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Rhizosphere effects of *Populus euramericana* Dorskamp on the mobility of Zn, Pb and Cd in contaminated technosols

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Abstract

Purpose: This study aimed at investigating the rhizosphere effects of *Populus euramericana* Dorskamp on the mobility of Zn, Pb and Cd in contaminated technosols from a former smelting site.

Materials and methods: A rhizobox experiment was conducted with poplars, where the plant stem cuttings were grown in contaminated technosols for two months under glasshouse conditions. After plant growth, rhizosphere and bulk soil pore water (SPW) were sampled together. SPW properties such as pH, dissolved organic carbon (DOC) and total dissolved concentrations Zn, Pb and Cd were determined. The concentrations of Zn, Pb and Cd in plant organs were also determined.

Results and discussion: Rhizosphere SPW pH increased for all studied soils by 0.3 to 0.6 units compared to bulk soils. A significant increasing was also observed for DOC concentrations regardless the soil type or total metal concentrations, which might be attributed to the plant roots activity. For all studied soils, the rhizosphere SPW metals concentrations decreased significantly after plant growth compared to bulk soils which might be attributed to the increase in pH and effects of root exudates. Zn, Pb and Cd accumulated in plant organs and the higher metals concentrations were found in plant roots compared to plant shoots.

Conclusions: **The restricted transfer of the studied metals to the plants shoots confirms the potential role of this species in the immobilisation of these metals**. Thus *Populus euramericana* Dorskamp can be used for phytostabilisation of technosols.

Keywords: Contaminated soil, *Populus euramericana* Dorskamp, Rhizosphere, Rhizobox experiment, Smelting site, Soil pore water (SPW).

Abbreviations

CEC	Cation exchange capacity
DOC	Dissolved organic carbon
EC	Electrical conductivity
MDN	Mortagne-du-Nord
PTE	Potentially toxic elements
SPW	Soil pore water
TOC	Total organic carbon

1 Introduction

Metals and metalloids occur naturally at low concentrations in soils. Due to its potential ecotoxicogical effects, soil contamination by these potentially toxic elements (PTE) has become a critical environmental concern. Mining and smelting of metal ores activities have increased the spread and occurrence of PTE contamination by release of a huge amount of PTE into the environment through wind and water runoff erosion (Razo et al. 2004; Navarro et al. 2008).

In the literature, several studies have been conducted to assess and remediate contaminated sites. Some of these preliminary studies are based on monometallic strains and poplar trees (reconstituted soils spiked with monometallic solution) (Durand et al. 2010; 2011).

Phytoremediation is a developing technology that uses green plants to clean up contaminated soil by translocation of PTE from soils to plants or immobilization of PTE in the plants rhizosphere and has recently emerged as a perceived green alternative to expensive and ecologically instructive conventional remediation technologies (Reeves et al. 2000; Lim et al. 2004). It has the advantages of being environmentally friendly and less disruptive to the soil (Salt et al. 1995). The understanding of the factors that control plant accumulation or contribute to immobilization of metal contaminants is one of the basic principles of phytoremediation.

It is well reported that the rhizosphere is a dynamic region where multiple interactions occur in the plant roots-soil-microbe system (Darrah et al. 2006). Moreover it has been assumed that the rhizosphere parameters (i.e. chemical, physical and biological root-modifications) were entirely different from that of bulk soil parameters (Curl and Truelove 1986). The factors influencing metal(loid)s mobility and bioavailability include root-induced pH changes, root-induced microbial activity and metal binding by root exudates that are considered as the most important factors of differences between rhizosphere and bulk soil (Ernst 1996). Therefore, the dynamic, transformations, bioavailability and toxicity of PTE in the rhizosphere are expected to differ noticeably from those in bulk soil.

The soil solution is the medium through which the PTE exert their effects on organisms (Knight et al. 1998). At the rhizosphere which includes the interface between plant roots - soil system, the behavior of PTE in the soil solution should be demonstrated to better understand their soil to plant transfer. The concentration of PTE in the rhizosphere soil pore water (SPW) is often considered to reflect accurately the plant-available concentration of PTE in the soil, which is influenced by properties as the dissolved organic carbon (DOC) and pH (Sauvé et al. 1996; Vijver et al. 2003).

It is well reported that the nature of the metal species and metal speciation in soil SPW is an important factors which dictate the plant metal uptake (Kim et al. 2010b).

Root activity (e.g. excretion of H^+ and HCO_3^- , respiration uptake of water and nutrients and exudation of organic compounds) can alter the chemical conditions in the rhizosphere SPW (Hinsinger 1998, 2001). Due to its solubility and lability, DOC plays also a key role in sustaining soil microbial activity (Kalbitz et al. 2000; Chantigny 2003). Therefore, the analysis of the rhizosphere SPW during the cultivation period may provide useful information concerning the potential toxicity of the PTE (Lorenz et al. 1994; Yanai et al. 1995, 1998; Moritsuka et al. 2000b). In fact soil pH, soil organic matter (SOM), DOC and the presence of organic acids (OAs) significantly influence metal solubility (Sauvé et al. 1997; Hees et al. 2000; Weng et al. 2002) and may be considerably different from that in the bulk soil. In particular, DOC compounds present in root exudates such as carboxylic acids contribute in the formation of soluble metal chelate complexes with P and changing soil pH resulting in the mobilisation of inorganic P in the rhizosphere (Kirk et al. 1999).

The aim of this study was to investigate the early responses of Zn, Cd and Pb mobility to rhizospehere effect of *Populus euramericana* Dorskamp in contaminated technosols after two months of cultivation of woody plant stem cuttings using a rhizobox system. This duration time is important (Di Baccio et al. 2014) according to the fact that poplar trees are mainly propagated by cutting rooting. Moreover this genotype has demonstrated an important metal accumulation (Pottier et al. 2015) and to grow on contrasting sites (Bonhomme et al. 2008). Total dissolved concentrations of PTE in SPW were determined together with control factors such as pH and DOC concentration in comparison with bulk soil. Additionally, the concentrations of these metals in plant organs were determined to investigate the translocation or immobilization capability of *Populus euramericana* Dorskamp.

2 Materials and methods

2.1 Soil sampling

Soil samples used in this study were collected from a metallicolous grassland contaminated with Zn, Pb and Cd located at Mortagne-du-Nord (MDN) in Northern France (Fig. 1) This area is adjacent to a former metallurgical site occupied for over 60 years by a Zn smelter unit linked to a sulfuric acid production unit and a Pb smelting unit (Thiry and van Oort 1999).

The site has been back-filled with slags and smelting crucible to a thickness of about 3 meters. The mineralogy of these materials is highly varied. More than 30 heavy metal-bearing (Zn, Pb, Cd, Cu) mineral species have been identified; these are sulfides, sulfates, carbonates, oxides and silicates. The soil development at the metalliferous grassland site has been previously described (Thiry et al. 2002). The O, A and C horizons can be distinguished by abrupt transitions that provide a neat horizontal stratification of the horizons for the first 40 cm of soil.

The geological context is made of the Sand of Ostricourt (Paleocene/ lower Eocene). These are glauconious sands with a medium granulometry. On top of them is the clay alluvial material of the nearby River Scarpe, clays and fine sands rich in organic matter. The ancient soils have been buried under the wastes. Three soil sampling sites named (MDN1, MDN2, and MDN3) were selected for this study according to the level of PTE concentrations (Qasim and Motelica-Heino 2014; Qasim et al. 2015) and spatial distribution of the vegetation which essentially consists of *Arabidopses hallari* L. and *Avena sativa* L.

Surface soils (0-20 cm) were sampled from each location with a stainless steel spade, carefully transferred to clean polyethylene bags before transport to the laboratory, oven-dried for 72h at a constant temperature of 40°C, manually homogenized, then sieved using a 2mm mesh sieve to remove the gross matter.

2.2 Soil physico-chemical properties

Soil physico-chemical properties were characterized by routine soil testing laboratories according to standardized French (AFNOR 1999) or international (ISO 1999) procedures.

Soil pH and EC were determined in deionised water extracts (1:2.5 w/v) (NF ISO 10390 (2005)), using a combined pH-EC meter (WTW, ProfiLine 1970i, Germany), calibrated using pH 4.0 and pH 7.0 standards. Total organic carbon was determined according to ISO (1999). Cation exchange capacity (CEC) was determined by the 0.05N cobalthexamine method (Aran et al. 2008). Total metals concentration were determined by aqua regia/hydrofluoric acid digestion (Zhang et al. 2008), then measured by inductively coupled plasma mass spectrometry (ICP–MS, Finnigan Element XR,Thermo Electron, Germany).

2.3 Reagents and standards

All the reagents used to prepare the extracting solutions were products of analytical-grade quality (Merck pro-analysis, Darmstadt, Germany). All solutions and dilutions were prepared using ultra-pure water (18.2MΩcm^{-1}) (Thermo Scientific Barnstead Easy pure II systems).

Standard stock solutions of 1000 mg l^{-1} of different elements were prepared from metal wires or salts of purity higher than 99.998% (VWR international, BDH Prolabo ICP Standards, Belgium). Diluted standard working solutions were prepared from these on a daily basis. All laboratory glassware and plastic ware were rinsed three times with double deionized water after being soaked in a HNO_3 (10%, v/v) batch for 24h.

2.4 Rhizobox and cultivation experiment

A rhizobox experiment was conducted in a greenhouse for two months. In this work, a rectangular rhizobox with dimensions of 150 × 180 ×260 (length ×width × height, in mm) were used. It was divided into three sections: a root compartment (60 mm in width), and left and right non-rhizosphere zones (100 mm in width). Root growth was limited to the central compartment within the nylon cloth 300 mesh (DIATEX sas, France). **1 kg of soil was placed in the rhizosphere zone. In each of the 2 non-rhizosphere zones 1.75 kg were placed.** *Populus euramericana* Dorskamp plant was cultivated in the rhizosphere zone and the experiment was made in three replicates. The plants were watered by distilled water throughout the study to keep the soil at approximately 80% of its water holding capacity. The plants were allowed to grow for 2 months in a greenhouse under controlled conditions with natural light and air temperature of 22°C and 55% relative air humidity. At the end of the experiment, the plants were harvested, washed thoroughly with tap water and then rinsed with distilled water. Each plant was separated into roots, stems, cuttings and leaves. Then the plant organs were oven dried in 70°C for 3days. Dried plant organs were ground with a laboratory grinder and 200mg (±0.5mg) of each plant organs were digested with a pressurized closed-vessel microwave system (Multiwave 3000, Anton Paar GmbH, Germany). Microwave polyfluoroacetylene (PFA)-teflon vessels were cleaned before each digestion using 10ml of aqua regia (HNO_3/HCl, 1:3v/v, Fisher scientific, UK), heated for 20min. at 200°C and then rinsed with double deionized water. Blanks were processed in a method identical to the samples. ICP–MS measurements were carried out by diluting 1:10 with Milli-Q water the plant digests to determine the studied PTE concentrations in plant organs.

2.5 Soil pore water collection and analysis

Soil pore water (SPW) was collected at the beginning and at the end of the experiment by Rhizon soil moisture samplers (Rhizosphere Research Products, Wageningen, The Netherlands). Water was priory added to the soil to obtain 80% of the WHC and the soil was allowed to equilibrate for 24 hr. The SPW were separated into several sub-samples and stored at 4°C until the analysis step. An aliquot (3 mL) of each SPW was acidified with nitric acid and ICP-MS measurement used to determine the PTE (Zn, Pb and Cd) concentrations in SPW.

The rest of the SPW solutions were used to measure pH using a combination pH-EC meter and DOC concentration using an automatic carbon analyzer (Shimadzu© TOC 5000A).

2.6 Statistical analysis

Results were analyzed with the SPSS statistical software package (SPSS, Chicago, IL, USA). Means are expressed with their standard error (± SE). Statistical comparison of the means resulted from Student's *t*-test. Levels of significance were $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. For dry weight and PTE concentrations in plant organs means are expressed with their standard error and were compared by ANOVA. In each case the number of replicates (n) is indicated. Statistical tests were considered significant at $P \leq 0.05$.

3 Results and discussion

3.1 Studied soil properties

The main physico-chemical properties of the soil samples are summarized in Table 1. Soil samples were different in their main physical and chemical properties. Soils pH was acidic to slightly acid and ranged from 6.1 to 6.9. Total organic carbon (TOC) ranged from 3.3 to 6.45%. Cation exchange capacity varied from 6.7 to 8.5 cmol(+)kg^{-1} for MDN1, MDN2 and MDN3 respectively. **The total PTE were also varied** among the studied soils. The highest PTE concentration was observed for MDN1. The PTE concentration in both MDN2 and MDN3 was almost similar but it remains less than that for MDN1. Among the studied metals, Zn has the highest concentration in all studied soils.

3.2 Soil pore water pH and DOC concentrations

It is well reported that the changes in the chemical soil properties occurred as a result of soil–plant interactions in the rhizosphere, and that the soil pH is one of the most important chemical factors controlling the metals availability (Fitz et al. 2003).

At the end of the experiment, as can be seen in Fig. 2 a significant increase in SPW pH was observed after plant growth in all studied samples compared to bulk soil. The plant growth increased rhizosphere SPW pH by 0.3 to 0.6 units.

The release of H^+ or OH^- by roots is the main process responsible for the pH changes in the rhizosphere resulted from imbalance uptake of anion and cation by plants roots (Haynes 1990; Sas et al. 2001; Bravin et al. 2009). Several studies have reported that plant growth reduced the rhizosphere soil pH resulting in acidification of the soils due to the release of H^+ and organic acids by plants (Bernal and McGrath 1994; Gonzaga et al. 2009). The rhizosphere pH reduction might be also attributed to the build-up of CO_2 in the rhizosphere by the respiration process of microorganisms and roots (Hinsinger et al. 2005).

Contrary to these findings, our results were however in agreement with Luo et al. (2000) who found that *T. caerulescens* rhizosphere soil had a higher pH compared to the non-rhizosphere soils.

Kim et al. (2010a, b) also reported that soil solution pH increased by 1.3 units after Indian mustard and sunflower growth in both acidic and alkaline soils.

It appears in our experiment that independent of the characteristics of studied soils, the anion uptake by plant roots might **increase of SPW pH of rhizosphere** to maintain electrical neutrality across the root soil interface and to counterbalance the excess negative charge taken up by the **roots (Nye, 1981; Gahoonia et al. 1992). This explains the results that the adjacent soil was more alkaline.** Kim et al. (2010b) suggested that the increase of pH in acidic soils compared to alkaline soils might be attributed to the variable buffering capacity of these soils. In the other hand, Roemkens et al. (1999) suggested that changes of the calcium concentration resulted in increasing of rhizosphere SPW pH. However, increases in SPW pH in the rhizosphere can affect metals solubility at the root-soil interface.

Fig. 3 showed irrespective of soil type, a significant increase in DOC concentrations in the SPW of rhizosphere of all studied soils after plant growth. However, the DOC concentrations were almost more than 1.5 times higher compared with those of bulk soils. Our findings are in agreement with those of (Lorenz et al. 1997; Cattani et al. 2006; Khalid et al. 2007) who reported that the presence of a range of grassland plants significantly increased the DOC concentrations in comparison with unplanted soil. Similarly, Fitz et al. (2003) found that DOC concentrations were increased by 86% in rhizosphere soil solution of As hyperaccumulator *P. vittata*.

In fact, these authors have attributed the reason for DOC increase to root exudation and to the solubilisation or mineralisation of soil organic matter (SOM) which caused by the microbial or rhizosphere activity. It is well known that the rhizodeposits such as root exudates serve as a carbon source; therefore, in this study, the increase in DOC concentrations after plant growth was attributed to the increasing release of root exudates (which consist of a complex mixture of organic acids, sugars, vitamins, nucleosides, inorganic ions).

3.3 Zn, Pb and Cd concentrations in rhizosphere soils

The total dissolved concentrations of Zn, Pb, and Cd in SPW at the end of the experiment are shown in **Fig. 4.** It can be seen that for all the studied soils, Zn concentrations in rhizosphere SPW decreased significantly compared to the bulk soil. After plant growth, the solubility of Zn was reduced by 12.3% to 24.9% for the rhizosphere soils compared to bulk soil **(Fig. 4A).** A similar picture was observed for Cd. The plant growth resulted in a significant decrease in rhizosphere SPW Cd concentrations relative to the bulk soil for MDN2 and MDN3 but not for MDN1. The solubility of Cd was reduced by 18.2% and 20% respectively (Fig. 4B).

A significant decrease in the rhizosphere SPW Pb concentrations was also observed in both MDN1 and MDN3 but not for MDN2. The solubility of Pb was reduced by 23% and 52% compared to bulk soil (Fig. 4C).

For all studied soils, the Zn, Pb, and Cd concentrations in the SPW were significantly $P \leq 0.05$ decreased after plant growth compared to bulk soil indicating that the plant growth affected the studied PTE concentrations in SPW.

It is well known that root exudates influence metals behavior directly or indirectly, resulting in increase or decrease of their mobility and bioavailability by acidification, alkalinisation, precipitation and their effects on physical and chemical properties of the rhizosphere (Tao et al. 2004; Uren and Reisenauer 1988). Additionally, several studies have reported that in contaminated soils, depending on the soil buffer capacity, the rhizosphere pH can increase up to 2 units compared to the bulk soil and may enhance metals bioavailability and increase metal uptake by plants (Wenzel et al. 2002; Vazquez et al. 2006) or remain unchanged (Martınez-Alcala et al. 2009).

However, the reduction of metal solubility with increasing pH might be due to adsorption on the soil surface or the precipitation of metals as phosphates or the formation of new aggregate structures within the repacked soil (Luster et al. 2008). Therefore, despite of the increase in DOC concentrations, the observed decrease in metal solubilities in the rhizosphere SPW compared to bulk soil might be attributed to the increase of soil solution pH, indicates that the metals solubilites in these studied soils was driven by soil pH mainly.

Despite the fact that DOC accounts for a small portion of SOM only, it is generally accepted that DOC can affect the nutrient and contaminant mobility, thus, facilitating metal release from the soil solid phase to the SPW through the formation of metals (i.e. Zn, Pb Cu, Cd and

Ni)-DOC complexes (Weng et al. 2002; Kaiser et al. 2002; Schwab et al. 2005). Similarly, Wenzel et al. (2003) found a significant correlation between Ni and DOC concentrations in SPW. This hypothesis is compatible only with the solubility of Zn. Linear regression analysis showed a significant positive relationship ($r^2 = 0.70$, $P < 0.01$) between DOC concentrations and solubility of Zn. Contrary to these findings, our results for Cd and Pb may be in line with the explanation of Benjamin and Leckie (1981a, b) who reported that complex formation by DOC can decrease the metal solubility through adsorption of metals–DOC complexes on the soil surface. Likewise, Kim et al. (2010a) found that Indian mustard growth has increased the soil solution pH and resulted in decreasing metal solubilites in spite of the increasing in DOC concentration, which was explained by the increase of metal binding onto the soil surface through deprotenation due to the increase in pH value (Naidu and Harter 1998).

Therefore, depending on the foregoing, it can be concluded that DOC concentrations increasing during plant growth might have an opposite effect by reducing the impact of increasing pH on the metal solubilites, given that the SPW pH is the most important chemical factor controlling the availability of metals and that the increase of the rhizosphere pH decreased the chemical activity of most metals. Rhizosphere alkalinisation may thus be an important mechanism for the immobilisation of the studied PTE by *Populus euramericana* Dorskamp plants in contaminated technosols. The general increase in SPW pH by this plant for the majority of soils studied here indicated the potential use of this plant for phytostabilization.

3.4 Plant growth and metals uptake

The rhizobox experiment of *Populus euramericana* Dorskamp grown in contaminated MDN soils for 60 days showed an accumulation of varying amounts of PTE in the different plant organs (Fig. 6).

Compared to other studied elements, the accumulation of Zn was found maximum, whereas, Cd was found to be minimum in all plant organs for all studied soils.

The plant organs (leaves, roots and stems) dry weight (DW) **is shown in (Fig.5).** The highest shoot DW was observed for MDN1, followed by MDN3 soil and the lowest shoot DW was observed for MDN2 soil. Contrary to shoot biomass, root biomass for MDN3 was found maximum followed by MDN1 and the lowest was recorded also for MDN2 soil.

It can be seen from Fig. 6A that plant roots contained higher concentration of Zn than shoots for both MDN1 and MDN2 and the opposite was observed for MDN3. This might be attributed to the high DOC concentrations and the lowest rhizosphere pH value for MDN3 compared to the other soils. Therefore, Zn uptake by plants leaves was related to SPW Zn concentrations (r^2 = 0.72, P< 0.01). It is well reported that the large Zn concentrations in plant leaves associated with small concentrations in roots, indicating high metal uptake and efficient translocation from root to shoots (Utmazian et al. 2007). In contrast, the low Zn concentrations in plant shoots for both MDN1 and MDN2 samples might be attributed to the fact that a significant amount of Zn accumulated in plant roots, indicating that plant translocation from root to shoots is poor.

Leaves Cd uptake was more closely related to the Cd SPW concentration (r^2 = 0.45, P< 0.05). Fig. 6B showed that the highest Cd plant shoots and root concentration was observed for MDN1 compared to other soils. This indicated that plant Cd uptake and translocation was dependent on both SPW Cd and total soil Cd concentration, implying the dependence of metal uptake by plants on the change in dissolved Cd concentration induced by pH increase related to plant growth. Our results were in agreement with that of Kim et al. (2010b) which found the same trend in green house potted experiment. Our results were also in line with that obtained by Bose and Bhattacharyya (2008), who found that Zn, Cd and Pb, concentrations were highest in roots and lower in shoots in wheat plant. The low Cd concentrations in plant shoots compared to Zn and Pb might be attributed to the chemical similarity between Cd and Zn which influences the Cd plants contamination (Hamon et al. 1998). Therefore, the higher Zn concentration in SPW competes directly with Cd resulting in the reduction of Cd uptake by plants (McLaughlin et al. 1995; Grant and Bailey 1997).

As for Zn, plant roots contained higher concentration of Pb than shoots (Fig. 6C) the content of Pb in plant roots was linearly related to Pb concentration in rhizosphere soil solution (r^2 = 0.66, P< 0.01). In the other hand, no significant correlations was observed between Pb concentrations in plant leaves with rhizosphere SPW Pb concentrations indicating that the translocation of Pb from roots to shoots is limited and thus Pb accumulated in roots. This result is in agreement with Yang et al. (2010) who reported that irrespective of plant species, roots accumulated more Pb and Zn than plant shoots. Murillo et al. (1999) and Liu et al. (2000) observed similar results for Pb and other metals translocation from roots to shoots in sunflower and Indian mustard plants.

From the foregoing, the difference in studied metals concentration between plant roots and shoots might be attributed to the explanation given by Cobbett and Goldsbrough (2002). It shows the ability of plant to develop many strategies such as chelation with phytochelatins (PCs) and compartmentation into cytosol to reduce metals uptake. It might be also attributed to the ability of plant root cell wall to retain and/or actively transport Pb from the cell wall back to soil, resulting in the decrease of metals uptake and accumulation by the root (Nishizono et al. 1987).

As for Cd, the low Pb plant uptake compared to Zn might be attributed to that of multi metal contamination as the presence of some metals may affect the solubility and plant uptake of the other metals. This is in line with Nan et al. (2002) who found that the total Zn content of soil lowered the accumulation of Pb in the wheat grains.

4 Conclusions

The present study demonstrated that a significant change in the SPW pH and DOC concentrations was observed after *Populus euramericana* Dorskamp growth in contaminated technosols. The presence of these plants increased rhizosphere SPW pH by 0.1 to 0.6 units compared to bulk soil. DOC concentrations also increased irrespective of soil type or total metal concentrations compared to bulk soil. Plant growth reduced the solubility of Zn, Pb and Cd concentrations due to pH increases or through the formation of metals-DOC complexes. These changes in rhizosphere SPWproperties influenced the plant metals uptake. The higher concentrations of studied metals were found in plant roots compared to plant shoots. The restricted transfer of metals to the plant shoots confirms the potential role of this species in the immobilisation of metals and phytostabilisation.

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References

Benjamin MM and Leckie JO (1981a) Multiple-site adsorption of Cd, Cu, Zn, and Pb on amorphous iron oxyhydroxide. J Colloid Interface Sci 79:209–221

Benjamin MM and Leckie JO (1981b) competitive adsorption of Cd, Cu, Zn, and Pb on amorphous iron oxyhydroxide. J Colloid Interface Sci 83:410-419

Bernal MP, McGrath MP (1994) Effects of pH and heavy-metal concentrations in solution culture on the proton release, growth and elemental composition of Alyssum murale and Raphanus sativus L. Plant Soil 166:83–92

Bonhomme L, Barbaroux C, Monclus R, Morabito D, Berthelot A, VillarM, Dreyer E, Brignolas F (2008) Genetic variation in productivity, leaf traits, and carbon isotope discrimination in hybrid poplars cultivated on contrasting sites. Ann For Sci 65:2–8

Bose S, Bhattacharyya AK (2008) Heavy metals accumulation in wheat plant grown in soil amended with industrial sludge. Chemosphere 70:1264–1272

Bravin MN, Marti AL, Clairotte M, Hinsinger P (2009) Rhizosphere alkalisation – a major driver of copper bioavailability over a broad pH range in an acidic, copper contaminated soil. Plant Soil 318:257–268

Cattani I, Fragoulis G, Boccelli R, Capri E (2006) Copper bioavailability in the rhizosphere of maize (Zea mays L.) grown in two Italian soils. Chemosphere 64:1972-1979

Chantigny MH (2003) Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. Geoderma 113:357-380

Cobbett C, Goldsbrough P (2002) Phytochelation and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

Curl EA, Truelove B (1986) The Rhizosphere. Springer, Berlin, New York, p 286

Darrah PR, Jones DL, Kirk GJD, Roose T (2006) Modelling the rhizosphere: a review of methods for ‘upscaling’ to the whole-plant scale. Eur J Soil Sci 57:13-25

Di Baccio D, Castagna A, Tognetti R, Ranieri A, Sebastiani L (2014) Early responses to cadmium of two poplar clones that differ in stress tolerance. Plant Physiol 171: 1693-1705

Durand TC, Hausman JF, Carpin S (2010) Zinc and cadmium effects on growth and ion distribution in *Populus tremula* x *Populus alba.* Biol. Plant. 54 : 191-194

Durand TC, Baillif P, Albe’Ric P, Carpin S, Label P, Hausman JF, Morabito D (2011) Cadmium and zinc are differentially distributed in Populus tremula x P. Alba exposed to metal excess. Plant Biosys 145:397–405

Ernst WHO (1996) Bioavailability of heavy metals and decontamination of soils by plants. Appi Geochem 11:163–167

Fitz WJ, Wenzel WW, Zhang H, et al. (2003) Rhizosphere characteristics of the arsenic hyperaccumulator Pteris vittata L. and monitoring of phytoremoval efficiency. Environ Sci Technol 37:5008-5014

Gahoonia TS, Claassen N, Jungk A (1992) Mobilization of phosphate in different soils by ryegrass supplied with ammonium or nitrate. Plant Soil 140:241–248

Gonzaga MIS, Ma LQ, Santos JAG, Matias MIS (2009) Rhizosphere characteristics of two arsenic hyperaccumulating Pteris ferns. Sci Total Environ 407:4711–4716

Grant CA, Bailey LD, Harapiak JT, Flore NA (2002) Effect of phosphate source, rate and cadmium content and use of Penicillium bilaii on phosphorus, zinc and cadmium concentration in durum wheat grain. J Sci Food Agric 82:301–8

Hamon RE, McLaughlin MJ, Naidu R, Correll R (1998) Long-term changes in cadmium bioavailability. Environ Sci Technol 32:3699–703

Haynes RJ (1990) Active ion uptake and maintenance of cation–anion balance: a critical examination of their role in regulating rhizosphere pH. Plant Soil 126:247–264

Hees PAWV, Lundstrom US, Giesler R (2000) Low molecular weight organic acids and their complexes in soil solution-composition, distribution and seasonal variation in three podzolized soils. Geoderma 94:173–200

Hinsinger P (1998) How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. Adv Agron 64:225-265

Hinsinger P (2001) Bioavailability of trace elements as related to root-induced chemical changes in the rhizosphere. In: Gobran GR, Wenzel WW, Lombi E (eds) Trace Elements in the Rhizosphere. CRC Press, Boca Raton, pp 25-41

Hinsinger P, Plassard C, Jaillard B (2005) Rhizosphere: a new frontier for soil biogeochemistry. J Geochem Explor 88:210–213

Kaiser K, Guggenberger G, Haumaier L, Zech W (2002) The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil, Org. Geochem 33:307–318

Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: a review. Soil Science 165:277-304

Khalid M, Soleman N, Jones DL (2007) Grassland plants affect dissolved organic carbon and nitrogen dynamics in soil. Soil Biol Biochem 39:378–381

Kim KR, Owens G, Kwon SIK (2010a) Influence of Indian mustard (Brassica Juncea) on rhizosphere soil solution chemistry in long – term contaminated soils: A rhizobox study. J Environ Sci 22:98–105

Kim K R, Owens G, Naidu R (2010b) Effect of Root-Induced Chemical Changes on Dynamics and Plant Uptake of Heavy Metals in Rhizosphere Soils. Pedosphere 20:494 504

Kirk GJD, Santos EE, Findenegg GR (1999) Phosphate solubilisation by organic anion excretion from rice (Oryza sativa L.) growing in aerobic soil. Plant Soil 211:11–8

Knight B, Zhao FJ, McGrath SP, Shen ZG (1997) Zinc and cadmium uptake by the hyperaccumulator Thlaspi caerulescens in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution. Plant Soil 197:71-78

Knight BP, Chaudri AM, McGrath SP, Giller KE (1998) Determination of chemical availability of cadmium and zinc in soils using inert soil moisture samplers. Environ Pollut 99:293–298

Lim JM, Salido AL, Butcher D (2004) Phytoremediation of lead using Indian mustard (Brassica juncea) with EDTA and electrodics. Microchem J 76:3–9

Liu D, Jiang W, Liu C, Xin C, Hou W (2000) Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard [*Brassica juncea (L.)*]. Bioresour Technol 71:273 277

Lorenz SE, Hamon RE, Holm PE, Domingues HC et al. (1997) Cadmium and zinc in plants and soil solutions from contaminated soils. Plant Soil 189:21–31

Lorenz SE, Hamon RE, McGrath SP (1994) Differences between soil solutions obtained from rhizosphere and non-rhizosphere soils by water displacement and soil centrifugation. Eur J Soil Sci 45:431-438

Luo YM, Christie PAJ, Baker M (2000) Soil solution Zn and pH dynamics in non rhizosphere soil and in the rhizosphere of Thlaspi caerulescens grown in a Zn/Cdcontaminated soil, Chemosphere 41:161–164

Luster J, Menon M, Hermle S, Schulin R, Georg-Gunthardt MS, Nowack B (2008) Initial changes in refilled lysimeters built with metal polluted topsoil and acidic or calcareous subsoils as indicated by changes in drainage water composition. Water Air Soil Pollut 8:163‐176

Martınez-Alcala I, Clemente R, Bernal MP (2009) Metal availability and chemical properties in the rhizosphere of Lupinus albus L. growing in a high- metal calcareous soil. Water Air Soil Pollut 201:283–293

McLaughlin MJ, Maier NA, Freeman K, Tiller KG, Williams CMJ, Smart MK (1995) Effect of potassic and phosphatic fertilizer type, fertilizer Cd concentration and zinc rate on cadmium uptake by potatoes. Fert Res 40:63–70

Moritsuka N, Yanai J, Kosaki T (2000b) Non-destructive method for determining temporal and spatial changes of the soil solution chemistry in the rhizosphere. Soil Sci Plant Nutr 46:713-719

Murillo JM, Marañó T, Cabrera NF, Lopez R (1999) Accumulation of heavy metals in sunflower and sorghum plants affected by the Guadiamar spill. Sci Total Environ 242:281 292

Naidu R, Harter RD (1998) Effect of different organic ligands on cadmium sorption by and extractability from soils. Soil Sci Soc Am J 62:644–650

Nan Z, Zhao C, Li J, Chen F, Sun W (2002) Relations between soil properties and selected heavy metal concentrations in spring wheat grown in contaminated soils. Water Air Soil Pollution 133:205–213

Navarro MC, Pe'rez-Sirvent C, Martı'nez-Sa'nchez MJ, Vidal J, Tovar PJ, Bech J (2008) Abandoned mine sites as a source of contamination by heavy metals: a casestudyinasemi aridzone. J Geochem Explor 96:183–193

Nishizono H, Ichilawa H, Suzuki S, Ishii F (1987) The role of the root cell wall in the heavy metal tolerance of *Athyrium yokoscense*. Plant Soil 101:15–20

Nye PH (1981) Changes of pH across the rhizosphere induced by roots. Plant Soil 61:7–26

Pottier M, García de la Torreb SV, Victora C, Davida CL, Chalot M, Thominea S (2015) Genotypic variations in the dynamics of metal concentrations in poplar leaves: A field study with a perspective on phytoremediation. Environ Pollut 199: 73-82

Qasim B, Motelica-Heino M (2014) Potentially toxic element fractionation in technosoils using two sequential extraction schemes. Environmental Science and Pollution Research 21:5054 – 5065

Qasim B, Motelica-Heino M, Joussein E, Soubrand M, Gauthier A (2015) Potentially toxic elements phytoavailability assessment in Technosols from former smelting and mining areas. Environ Sci Pollut Res 22:5961–5974

Razo I, Carrizales L, Castro J, Dıaz-Barriga F, Montoroy M (2004) Arsenic and heavy metal pollution of soil, water and sediments in a semi-aridclimate mining areain Mexico. Water Air Soil Pollut 152:129–152

Reeves RD and Baker AJM (2000) Metal-accumulating plants. In: Raskin I, Ensley BD (eds) Phytoremediation of Toxic Metals. Wiley-Interscience, New York, pp 193‑230

Roemkins PFAM, Bouwman LA, Boon GT (1999) Effect of plant growth on copper solubility and speciation in soil solution samples. Environ Pollut 106:215–231

Salt DE, Blaylock M, Kumar PBAN, Dushenkov V, Ensley BD, Chet I et al. (1995) Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13:468–474

Sas L, Rengel Z, Tang C (2001) Excess cation uptake and extrusion of protons and organic acid anions by Lupinus albus under phosphorus deficiency. Plant Science 160:1191-1198

Sauvé S, McBride MB, Norvell WA, Hendershot WH (1997) Copper solubility and speciation of in situ contaminated soils: effects of copper level, pH and organic matter. Water Air Soil Pollut 100:133–149

Schwab AP, He Y, Banks MK (2005) The influence of organic ligands on the retention of lead in soil. Chemosphere 61:856–866

Tao S, Liu WX, Chen YJ, Xu FL et al. (2004) Evaluation of factors influencing root-induced changes of copper fractionation in rizosphere of a calcareous soil. Environ Pollut 129:5–12

Thiry M, van Oort F (1999) Les phases minérales majeures et mineures d'une friche industrielle de métallurgie des métaux non ferreux: état d'altération, évolution géochimique et devenir des métaux polluants du site de Mortagne-du-Nord, In: Les cahiers des clubs CRIN, acte du colloque 'La Spéciation des Métaux dans le Sol. Association ECRIN Paris pp. 108–159

Thiry M, Huet-Taillanter S, Schmitt JM (2002) La friche industrielle de Mortagne-du-Nord (59) -I- Prospection du site, composition des scories, hydrochimie, hydrologie et estimation des flux. Bull. Soc. géol. France 173: 369-381

Uren NC, Reisenauer HM (1988) The role of root exudates in nutrient acquisition. In: Tinker PB, Lauchli A (eds) Advances in Plant Nutrition, vol. 3. Praeger, New York, pp 79–114

Vazquez S, Agha R, Granado A, Sarro MJ, et al. (2006) Use of whit lupin plant for phytostabilization of Cd and as polluted acid soil. Water Air Soil Pollut 177:349–365

Vijver M, Jager T, Posthuma L, Peijnenburg W (2003) Metal uptake from soils and soil-sediment mixtures by larvae of Tenebrio molitor (L.) (Coleoptera). Ecotox Environ Safe 54:277–289

Weng L, Temminghoff EJM, Lofts S, Tipping E, Van Riemsdijk WH (2002) Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. Environ Sci Technol 36:4804–4810

Wenzel W W, Brandstetter A, Wutte H, Lombi E, Prohaska T, Stingeder G et al. (2002) Arsenic in field-collected soil solutions and extracts of contaminated soils and its implication to soil standards. J Plant Nutr Soil Sci 165:221–228

Wenzel WW, Bunkowski M, Puschenreiter M, Horak O (2003) Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ Pollut 123:131–138

Yanai J, Araki S, Kyuma K (1995) Effects of plant growth on the dynamics of the soil solution composition in the root zone of maize in four Japanese soils. Soil Sci Plant Nutr 41:195-206

Yanai J, Robinson D, Young IM, Kyuma K, Kosaki K (1998) Effects of the chemical form of inorganic nitrogen fertilizers on the dynamics of the soil solution composition and on nutrient uptake by wheat. Soil Sci Plant Nutr 202:263-270

Yang J, Ma Z, Ye1 Z, Guo X, Qiu R (2010) Heavy metal (Pb, Zn) uptake and chemical changes in rhizosphere soils of four wetland plants with different radial oxygen loss. J Environ Sci 22:69–702

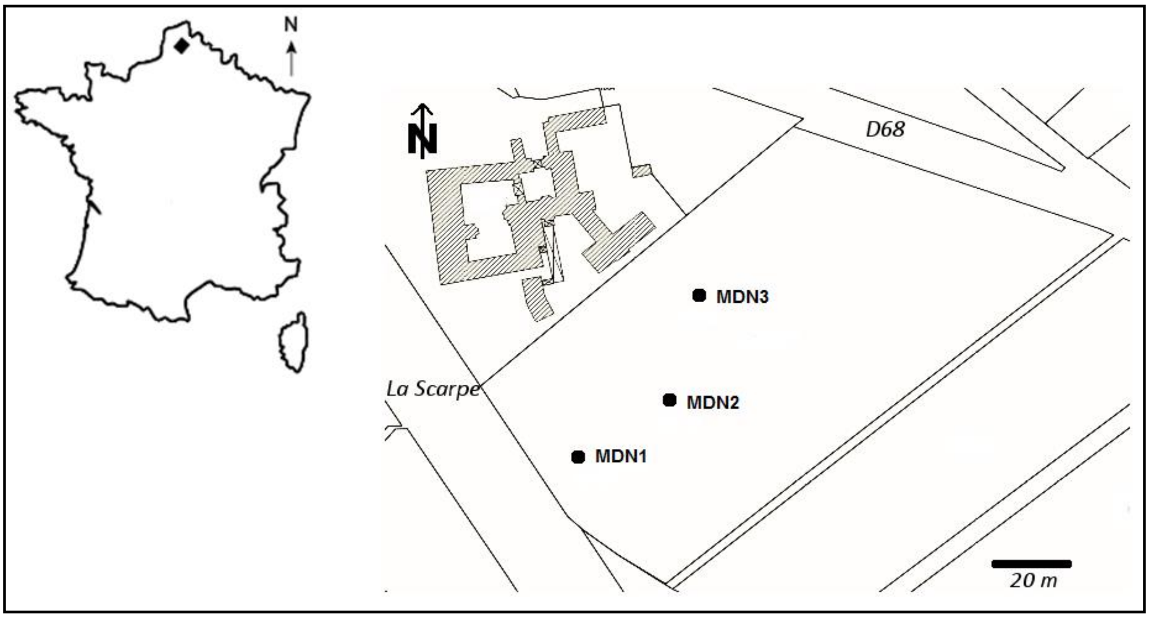

Fig. 1 Description map of the locations of the three selected soils collected from Mortagne-du-Nord (MDN) site.

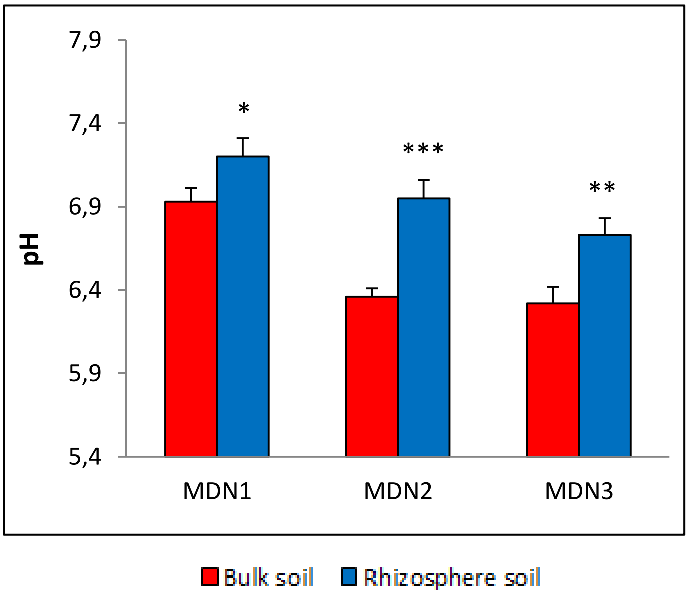

Fig. 2 pH in the bulk soil solution and in the rhizosphere soil solution of *Populus euramericana* Dorskamp grown in a contaminated technosol during 60 days. Bars represent standard errors (n = 3). Significantly different values by comparison with the bulk soil, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

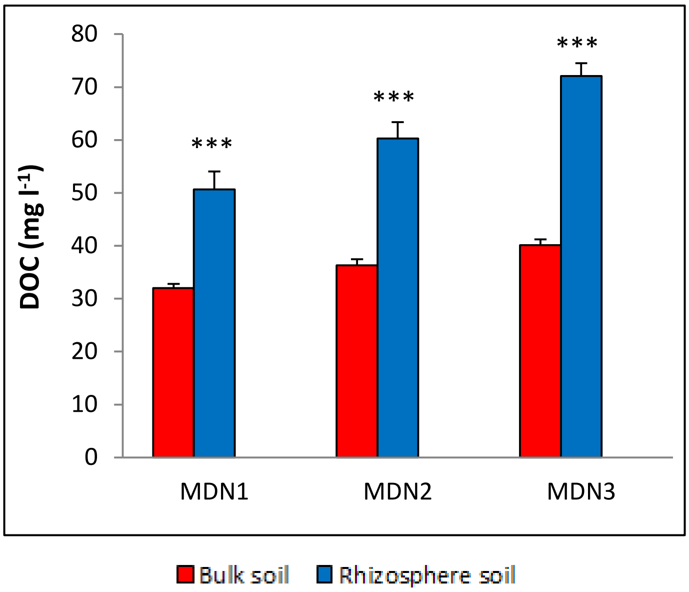

Fig. 3 DOC concentrations in the bulk soil solution and in the rhizosphere soil solution of *Populus euramericana* Dorskamp grown in a contaminated technosol during 60 days. Bars represent standard errors (n = 3). Significantly different values by comparison with the bulk soil, * P < 0.05; ** P < 0.01; *** P < 0.001.

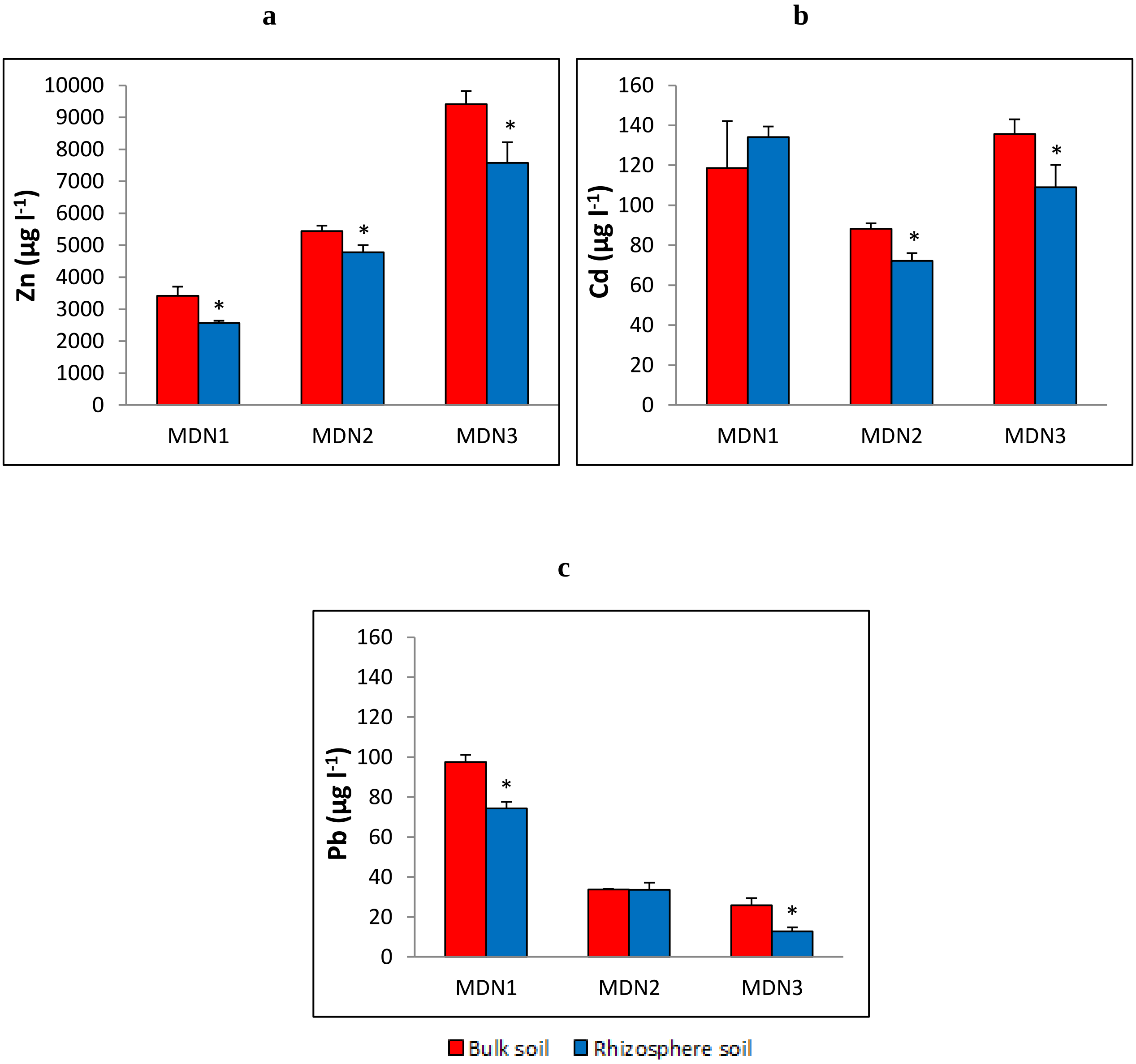

Fig. 4 Total dissolved concentrations of Zn (a), Cd (b) and Pb (c) in the bulk soil solution and in the rhizosphere soil solution of *Populus euramericana* Dorskamp grown in a contaminated technosol during 60 days. Bars represent standard errors (n = 3). Significantly different values by comparison with the bulk soil, * $P < 0.05$.

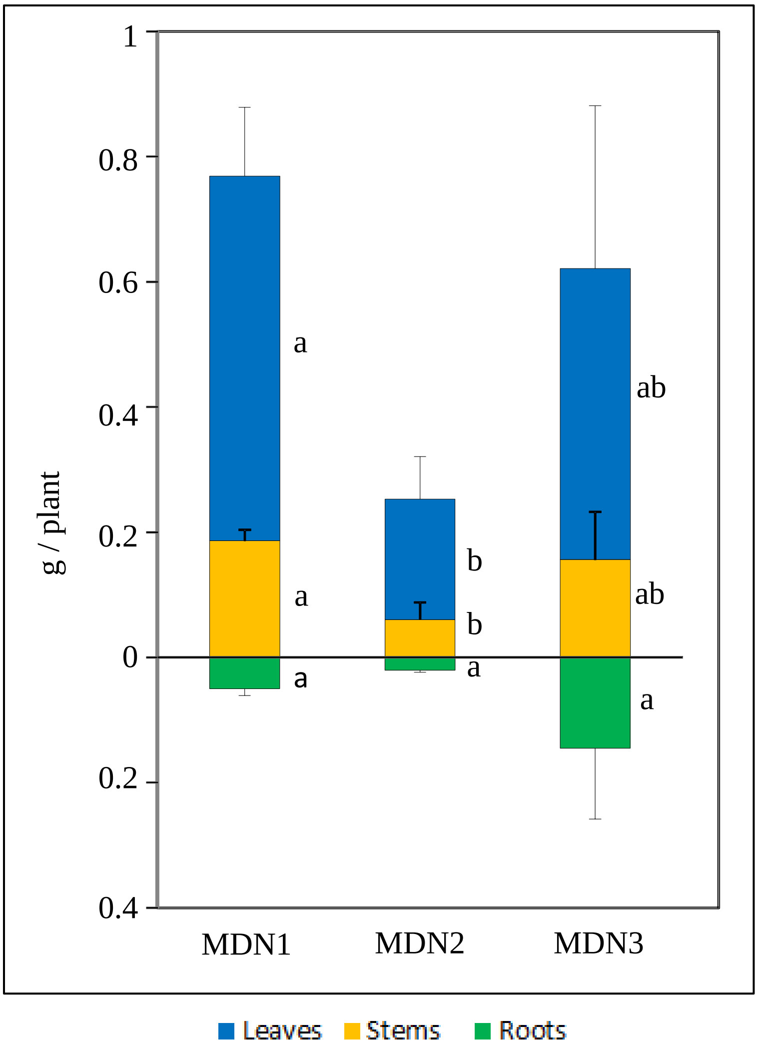

Fig. 5 Biomass (dry weigth) of the different plant organs (leaves, stems, roots) of *Populus euramericana* Dorskamp grown in a contaminated technosol during 60 days. Bars represent standard errors (n = 3). Treatments assigned the same letter indicate no significant difference between the means ($P \leq 0.05$).

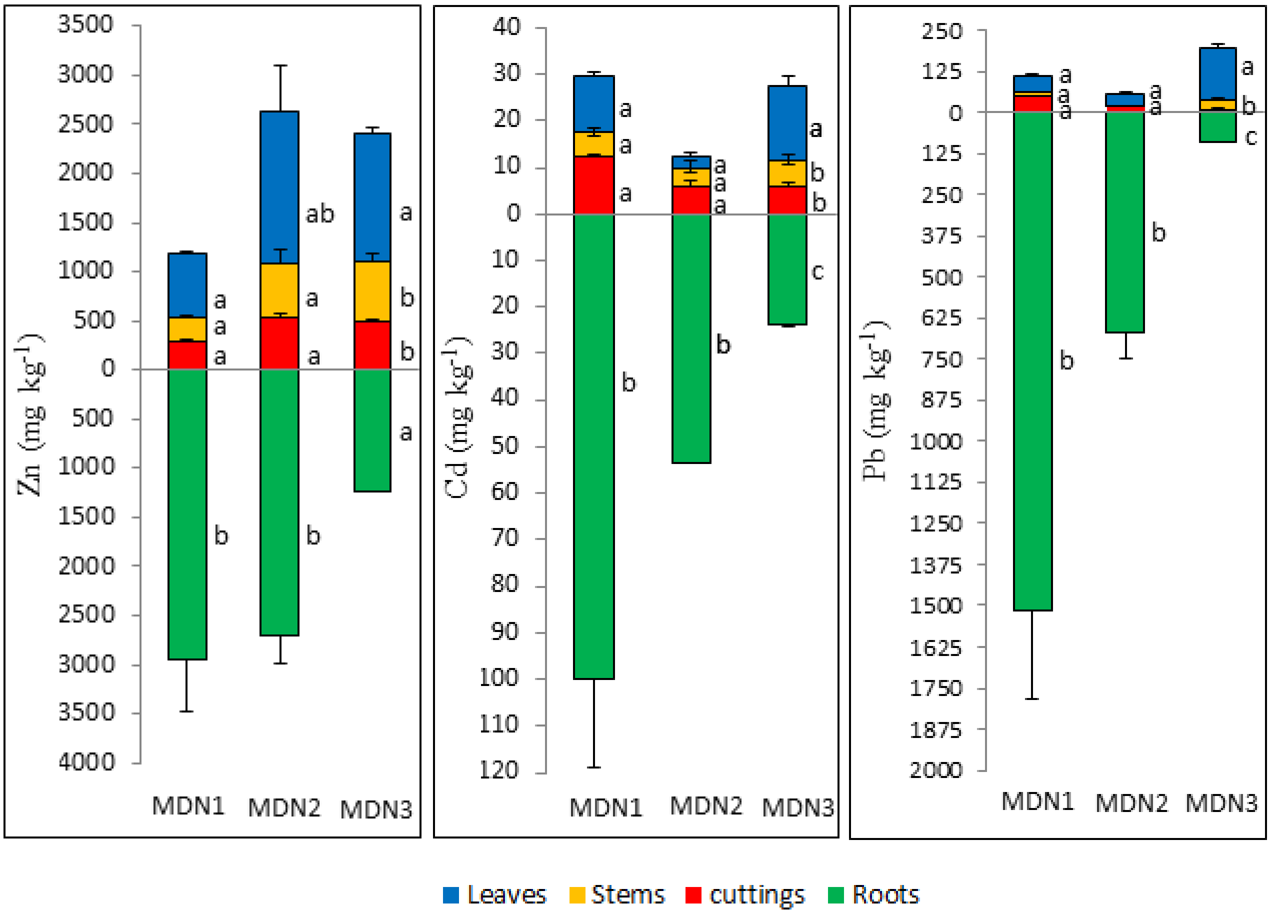

Fig. 6 Concentrations of Zn (a), Cd (b) and Pb (c) in the different organs (leaves, stems, cuttings and roots) of *Populus euramericana Dorskamp* grown in a contaminated technosol during 60 days. Bars represent standard errors (n = 3). Treatments assigned the same letter indicate no significant difference between the means ($P \leq 0.05$).

Table 1: Physico-chemical characteristics of the selected samples from Mortagne-du-Nord (MDN) (n=3; ± standard deviation)

Parameters	**MDN1**	**MDN2**	**MDN3**
pH-H_2O	6.92±0.12	6.35±0.34	6.14±0.17
EC (µs.cm^{-1})	112.27±3.85	112.64±7.41	113.71±1.93
CEC (c mol(+) kg^{-1})	7.21±0.70	8.53±0.25	6.74±1.21
TOC %	3.35±0.94	4.39±0.14	6.45±0.10
Clay %	2.2± 0.24	1±0.01	1.2±0.24
Silt %	22.34±0.35	22.16±0.41	22.13±0.34
Sand %	75.45±0.12	76.84±1.03	76.66±0.28
Tot. Zn (mg kg-1)	7726±12	3114±11	3127±9
Tot. Pb (mg kg-1)	3551±10	881±8	874±5
Tot. Cd (mg kg-1)	72±11	64±5	51±6

EC: electrical conductivity, CEC: cation exchange capacity, TOC: total organic carbon