

**BIOREMEDIATION OF AGRICULTURAL SOIL CONTAMINATED WITH LEAD USING
INTERACTION: COMMON BARLEY *HORDEUM VULGARE* AND EARTHWORM
*LUMBRICUS SP***

**BIOREMEDIATION D'UN SOL AGRICOLE CONTAMINÉ AU PLOMB A L'AIDE
D'INTERACTION: ORGE COMMUNE *HORDEUM VULGARE* ET VER DE TERRE
*LUMBRICUS SP***

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ABSTRACT

The aim of this work is to study the possibility of remedying polluted agricultural soils by lead with the association: earthworms / barley. An experimentation of sixty pots was conducted in controlled conditions containing artificially contaminated soil by lead. It is divided into three systems: S1: soil-plant; S2: soil-earthworms; S3: soil-plant-earthworms, and five blocks representing lead concentrations: control; 500 $\mu\text{g g}^{-1}$; 1000 $\mu\text{g g}^{-1}$; 1500 $\mu\text{g g}^{-1}$ and 2000 $\mu\text{g g}^{-1}$ with 4 replicas each.

The results show that the S3 system (soil-plant-earthworm) has the highest remediation rate compared to the two other: S1 (soil-earthworm), S2 (soil-plant). The presence of earthworms *Lumbricus sp* decreases the bioaccumulation of lead by *Hordeum vulgare*, while concentrations recorded in earthworm tissues suggests that the presence of the plant considerably increase those concentrations.

The concentrations of lead in soil, earthworms and plants are influenced by the physical and chemical soil parameters; however, other factors related to the pollutant, the species of both earthworm and plant and their interactions can increase or decrease retention of lead by the soil and its bioaccumulation.

RÉSUMÉ

Le but de ce travail est d'étudier la possibilité de remédier les sols agricoles pollués au plomb à l'aide d'une association vers de terre / orge. Une expérimentation de soixante pots comportant des sols artificiellement contaminés au plomb a été menée dans des conditions contrôlées. Elle est répartie en trois systèmes : S1: sol-plante ; S2: sol-ver de terre; S3: sol-plante-ver de terre et cinq blocs représentant les concentrations de plomb : 500 $\mu\text{g g}^{-1}$; 1000 $\mu\text{g g}^{-1}$; 1500 $\mu\text{g g}^{-1}$; 2000 $\mu\text{g g}^{-1}$ et témoin avec 4 répliques chacun.

Les résultats montrent que le système S3 (sol-plante-ver de terre) présente le taux de remédiation le plus élevé comparé aux deux autres : S1 (sol-ver de terre), S2 (sol-plante). La présence des vers de terre *Lumbricus sp* diminue la bioaccumulation du plomb par *Hordeum vulgare*, alors que les concentrations enregistrées dans les tissus des vers de terre suggère que la présence de la plante accroît considérablement ces concentrations.

Les concentrations du plomb dans le sol, les vers de terre et la plante sont influencées par les paramètres physico-chimiques du sol cependant d'autres facteurs liés au polluant, à l'espèce du ver de terre et à celle de la plante et leurs interactions peuvent augmenter ou diminuer la rétention du plomb par le sol et sa bioaccumulation.

INTRODUCTION

Earthworms are one of the dominant groups of macro invertebrates of ground in several terrestrial ecosystems representing nearly 80% of the biomass of soil. They are identified as ecosystem engineers for their long-term effects on soil physical, chemical and biological properties (Edwards and Bohlen, 1996; Blouin et al, 2013; Bityutskii et al, 2016).

Due to their constant contact and their strong interaction with soil, earthworms can be profoundly affected by the soil pollution and accumulated contaminants in their bodies. Those characteristics among others allowed their use as indicator organisms of soil contamination (Lanno *et al*, 2004; Xiao *et al*, 2006).

Earthworms can concentrate some chemical products by involving selective absorption and excretion mechanisms, which vary according to species and families of chemicals. The toxic effects of a large number of chemical substances and the analysis of their absorption and metabolism have been identified to underline the importance of earthworms in biomonitoring of soil quality (De Vaufleury *et al*, 2013).

Human activity is the source of soil contamination by various organic and inorganic compounds. Heavy metals pollution caused by industrial enterprises activity and road traffic has reached high levels in the soil in some regions (Ha *et al*, 2011; Jiang, Z.F., 2012; Adriano, D.C., 2001; Alkorta *et al*, 2004).

It is established that such heavy metals as copper (Cu), zinc (Zn), lead (Pb) and cadmium (Cd) show a different effect of acute and chronic toxicity to animals and plants (Cheng *et al*, 2002; Li M, *et al*, 2009; Li N *et al*, 2009). Excessive levels of lead in soil inhibit the normal plant growth, disturb the ecosystem equilibrium and have an extremely negative impact on the environment and human health (Mishra *et al*, 2006 Zeng *et al*, 2006).

Studies on the ability of certain plant to accumulate heavy metals propose them as an alternative to the physical and chemical methods of decontamination.

Several studies were conducted using the plant / earthworms association for soil remediation. Earthworms increase the availability of heavy metals in some situations and aid in maintaining the structure and the quality of soil. The introduction of earthworms into metal contaminated soils has been suggested as an aid for the phytoremediation processes (Lemtiri *et al*, 2015; Jusselme *et al*, 2012).

The main objectives of this study are: i) to study the possibility of decontaminating a polluted by lead arable land using the association earthworm / plant; ii) to assess these two organisms impact on soil physics-chemical parameters.

MATERIAL AND METHODS

Samples and characterization of experimental soil

The soil samples were taken in uncontaminated arable land to the depth of 0 - 30 cm. All soil samples were air dried, crushed, sieved at 5 mm and mixed. This sieved soil was used for the pot experiment.

The soil samples were sieved at 2 mm additionally to provide physics-chemical analyses. The distribution of particles according to their size, or particle size analysis was determined by sedimentation using the Robinson pipette. Soil pH was measured using a soil suspension in a ratio (M/V) 1/5. The organic matter (OM) was determined by calcination in the oven at 500 °C according to NF ISO10694. The cation exchange capacity (CEC) was determined by percolation according to NF X 31-130. The electrical conductivity was measured in a soil suspension (M/V) 1/5 according to NF ISO 11265. The total lead concentration was determined by tri-acid attack following standard NF X 31-147, and measured by atomic absorption spectrometry (Agilent Technology. 55 AA). The characterization of experimental soil and the averages of lead concentrations in the soil, barley and earthworms ($\mu\text{g g}^{-1}$ dry weight) are represented in Table 1.

Lead contamination of soil

The soil was artificially contaminated with four levels of lead (500 $\mu\text{g/g}$, 1000 $\mu\text{g/g}$, 1500 $\mu\text{g/g}$ and 2000 $\mu\text{g/g}$) using lead nitrate powder [Pb (NO₃)₂] dissolved in distilled water, in addition to uncontaminated soil (control) with a lead concentration (<100 $\mu\text{g/g}$) in the international norms described by (AFNOR X 31 in 1996).

Experimental procedure

Three factors were involved in the experimental design: (i) lead concentration in soil; (ii) Presence / Absence of the earthworms and (iii) Presence / Absence of the plant. The experimentation is divided into three systems: S1: soil-plant; S2: soil-earthworms and S3: soil-plant-earthworms, five blocks representing lead concentrations: control, 500 $\mu\text{g g}^{-1}$, 1000 $\mu\text{g g}^{-1}$, 1500 $\mu\text{g g}^{-1}$ and 2000 $\mu\text{g g}^{-1}$ with 4 replicas each (see fig. 1). This disposition will allow to study and compare the effect of each organism alone on the soil and the effect of their association at different levels of pollution.

Table 1

Lead concentrations in the soil, plants and earthworms ($\mu\text{g g}^{-1}$ DW), and chemical parameters measured in the experimental soils

Parameters	Mean \pm SD	Minimum	Maximum
Soil (N=60)			
Clay (%) ^a	24,51 \pm 3,73	15,30	34,35
Silt (%) ^a	9,54 \pm 5,90	0,77	25,54
Sand (%) ^a	65,98 \pm 5,49	45,95	74,49
OM (%) ^b	2,30 \pm 0,37	1,50	3,12
pH _{water} ^c	7,42 \pm 0,57	6,39	8,34
CEC(meq100 ⁻¹ g) ^d	11,93 \pm 3,20	0,13	18,55
EC ($\mu\text{s/cm}$) ^e	831,27 \pm 273	335,00	1485,00
System 1(N=16)			
Pb soil ($\mu\text{g/g}$) ^f	331.86 \pm 66.95	200,00	430,00
Pb plant ($\mu\text{g/g}$) ^f	59.44 \pm 13.16	38,00	75,00
Remediation (%) ^g	17.93 \pm 1.87	14,85	21,50
Control 1 (N=4)			
Pb soil ($\mu\text{g/g}$) ^f	50.00 \pm 21.60	20,00	70,00
Pb plant ($\mu\text{g/g}$) ^f	11.75 \pm 3.10	9,00	16,00
Remediation (%) ^g	27.13 \pm 12.34	16.67	45.00
System 2 (N=16)			
Pb soil ($\mu\text{g/g}$) ^f	342.5 \pm 54.59	250,00	440,00
Pb EW ($\mu\text{g/g}$) ^f	24.01 \pm 10.97	12.55	54.54
Biomass (g)	2.11 \pm 0.45	1.40	2.90
Remediation (%) ^g	7.54 \pm 1.95	5,32	12.99
Control 2 (N=4)			
Pb soil ($\mu\text{g/g}$) ^f	70.00 \pm 16.33	50.00	90.00
Pb EW ($\mu\text{g/g}$) ^f	8.20 \pm 0.48	7.50	8.59
Biomass (g)	3.13 \pm 0.1	3.00	3.20
Remediation (%) ^g	12.29 \pm 1.49	10.38	14.00
System 3 (N=16)			
Pb soil ($\mu\text{g/g}$) ^f	316.25 \pm 87.25	150,00	430,00
Pb plant ($\mu\text{g/g}$) ^f	38.00 \pm 5.68	31.00	50.00
Pb EW ($\mu\text{g/g}$) ^f	26.01 \pm 6.66	16.53	39.00
Biomass (g)	1.18 \pm 0.47	0,90	2,50
Remediation (%) ^g	18.79 \pm 2.14	15.70	22.69
Control 3 (N=4)			
Pb soil ($\mu\text{g/g}$) ^f	67.5 \pm 9.57	60,00	80,00
Pb plant ($\mu\text{g/g}$) ^f	20.25 \pm 2.25	17.00	23.00
Pb EW ($\mu\text{g/g}$) ^f	12.84 \pm 1.09	11.36	13.93
Biomass (g)	2.78 \pm 0.13	2.60	2,90
Remediation (%) ^g	49.01 \pm 10.81	40.29	64.72

^a Size particles by sedimentation using the Robinson pipette method.
^b Organic matter was determined by calcination in the oven at 500°C according to NF ISO10694
^c pH_{water} with distilled water (w:v 1:5 ratio).
^d CEC determined by percolation according to NF X31-130.
^e Electrical conductivity water suspension with ratio 1:5.
^f Digestion with mixture of three acids inspired by the NF X 31-147.
^g calculated according to $:(R_1/R_0)*100$; [R₁: accumulated Pb in the system, R₀: accumulated Pb in soil].

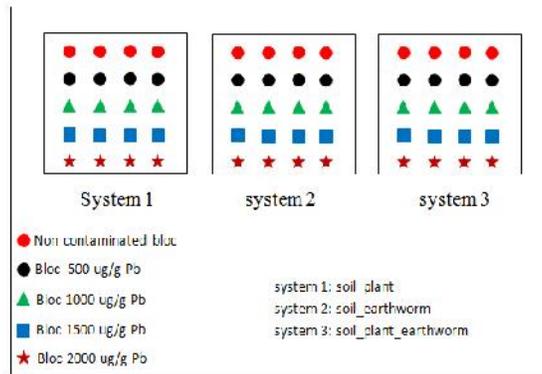


Fig. 1 - Experimental design

Sixty pots (18 cm diameter x 20 cm height) were prepared with 3 kg of dry soil, contaminated and mixed. The plant used in this experiment is a grass, common barley *Hordeum vulgare* L. Ten seeds were sown in the pots of the system S1 and S3. The experiment was conducted in greenhouses (temperature of $25 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ relative humidity) and germination was determined visually. The pots of the three systems are watered daily to keep the soil moist. After 4 months, adult anecic earthworms *Lumbricus* sp were collected in an uncultivated and unpolluted soil of Tiaret. They were rinsed to remove the soil, kept in plastic box in the laboratory (24 h) for cleaning their gut. 2 - 3 earthworms (mass: 3 ± 0.5 g) were introduced into each pot of system S2 and S3; to provide a density of about 100 g m^{-2} (Blouin et al, 2006; Jusselme et al, 2013). The experiment was conducted under controlled conditions during 21 days.

Harvesting and treatment of samples

At the end of the experiment the samples were harvested, the aerial part of the plant was cut at ground level. The stems and leaves were separated and preserved in paper bags. Then the pots were overturned on plastic. Earthworms were gathered, their survival was determined by observing their activities in the hand; afterward they were rinsed, weighed and conserved for 48 hours in boxes containing moistened paper with distilled water to clean their gut (Lemtiri et al, 2015; Bityutskii et al, 2016).

The roots were collected, washed with distilled water to remove the soil and kept in paper bags, the soil dried in the open air for 48 h. The samples were the subject of a series of operations as follows:

- dehydration: the usual method is dehydration in an oven at $105^\circ \pm 2^\circ \text{C}$ for 72 hours, and the earthworms for 24 h.
- grinding: This step is highly critical as it can be source of contamination or loss. For this, the grinder used is an agate mortar; 0.5 to 1 g of the powder obtained is placed in quartz capsules and calcined in an oven, the temperature of which being gradually increased to 450°C for 3 h.
- mineralization and dissolution: after calcination, the sample are placed in an acid solution (10 ml of hydrofluoric acid HF 40% and 3 ml of perchloric acid ClHO_4 70%) and heated in a sand bath until total evaporation of the solution. Outside of the sand bath, we added 1 ml of nitric acid HNO_3 and 10 ml of distilled water and allowed 30 min, after that, it was placed in the sand bath for 30 min to 1 h.
- filtration and dilution: After filtration, the obtained extracts are diluted with 100 ml of distilled water (Durand, C., 2003). The lead concentration was determined by atomic absorption spectrometry.

Statistical analysis

The statistical treatment was performed using two software packages STATISTICA 8 and SPSS 20. The data obtained were subjected to several analyses: Descriptive statistics, ANOVA, ANOVAR and correlation analysis. Firstly, the effects of soil lead levels on the concentration of lead in earthworm's tissues and plant were evaluated through analysis of variance (ANOVA). Secondly, the effects of physicochemical parameters of soil on the concentration of lead in earthworm's tissues and plant were investigated. Finally, the effects of lead levels (block T, B1, B2, B3 and B4) and the systems (S1, S2 and S3) on Pb concentration in earthworms' tissues and plant were evaluated. Differences were considered significant at $P 0.05^*$, highly significant at $P 0.01^{**}$ and very highly significant at $P 0.001^{***}$.

RESULTS

Levels of lead in soil

The concentrations of lead in soil within blocks of each system before and at the end of the experiment are represented in fig. 2.

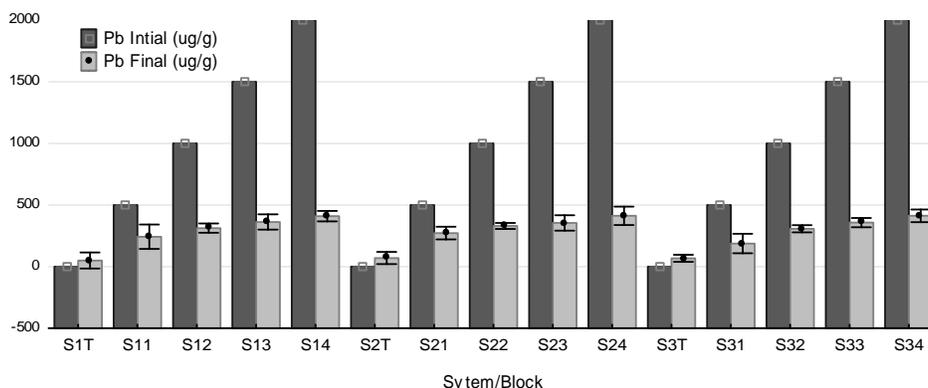


Fig.2 - Lead levels in the experimental soil

We see that the final concentrations of the soil have greatly reduced compared to the initial concentrations, a positive correlation was observed between the levels of contamination and soil concentrations with a correlation coefficient $r=0,94^{***}$ and a highly significant effect $p<0.001^{***}$.

The toxicity of metals does not depend only on the total concentration but also on their mobility and reactivity with other components of the ecosystem (Abollino et al, 2002). Many authors classify this reactivity in the order: $Ni>Zn>Cu>Pb$ (Harter, R. D., 1983; Kabala and Szerszen, 2002). Lead is generally found in surface horizons 0-20 cm than in deeper soil layers (Contat et al, 1991). Several factors affect its mobility and bioavailability: pH, soil texture especially clay content and organic matter content.

Concentrations of lead in the three systems

Remediation percentages of the three systems (including the five blocks) are shown in fig. 3. We observe that the association plant-earthworm (S3) shows the highest rate of remediation (accumulation) $24.83 \pm 13.26 \%$, a highly significant difference was observed between the three systems $p<0.001^{***}$. The concentrations of lead accumulated in the plant and earthworm increased significantly with the levels of lead in soil $p <0.001^{***}$.

The accumulation of lead by barley varies depending on the concentration of lead in the soil and the physic - chemical parameters of the soil (Maatoug et al, 2013). This concord with the results found in our study. Bioaccumulation of metals in earthworms depends a lot on the species and the characteristics of their environment, including soil composition and pH (Van Gestel and Ma 1988; Morgan J.E. and Morgan A.J. 1991; 1999). De Vauflery A. et al, 2013). The presence of the plant and earthworms can create a competition between them for the accumulation of lead. By producing exudates, plants can modify metal speciation and their behaviour in soil, particularly in the *rhizosphere* (Chaignon and Hinsinger, 2003; Uzu, G. et al, 2009). Therefore, plants can change the metal accumulation by earthworms (Lemtiri et al, 2015).

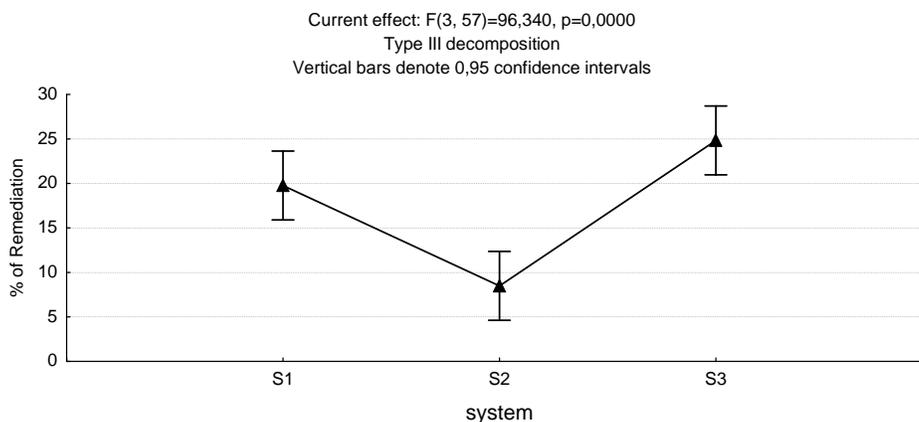


Fig. 3 - Percentage of remediation in the three systems S1: soil-plant; S2: soil-earthworm; S3: soil-plant-earthworm

Effects of the interaction plant / earthworms on the contaminated soil

Our study focused on the interaction between earthworm *Lumbricus sp* and the plant *Hordeum vulgare* for bioremediation of artificially contaminated soil with lead. In order to study the effects of the five lead concentrations used in this experiment which are divided into blocks, on the recorded soil concentrations, an ANOVA test was performed (fig. 4). A very highly significant difference is observed for the effect of lead dose added to the ground on the lead concentration in the soil, plants and earthworms $P<0.000^{***}$.

We notice an important fixative power of lead by the ground following the doses added at the beginning of the experiment.

The system S3 presents the highest concentration of lead extracted from the soil. We also observe that the plant accumulates more lead on its own $49.9 \pm 22.83 \mu\text{g g}^{-1}$ than in the presence of earthworms $34.45 \pm 8.92 \mu\text{g g}^{-1}$. The correlation matrix shows a positive correlation between Pb soil and Pb plant $r=0.688^{**}$.

The concentrations of lead in earthworm tissues after 21 days of exposure are positively correlated with soil concentrations with a correlation coefficient $r=0.919^{**}$ and a highly significant effect $p <0.000^{**}$. We observed that concentrations of lead in earthworm tissues are higher in the presence of the plant $23.37 \pm 8.02 \mu\text{g g}^{-1}$ against $20.85 \pm 11.71 \mu\text{g g}^{-1}$ in its absence.

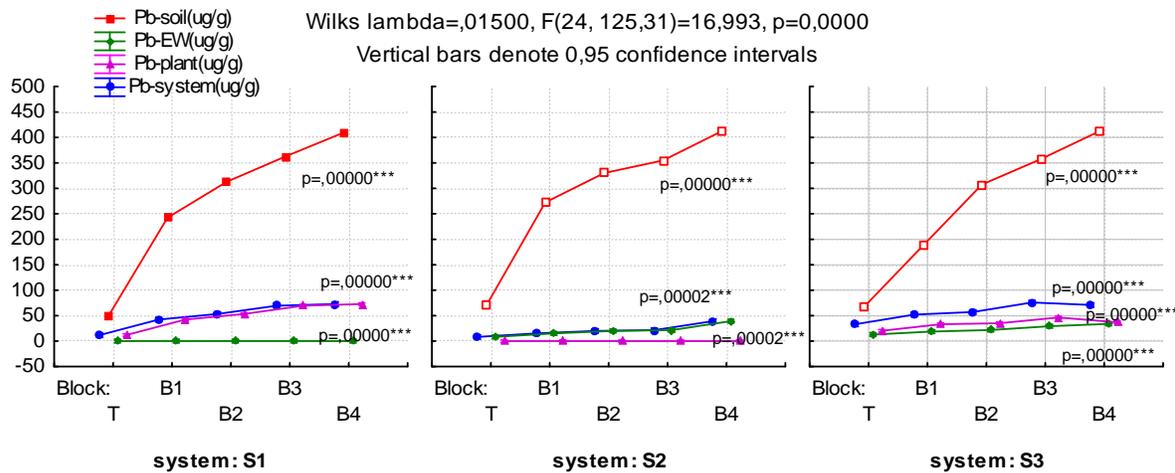


Fig. 4 - Effect of the interaction between bloc and Pb concentrations in plant, earthworm and soil for the three systems

Barley accumulates the trace elements to different degrees depending on the metal and its concentration in the soil. (Maatoug et al., 2013) reported that in an agricultural soil close to a highway contaminated with lead $1714.39 \pm 512.62 \mu\text{g g}^{-1}$, barley accumulates until $36.28 \pm 14.90 \mu\text{g g}^{-1}$. In our study, we find that for lead concentrations in the soil of the order of $331.86 \pm 66.95 \mu\text{g g}^{-1}$ barley accumulates $59.44 \pm 13.16 \mu\text{g g}^{-1}$.

Lead contrary to the other elements (Zn, Cu) is not an essential element, although present in the plants, it does not participate to any known physiological or biochemical function (Marschner H., 1995). Pb is accumulated by the plant according to another uptake pathway than those of the essential elements Zn and Cu (Lemtiri et al 2015). In this study, lead being added in a highly soluble form may be more available and easily absorbed by the plant, which explains the high concentrations accumulated by barley. Earthworms (of various species) that live in soils polluted by metals, mainly of anthropogenic source, have heavy metal contents much higher than those which develop in unpolluted areas (Ireland M.P., 1983; Morgan J.E. & Morgan A.J., 1988; Dai et al, 2004; De Vauffleury et al, 2013). Other studies report that concentrations in earthworms were weakly correlated with those of soils (Beyer and Cromartie, 1987; Abdul Rida and Bouché, 1995). The results found in this study indicate a very significant correlation between lead levels in soil and lead concentrations in earthworms *Lumbricus sp.*

An increase of metal concentration in plants was observed in the presence of earthworms (Wen and Hu, 2004). These results are opposite of what we observed in our study. Lead concentration in barley decreases greatly in the presence of *Lumbricus sp.* It can be explained by a different accumulation capacity and affinity according to the plant and the metal.

Earthworms can concentrate some chemicals involving selective absorption and excretion mechanisms, which vary according to earthworm species and chemical families (De Vauffleury A, 2013).

The ability of earthworms to accumulate heavy metals is widely studied (Morgan et al, 1986; Beyer W.N. et al, 1987; Morgan J.E. and Morgan A.J., 1992). Some differences in bioaccumulation were reported between ecophysiologicaly distinct earthworms (Morgan et al, 1993; Van Vliet et al, 2005; Kamitani and Kaneko, 2007). The presence of the plant increases the concentration of lead in earthworm tissues.

Effect of physicochemical soil parameters on Pb soil, Pb plant and Pb earthworms

In order to investigate the effect of physicochemical parameters of the soil on the accumulation of lead in the soil, plant and earthworms we performed a correlation analysis with a variance analysis (ANOVA).

Effect of pH

We observe a negative correlation between soil pH and lead soil, plants and earthworms with a correlation coefficient $r=-0.572^{**}$, $r=-0.396^*$, $r=-0.410^{**}$, respectively. ANOVA revealed a significant effect of soil pH on the Pb soil $p=0.037^*$ and Pb plant $p=0.002^{**}$, but no effect on the Pb earthworms $p=0.224$.

The pH is a factor whose role is crucial for the mobility of metal ions, because it influences the number of negative charges that can be brought into solution (McLaughlin, M.J., 2000). Generally, when

the pH increases, the cations are less soluble and less mobile (Blanchard C., 2000; Santillan-Medrano and Jurinak, 1975; Kabala and Szerszen, 2002). Different interpretations have been advanced to explain the influence of soil pH on the accumulation of lead (McBride, 1994). It was found that the Cd, Cu, Hg, Ni, Pb and Zn are strongly absorbed by the roots at pH<5.5 (Blaylock and Huang, 2000).

The accumulation of metals in earthworms is influenced by their concentration in the soil, and in the case of Cd, by the pH (Spurgeon et al. 2006). The low bioavailability of metals (Cu, Pb and Zn) in a contaminated soil with an alkaline pH, and the absence of toxic effects were confirmed experimentally in *Lumbricus terrestris*, in urban soils little rich in nutriment of Montreal (Kennette D., 2002). Among the reasons that may explain the limited effects of pH change on the concentration of earthworm tissue (Oste et al. 2001) suggest firstly, an effect of pH on the absorption by the skin, and secondly, the influence of soil particles ingested.

Effect of CEC

The cation exchange capacity is positively correlated with lead soil $r=0.221$ and earthworms $r=0.215^{**}$ and is weakly correlated with the lead of the plant with a correlation coefficient $r=0.023^{**}$. ANOVA shows no significant effect of CEC on the Pb soil, Pb earthworms and Pb plant $p > 0.05$.

The pH influences the CEC, the nature of the organic matter and its decomposition via the fauna and flora of the soil. (Allan and Jarrell, 1989) have shown that CEC decreases gradually as decreases the external pH. The soil constituents thus modified will register increase or decrease the complexation capacity of the elements and therefore the mobility and bioavailability of trace elements (McBride et al 1997; Sauvé et al, 1997 Sauvé and McBride, 1998; Venditti D., 2000). Simultaneous measurements of the cation exchange capacity suggest that this soil parameter is a better indicator of the bioavailability of Cd and Zn because it takes into account the type of clay and organic matter (Lock K. & Janssen C.R., 2001).

Effect of clay rate

Concerning the clay content it has a positive correlation with Pb soil $r=0.234^{**}$ and Pb earthworms $r=0.102$ and negatively correlated with the Pb plant $r=-0.054^{**}$, the analysis of variance indicated a highly significant effect for Pb soil and Pb plant $p<0.000^{***}$ and highly significant for Pb earthworms $p<0.005^{**}$.

Clays and organic matter play a predominant role in the adsorption of lead by the plant. Trace elements show a high affinity for the humic substances with which they form stable humic-clay complex, eventually soluble. This explains the abundance of the trace elements on the surface mainly in the presence of organic matter (Baize D., 1997).

Lock and Janssen (2001) conducted experiments with *Enchytraeus albidus*, which show that the toxicity of Cd and Zn depends on the nature of the clay used and the organic matter.

Effect of organic matter

A weak correlation was observed between the organic matter and the three variables, however it is negative with Pb soil $r=-0.029^{**}$, Pb earthworms $r=-0.038$ and positive with Pb plant $r=0.052^{**}$. ANOVA shows no significant effect $p>0.05$.

The assimilation of trace elements by plants is highly dependent on the bioavailability of these elements in the soil. The soil constituents, especially clays and organic matter can interact with the metal across different chemical interactions (electrostatic interactions etc.) All these interactions limit the bioavailability of the metals in the soil (Tanner and Headley, 2011).

The soil characteristics have a different impact from one earthworm species to another also depending on the studied metal (Peijnenburg W.J.G.M., 1999; Posthuma L., 1998). It was observed an increase of lead concentration in earthworms exposed in soil with high lead contamination (Grelle and Descamps, 1998; Scaps et al., 1997). Pb accumulation is proportional to the time of exposure. However the concentration in the earthworms remains much lower than that of soil. These results are similar with the results that we found in our experiments.

CONCLUSIONS

The present study shows that using the association *Hordeum vulgare* and *Lumbricus sp* significantly increases the concentrations of lead extracted from the soil. The concentrations of lead in *Lumbricus sp* tissues and *Hordeum vulgare* increase with increasing levels of lead in the soil.

The lead concentrations in the soil depend on the physico-chemical parameters. However, other factors (element content and mobility) may increase or decrease retention of lead by the soil. The system S3

(soil-plant-earthworm) has the highest rate of remediation 24.83 ± 13.26 % compared to the two others S1 (soil-earthworm) 8.49 ± 2.67 % and S2 (soil-plant) 19.77 ± 6.41 %.

The presence of earthworms *Lumbricus sp* decreases the bioaccumulation of lead by *Hordeum vulgare*, while content recorded in tissues of earthworms suggests that the presence of the plant significantly increases the element concentration. This can be explained by a competition between the two organisms in the absorption of trace elements.

Several studies report that the accumulation of trace elements differs from one species to another. However, the interaction between two organisms such as earthworms and plants can be complex and influenced by many factors: the species of the plant and the earthworm, the physico-chemical parameters of the soil, the levels of pollution and the nature of the pollutant.

The results of this study suggest that it is possible to use the association plant/earthworms for the bioremediation of agricultural soils polluted by lead.

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