Decontamination of Agricultural Soil Polluted with Lead using the Common Barley (*Hordium vulgare*)

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ABSTRACT

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KEYWORDS

Soil pollution, Decontamination, Hordeum vulgare, Lead, Bioavailability, Algeria.

The present work aimed at studying the possibility of decontaminating polluted soil by lead from traffic road, using Hordeum vulgare as a heavy metals hyperaccumulative plant. High concentrations of lead stored at the horizons of soil surface (1714.39 \pm 512.62 µg g⁻¹), have revealed that the traffic and road infrastructures are important outcomes of toxic heavy metals to the environment. In addition, we noticed that Hordeum vulgare may hold a total of $36.28 \pm 14.90 \,\mu g \,g^{-1}$, which correspond to 2% of that to of that accumulated in the soil. Importantly, highest values were observed in the roots $(18.32 \pm 8.38 \,\mu g \,g^{-1})$, which means 50,50%, whereas, the rate of heavy metals in the stems and leaves were $10.83 \pm 5.86 \,\mu g \, g^{-1} \, (29.95\%)$ and $7.71 \pm 3.74 \,\mu g \, g^{-1} \, (29.95\%)$ g⁻¹ (21,25%), respectively. Otherwise, the accumulation of lead was influenced by physical and chemical properties of the soil (pH, cation-exchange capacity CEC, percentage of clay). A high content of lead was observed in the plant at low pH; in contrast, the CEC of the soil and the percentage of clay, positively correlated with plant Pb. However, a strong correlation between soil Pb and plant Pb was noticed. Accordingly, lead uptake by Hordeum vulgare seems to be influenced by its bioavailability in soil.

تنقية تربة زراعية ملوثة بعنصر الرصاص باستعمال نبات الشعير (Hordeum vulgare)

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المستلخص

يه دف هذا العمل الى تقييم امكانية تطهير التربة الملوثة بعنصر الرصاص (Pb) باستعمال نبات الشعير Hordeum vulgare. بينت النتائج المتحصل عليها وجود تراكيز من الرصاص (Pb) في الأفاق العليا للتربة بحدود $^{\rm Pg}$ $^{\rm Pg}$

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الكلمات الدالة

تلوث التربة، تنقية التربة، الشعير Hordeum vulgare، عنصر الرصاص (Pb)، التواجد الحيوي، الجزائر

Introduction

Heavy metals, found as trace in natural soils, can reach very high concentrations in certain substrates polluted by human activities. Particularly, zinc, lead, cadmium and copper are present in emissions of different industrial and agricultural activities and are also emitted by the exhaust fumes of motor vehicles. High concentrations in soils modify considerably the composition of the flora, allowing only a limited number of species tolerating their toxicity (Antonovics *et al.*,1971); (Gartside *et al.*,1974).

According to (Martirnelli, 1999), the main sources of lead are exhaust fumes and brake linings. The lead is deposited on pavements and is conveyed by storm water runoff, resulting in significant contamination of these waters. As a result, during their infiltration into the soil at the roadside, accumulation of pollutants in the surface layers of soil is observed. Piggott, (1999) noticed that the levels of trace metals decrease sharply with soil depth.

Lead is generally more abundant in the surface humus horizons than in deeper horizons. This may be a natural cause but it is greatly enhanced by all anthropogenic contamination reaching the ground by the surface (Baize, 1997). According to (Doelman and Haanstra., 1979) soils are not the final trap for this element. When changes in physico-chemical conditions of the environment occur, lead can be remobilized by various mechanisms, migrated to ground-water, contaminating water resource or being assimilated by plants.

Mcbride (1994) showed that metal particles deposited on the surface of leaves do not enter inside and can be easily washed by rain. On the other hand, pollutants are in a soluble form usable (bioavailable) by the plant. Once absorbed by the roots, it may be sent to the aerial parts.

Soil contamination can lead to phytotoxicity problems that impact the productivity, the composition of plant communities, constituting the natural cover, and the ecosystems functions (Prasad, 2004). If the presence of trace element in soils does not systematically induce phytotoxicity, it may constitute a risk of contamina-

tion of the food chain due to the possible absorption of these elements by plants. However, this absorption is highly variable depending on the species.

The use of physico-chemical techniques for the restoration of heavy metals polluted soils is very expensive compared to their extractions at the laboratory scale. However, in recent years more and more studies are conducted on the rehabilitation of soils contaminated with heavy metals. The ability of some plants to tolerate or even to accumulate metals has opened new paths of research on soil treatment whose (Raskin *et al.* 1994); (Salt *et al.* 1995). Concerning traffic pollution in the soil, (Garcia, 1996) found a value of 1453mg.g-1 lead, in Spain; 479 mg.g-1 in France (Remy. 1998) and 1564 mg.g-1 in New Zealand (Ward *et al.*1977).

Phytoremediation, which is both effective and low cost, is a new technology (10 or 15 years old) for the decontamination of polluted soils, groundwater and wastewater. It is defined as the use of green plants (including grasses, herbaceous and woody) these plants have the capacity to remove, contain, or render environmental contaminants harmless, such as heavy metals, trace elements, organic compounds and radioactive compounds found in soil or water. (Morel, 1997) noted that barley, sunflower, dandelion, various Cruciferae, nettle and rapeseed were heavy metals hyper-accumulative plants. These plants are capable, due to their adapted physiologies, to accumulate up to 1% of their dry matter in heavy metal, which is a rate much higher than normal.

This work aimed to use barley, as a hyper-accumulating plant, to clean an agricultural soil contaminated with lead from traffic road. The goal was to estimate the concentration of accumulated lead, by *Hordeum vulgare*, in the vicinity of a national road with high traffic pressure, in the region of Tiaret (Algeria).

Material and Methods

(1) Selection of Sampling Sites

The study was conducted in Tiaret which is located in Northwest of Algeria between the Tellian Chain on the North and Atlassienne Chain on the

South, at an average altitude of 980 m (Fig.1). The climate is Mediterranean semiarid with an average annual rainfall of 400 mm/year. The prevailing winds come from West and Northwest, their average speeds range from 3 m to 4m/s.

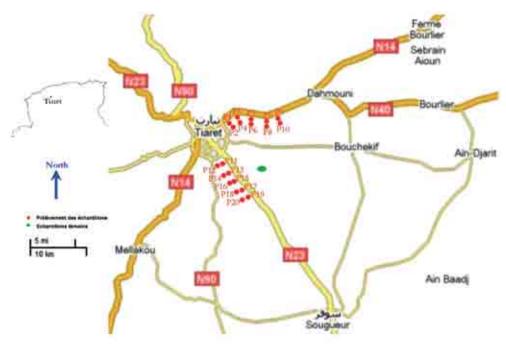


Figure 1 : Location of Sampling Points in the Study Area (*legend:* P1, P3-P19 at a distance of 2 m from the road, P2, P4-P20 at a distance of 4m of the road)

Tiaret comprises over 200 km of urban road networks. Its car fleet consists of 6,284 vehicles of all types; from which 11% are new. In 2006, the car fleet of Tiaret department consisted of 8015 registered vehicles. New cars (0 to 5 years) represent only 11% of the park, however, the cars over 11 years old account for 74%. Yet, it is precisely those older vehicles that are more polluting.

(2) Sampling

Initially, 20 surface horizons were chosen in soils located near two national roads (national roads No. 14 and No. 23) to determine the levels of lead in them, knowing that lead is an element of very low mobility and a strong tendency to accumulate in surface horizons (Fernandez *et. al.*2006). It is important to note that the daily traffic recorded on Road No. 14 and No. 23

is respectively 600 and 800 vehicles per day (Amirat *et al.* 2008). During the second year, 20 plants of common barley (*Hordeum vulgare*) were harvested at the same points of soil sampling (see, figure 1). Three control plants of common barley at a 5km distance away from any source of contamination were used as references when comparing with contaminated plants, in ecological conditions that were as similar as possible.

(3) Treatment of Samples

In most cases, methods of preparation and analysis of metals in soil are the same as for the leaves, lichens and fungi (Flückiger *et al.* 1978); (Deletraz and Paul. 1998).

(3.1) Soil Samples

Twenty (20) soil samples were collected from each point of the study area, near the road at a distance of 2m. The first set consists of 10 horizons at a

distance of 2m from the pavement, in contrast, the second set, parallel to the first, is at a distance of 4m (see, figure 1). In the laboratory, samples were air dried, sieved (<2 mm) and grounded. Physicochemical parameters of soil, including pH, CEC and particle size were determined by standard methods (Duchaufour.2001).

(3.2) Plant Samples

On the same sampling points of the soil, samples of *Hordeum vulgare* were collected (three plants per horizon) during the second year before seed formation (in April). The harvested plants required a preliminary washing with distilled water to remove possible atmospheric deposits and were separated into roots, stems and leaves. They were subjected to a set of operations:

(3.2.1) Dehydration

The usual method was dehydration in an oven at $105 \, ^{\circ} \pm 2 \, ^{\circ} \, \text{C}$ for 72 hours. The dehydrated organs were weighed separately to obtain the dry matter. DM was $0.2 \, \text{to} \, 0.3 \, \text{g}$.

(3.2.2) Grinding

This step is highly critical as it can be a source of contamination or loss. For this, the grinder used was an agate mortar. The grinder was made of titanium and steel guaranteed, without heavy metals. The resulting powder was calcined in an oven whose temperature was gradually increased to 500C, using quartz capsules.

(3.2.3) Mineralization and Dissolution

The fine powder obtained after calcination, was placed in an acid and an oxidizing solution (0.5 ml mixture of nitric acid HNO₃, hydrofluoric acid HF and perchloric acid ClHO₄) and then heated in a water bath for 24 h until the complete destruction of organic matter. Tubes that have been put to boiling were supplemented with 10 ml distilled water. This method allows the determination of trace elements (Regional Laboratory of Civil Engineering of Lille (French) - Environment / Water and Soil No. 01 80 00695 August 2004. (Certu. 2004).

(3.2.4) Quality Control and Assurance

For the determination of lead by Atomic Absorption Spectrometer (with the following specifications: analytical line: 283.3 nm, slit width: 0.5 nm, correction system: Zeeman effect, systematic addition of 5 ul of diluted modifier matrix 1/5), the detection limit was 0.2 ug/g and the limit of quantification is 0.3 mg/g with a standard deviation of the blanks estimated at 0.2 ug/g (Chiffoleau et al.2003). The standard solution used in this experiment was the PbCl 2 (10 mg / l) and the calibration curve was plotted according to the values: 0.5, 2.0, 5.0, 10.0; 20.0 µg//ml. Initial control measurements included three assays the coefficient of variation was 14.4% as the maximum value. The mean average was used as the midpoint of the diagram and alert thresholds are set to +2 and -3 from standard deviations. On the basis of a normal repeat, 95% average measurements of later series should be within the range of +2 and -2 from standard deviations (0.00049 \pm 0.02088 percentage Mass.).

Results and Discussion

(1) Lead Levels in the Plant and Soil

All results are presented in Table (1) which summarizes the different concentrations of lead absorbed by *Hordeum vulgare*, in the three organs (roots, leaves and stems), as well as the levels of lead in soil bordering the pavements of the two roads studied.

Data from Table 1, reveal strong contamination of the soil by lead (1714.39 \pm 512.62 mg g-1) with a remarkable variability between the different sampling points; hence the recorded values of lead in *Hordeum vulgare* (36.28 \pm 14.90 mg g-1) greatly exceeded the control values (2.58 \pm 0.16 mg g-1) and standard values (1.38 \pm 1.24 mg g-1). Larger values were observed in the roots (18.32 \pm 8.38 mg g-1), concentrations were lower in stems and leaves whose recorded values were respectively10.83 \pm 5.86 mg g-1 and 7.71 \pm 3.74 mg g-1.

		N	Mean	Median	Minimum	Maximum	1er Quartile	3ème Quartile	Standard Deviation
Roots		20	18.32	15.75	07.00	30.90	11.25	11.25	08.38
Stems		20	10.83	08.55	04.10	28.50	06.85	06.85	05.86
Leaves		20	07.71	06.90	01.10	16.70	05.50	05.50	03.74
total		20	36.28	33.52	13.70	71.20	24.50	24.50	14.90
	Roots	03	1.17	1.20	1.20	1.20	1.13	1.20	0.04
	Stems	03	0.83	0.84	0.84	0.97	0.70	0.97	0.13
control	Leaves	03	0.57	0.56	0.56	0.65	0.50	0.65	0.07
	total	03	2.58	2.62	2.62	2.73	2.40	2.73	0.16
Norms (plants) (Kabata, 2001)		05	1.38	_	0.01	2.50	_	_	1.24
Soil		20	1714.39	1688.17	845. 60	2712.00	1326.30	2082.00	512.62
(soil not contaminated (Baize, 1997) (EU + French)		_	9- 50 100	-	_	_	_	-	_

Table 1: Descriptive Statistics on Levels of Lead (mg g-1) in Plant and Soil

Lead is mainly absorbed by root hairs and stored in the cell walls, which explains the high rates in roots (Zimdahl, 1975); (Hughes, et al. 1980). However, (Contat, et al. 1991) found much more Pb in the 0-20 cm layer than in deeper soil layers, where the roots are more abundant. (Jean, 2007) found that the presence of metals in different parts of the plant indicates that there is an accumulation and also translocation to aerial parts. Several researchers have demonstrated the possibility of plants in remediation of soils contaminated by trace metals; (Huang, et al. 1997) found that the leaves of leaf mustard 'Brassica juncea" can accumulate 1.5% of the 600 mg.kg lead, mixed with Ethylene Diamine Tetraacetic Acid (EDTA), in an artificially contaminated soil. They also estimated that approximately 0.1% of total lead in soil is available for extraction. As a consequence, it was necessary to apply chelating agents such as EDTA to mobilize the lead and to strengthen the phytoextraction. Ye-Tao Tang et al. (2009) found

that the rhizosphere of *Arabis paniculata* can absorb 2.8% Pb, 17.9% Zn and 0.42% of Cd; in this study, it was found to be 2%.

Many researchers have estimated the bioavailability of a metal, which can be done by analyzing the contents of this metal in a plant grown on a given soil. (Juste, 1988) noted that the plant is the best indicator of the actual bioavailability of an element because it includes the effect of all factors that affect it. However, in the case of soils contaminated by heavy metals, it is admitted that a relatively large proportion of Cu, for example, can accumulate in the roots without an increase of the concentration in aerial parts (Marschner, 1995); (Martirnelli 1999). Therefore, the assessment of bioavailability of Cu concentrations by analysing the roots in addition to the aerial parts is more relevant (Marie, 2008). Lead reacts with organic acids in soil solution according to the following reaction:

 Moreover, (Kabata and Alina. 2001) found that the lead threshold values for plants grown in noncontaminated areas ranged from 0.05 to 3.0 mg g-1 during the period 1970 to 1980, while the average lead content for cereals from different countries appeared to vary considerably from 0.01 to 2.28 mg g-1, in contrast, it was 2.1µg g-1 in the forages and 2.5µg g-1 in grasses.

(2) Effect of Soil Physicochemical Factors on Lead Absorption by the Plant

The transfer of Pb to the plant is certainly conditioned by the physicochemical parameters of soil, including pH, CEC, rate of clay and the plant itself. However, influencing plant sensitivity towards the gaseous pollutants, particularly mineral supply (some pollutants such as NOx and SO₂ may be involved in the sulfur and nitrogen supply) and water supply (which leads to dehydration of the plant, to a decrease of the stomatal opening and a reduction in pollutants absorption). In order to study these effects, we performed a correlation analysis with analysis of variance of these parameters. The adsorption of lead depends on the clay content, CEC, on soil that results and on pH.

(2.1) Effect of Soil pH

It is not the pH of the rhizosphere (root zone is

limited to the first few millimeters of soil located around the roots (Hinsinger, 1998), but according to (Marschner, 1995), variations in the rhizosphere pH units from 1 to 2 units compared to soil pH is common for many plant species. intensity and direction of this change depends mainly on the plant species, nutrient conditions and the soil's capacity to buffer the medium. for *Hordeum vulgare*, alkaline soils with slightly acidic and well-drained medium texture are preferred (Leonard and Martin, 1963).

This correlation is illustrated in Figure 2, where a negative relationship is observed; with a correlation coefficient r = -0.49**.

Furthermore, analysis of variance indicated a significant effect of pH on plant lead (p <0.01). It was observed that at low pH, metal cations are more available for absorption by the plant. Different interpretations have been advanced to explain the influence of soil pH on the accumulation of lead. (McBride, 1994); & (Blaylock and Huang, 2000) found that Cd, Cu, Hg, Ni, Pb and Zn are strongly absorbed by the roots at pH <5.5, so it is possible to increase the phytoextraction by adding an acidic agent to a contaminated soil.

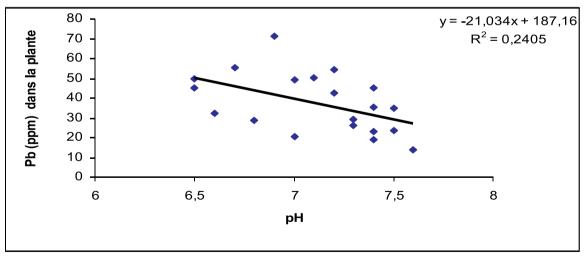


Figure 2: Correlation between pH of the Rhizosphere and Accumulation of Lead in Hordeum vulgare and the Accumulation of Lead in *Hordeum vulgare*

On a physiological level, (Jean, 2007) showed that the increase in pH favors protons elimination from aqueous complexes and surface functional groups of the solid phases. Due to the decrease of protons, competition between protons and metal cations is lower and therefore repulsions are decreased which then accelerates the formation of new phases. Thus, the solubility of metal cations decreases with increasing pH. For the plant to maintain an electrostatic equilibrium and a constant pH in root cells; cation samples are exactly compensated by the release of H + ions or the removal of anions; in contrast, the anion samples are compensated by the release of OH-, HCO3- ions or by the removal of cations (Hinsinger. 1998); (Straczek 2003).

(2.2) Effect of CEC

The cation exchange capacity of soil is positively correlated with concentrations of lead in the plant (r = 0.52**) as shown in Figure 3.

The ANOVA revealed a significant effect of this parameter (p <0.01). The cation exchange capacity is interdependent and determines much of the retained amounts of pollutants, including adsorption of pollutants that can be represented by the CEC, which is the maximum amount of cations that the soil can hold by physico-chemical adsorption. However, the root cation exchange capacity (rCEF) is defined as the number of binding sites for cations (anionic groups) localized on the cell walls (Wacquant, 1969); (Dufey and Braun,

1986). (Allan and Jarrell, 1989) showed that CEF decreases gradually as the external pH decreases.

Elements present in large quantities in the soil solution, such as calcium or magnesium, are transferred to the soil-root interface by mass flow which is often greater than the demand (Lorenz, et al. 1994); (Mc Laughlin, et al. 1998). These ions can then accumulate in the rhizosphere and lead in calcareous soils to precipitation of calcium around the roots (Jaillard, 1985). carbonate Conversely, the elements present in small amounts in the soil solution, as is typically the case of trace metals (Pb, Zn, Cd, etc. ...), are also transferred by the mass flux, but are insufficient compared to the removal of the plant (Hinsinger, et al. 2001). This therefore leads to a decrease in the concentration of metals such as Zn and Cd in soil solution near the roots (Lorenz, et al. 1994). This reduction creates a concentration gradient between the soil (mineral, soil solution) and roots and a diffusive transfer of these elements to the roots. (Mico, et al. 2008) investigated the accumulation of Co in Hordeum vulgare growing in a soil contaminated by this element. The results showed a significant correlation (r = 0.98) between the contamination of the plant and increasing soil CEC; in contrast, no relationship between contamination and organic carbon was found.

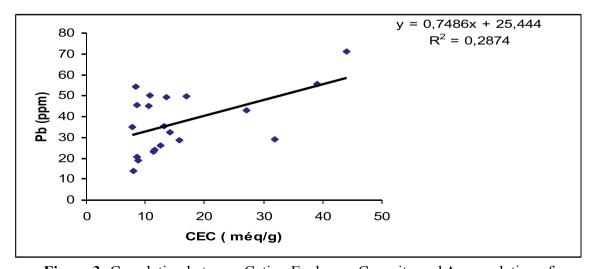


Figure 3: Correlation between Cation Exchange Capacity and Accumulation of Lead in *Hordeum vulgare*

(2.3) Effect of Clay

Clays are characterized by a non-neutral electrical surface, which determines their ability to exchange ions. These minerals have two types of charge.

- (2.3.1) The first, a negative charge, is structural and can not be changed. It is linked to Al⁺³ Si⁺⁴ or Mg⁺², Fe⁺², Al⁺³ substitutions, in the layers of clay.
- (2.3.2) The second, related to chemical reactions that occur on the surface of minerals, is a surface charge variable depending on the pH of the medium.

For a low pH, the majority of H⁺ ions bind to clay more than the OH-. The result is an overall positive charge and the clay is characterized by an anion exchange capacity. For high pH, the dominant OH- lead to an opposite phenomenon, and clay develops a cation exchange capacity (CEC).

A positive relationship exists between lead absorption by the plant, and the amount of clay; the more this content is important, the more the CEC is important, and the more the plant has a high accumulative power. Figure 4 shows this relationship (r = 0.80**).

Analysis of variance showed a highly significant correlation (p <0.01). Indeed, the more

the clay is the higher the CEC and the higher the plant capacity to absorbs metal; in this case, lead transfer to the plant is passive.

Organic matter and clays play an important role in the adsorption of lead by the plant. The trace elements show high affinity for humic substances with which they form stable humic clay complexes, possibly soluble. This explains the abundance of trace elements on the surface especially in the presence of organic matter where trace elements are absorbed specifically by iron oxides and manganese oxides (Baize, 1997). The lead will be available at the surface in this way.

(2.4) Correlation of Pb Plant to Pb Soil

The correlation of Pb plant to Pb soil is shown in Figure 5. This highly significant correlation (r = 0.72**) may indicate the high bioavailability of Pb to the plant (particularly by roots). The more the lead is bioavailable in the soil; the more it is easily absorbed by the plant. The best accumulation was recorded in the roots (r = 0.72).

In the case of *Hordeum vulgare*, Fig. 6 shows that about 2% of lead in soil is absorbed by the plant, without any phytotoxic symptoms.

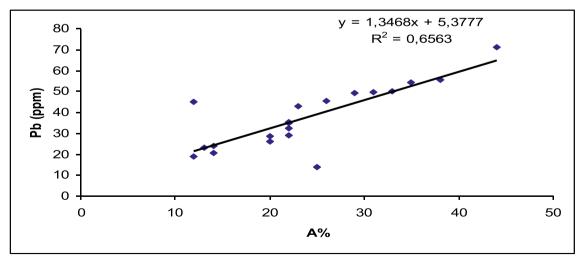


Figure 4: Correlation between Amount of Clay (%) and Accumulation of Lead in *Hordeum vulgare*

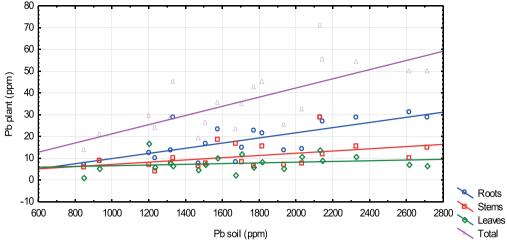


Figure 5: Correlation: Pb soil – Pb Plant (*Hordeum vulgare*) Equations:

y (Pb total) = 0.021x + 0.13, R2 = 0.52

y (Pb roots) = 0.011x - 2.00, R2 = 0.52

y (Pb stems) = 0.005x + 2.07, R2 = 0.19

y(Pb leaves) = 0.001x + 4.89, R2 = 0.05

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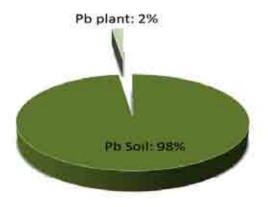


Figure 6: Percentage Accumulation of Lead in *Hordeum vulgare*

Several studies described the toxic effects of lead on the biological processes of the plant, such as photosynthesis, mitosis and water absorption, but toxic symptoms in plants are not very accurate; it was not easy to establish the concentrations of lead are toxic to the vital processes in plants. (Diehl, 1983) found that the particles of Pb in soils are rapidly converted into lead compounds soluble in water, easily available to plants.

A growing relationship between the total metal concentration in soil and the concentration accumulated in the plant after an artificial contamination of a soil by salt solutions of metals (Bingham et al. 1978); (Mitchell 1978), be explained by the fact that the metal, which is an ionic form, may be absorbed from the soil surface sites. (Tyler, 1975); & (Doelman and Haanstra, 1979) have shown that the accumulation of lead in surface soils has a great ecological importance because this metal is known to affect greatly the biological activity of soil and its absorption by plants. The concentrations of Cd, Zn, and Pb in the straw and wheat grain are directly dependent on those of the ground (Hagemeyer, 1999).

Empirical models were developed to describe linear or nonlinear relationships between the plant tissues content of trace metals and the soil content of these bioavailable elements, and showed that bioavailability is highly dependent on the nature of the extractant used, so the removal of trace elements is related to supply of the soil (Nguyen, 2007). At the xylem level, trace elements are transported to aerial parts through the transpiration stream (Salt, et al. 1995); & (Alloway, et al. 1995). The transport of metal components through the cell wall is

done passively (not metabolically) and therefore in the direction of concentration gradient (Briat and Lebrun 1999). (Malone, et al. 1974) pointed out that lead is transported and deposited in the same way in all plant tissues, which is why it is observed in roots, stems and leaves. Nigam et al. (2001) found that higher levels are often found in the roots.

Conclusion

The objective of this work is to show that common barley (Hordeum vulgare) has the potential to decontaminate effectively soil, contaminated with lead. The experimental protocol consisted of determining the concentrations of this metal in 20 soil samples and 60 samples of Hordeum vulgare (roots, stems and leaves). The results showed that the surface horizons of road soils were highly contaminated by lead $(1714.39 \pm 512.62 \text{ mg g-1})$ and exceeded the international standards. However, 2% of this value can be absorbed by *Hordeum vulgare*, that is 36.28 ± 14.90 mg g-1. Larger values were observed in roots with 18.32 ± 8.38 mg g-1; while, the values observed in the stems and leaves were. respectively: 10.83 ± 5.86 mg g-1 and 7.71 ± 3.74 mg g-1. The plant Pb's accumulation is influenced by physicochemical parameters of soil such as soil pH, CEC and the amount of clays. At low pH, lead is available to be absorbed by the plant (r = -049)**) while CEC and the amount of soil clays are positively correlated with levels of lead in the plant (r = 0.52 ** ** and r = 0.80, respectively).

The effects of heavy metals on local ecosystems and the mechanisms of their transfer, from soil to plants, are still not well understood. It is therefore very difficult to predict their long-term effects, to model the fate of pollutants from a contaminated site, to assess risks and hence choose a remediation strategy. However, plants grown for consumption are a potential hazard to public health because of a possible accumulation of these elements in their tissues. Risks associated with contaminated soils are in fact closely related to the bioavailability of the metallic elements. Once absorbed, these metallic elements can return to the soil (biogeochemical cycles). Moreover, these contaminated plants, can not and should not be used as human or animal food.

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