضحت النتائج بشكل معنوي ان معظم الرصاص الممتص بواسطة أنواع النجيلية المختلفة قد تراكم في الجذور أكثر من الساق.
- ٢- زيادة رقم الحموضة بمقدار وحدة واحدة خاصة في المعاملات ذات التركيز العالي (٥٠٠ جزء في المليون) أدى إلى زيادة النسبة بين الجذر / الساق بمقداره وصل إلى ١٢ مرة تقريباً.
- ٣- أوضحت النتائج ان زيادة تركيز الرصاص أدى إلى زيادة تدريجية في محتوى الرصاص في كل من الجذر والساق للأنواع المختلفة.
- ٤- أتضح أن النوع (D) أعطى أعلى محتوى من الرصاص بالمقارنة بباقي الأنواع المستخدمة في هذه الدراسة.
- ٥- زيادة تركيز الرصاص في المعاملات أدى إلى زيادة المادة الجافة لكل من صنفى (B)Buffalo grass, (D)Centipede grass
- ٦- أظهرت الدراسات الكينيتيكية ان الثابت  $\beta$  المعبر عن التيسر الحيوي للعنصر قد تأثر بنوع النبات المستخدم كذلك الجزء المدروس من النبات سواء كان جذر أم ساقاً.
- ٧- معدل انتقال العنصر من الجذر إلى الساق في الأنواع المختلفة من النجيليات ارتبط ارتباطاً معنوياً بزيادة رقم حموضة الوسط وإن زيادة الحموضة ارتبطت أيضاً بزيادة الانتقال من الجذر إلى الساق.

يتضح من النتائج المتحصل عليها إمكانية استخدام Turfgrass من النوع Spartina patens في معالجة الأراضي المتأثرة بالتلوث بالعناصر الثقيلة لاسيما المناطق الصناعية .