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Bashar Qasim, Mikael Motelica-Heino, Sylvain Bourgerie, Arnaud Gauthier, Domenico Morabito. Effect of nitrate and ammonium fertilization on Zn, Pb, and Cd phytostabilization by *Populus euramericana* Dorskamp in contaminated technosol. *Environmental Science and Pollution Research*, 2015, 22 (23), pp.18759-18771. 10.1007/s11356-015-5071-9 . insu-01181743

**HAL Id: insu-01181743**

**<https://insu.hal.science/insu-01181743v1>**

Submitted on 28 Sep 2015

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# Effect of nitrate and ammonium fertilization on Zn, Pb and Cd phytostabilization by *Populus euramericana* Dorskamp in contaminated technosol

Bashar Qasim<sup>1,2,\*</sup>, Mikael Motelica-Heino<sup>1</sup>, Sylvain Bourgerie<sup>3</sup>, Arnaud Gauthier<sup>4</sup>, Domenico Morabito<sup>3</sup>

<sup>1</sup>Institut des Sciences de la Terre d'Orléans (ISTO), UMR-CNRS 7327 Campus Géosciences, Université d'Orléans, France. *Mail:* [bhq\\_chem@yahoo.com](mailto:bhq_chem@yahoo.com) *Tel:* +33 (0)2 38 49 43 48

<sup>2</sup>Applied Sciences Department, University of Technology, Baghdad, Iraq.

<sup>3</sup>LBLGC EA 1207, INRA USC1328, Université d'Orléans, France.

<sup>4</sup>Laboratoire de Génie-Civil et géoEnvironnement (LGCgE), Université de Lille1, 59655 Villeneuve d'Ascq Cedex, Lille, France.

## Abstract

This study aimed at assessing the effect of nitrogen addition under two forms, nitrate and ammonium on the stabilization of Zn, Pb and Cd by *Populus euramericana* Dorskamp grown in contaminated soils for 35 days under controlled conditions. Temporal changes in the soil pore water (SPW) were monitored for pH, dissolved organic carbon (DOC) and total dissolved concentrations of metals in the soils rhizosphere. Rhizospheric SPW pH decreased gradually with  $\text{NH}_4^+$  addition and increased with  $\text{NO}_3^-$  addition up to one unit, whilst it slightly decreased initially then increased for the untreated control soil. DOC increased with time up to 6 times, the highest increase occurring with  $\text{NH}_4^+$  fertilization. An increase in the metal concentrations in the rhizospheric SPW was observed for  $\text{NH}_4^+$  addition associated with the lowest rhizospheric SPW pH, whereas the opposite was observed for the control soil and  $\text{NO}_3^-$  fertilization. Fertilization did not affect plant shoots or roots biomass development compared to the untreated control (without N addition). Metals were mostly accumulated in the rhizosphere and N fertilization increased the accumulation for Zn and Pb while Cd accumulation was enhanced for  $\text{NH}_4^+$  addition. Collectively our results suggest metal stabilization by *Populus euramericana* Dorskamp rhizosphere with nitrogen fertilization and are potential for phytostabilisation of contaminated technosol.

Keywords: potentially toxic elements, technosol, phytostabilisation, *Populus euramericana* Dorskamp, nitrogen fertilizers.

## 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
WHC	Water holding capacity

## 1. Introduction

Industrial operations such as smelting, mining, combustion of fossil fuel and waste disposal cause contamination of soil ecosystems with the release of huge amounts of potentially toxic elements (PTE)(Komarnicki, 2005; Jamali et al., 2009). The exposure to these PTE is considered as a serious threat to plants, humans and the whole environment through its entry into the food chain resulting in phytotoxicity (Pinto et al., 2004). Therefore, one of the main primary challenges for researchers in the field of environmental sciences is to reduce the environmental contamination to limit human health and ecosystems risks (Chigbo et al., 2013; Zhu et al., 2014).

Several traditional methods have been extensively used to remediate contaminated soils and technosol such as ‘dig and dump’, soil washing and sieving. These approaches are effective but destructive thus not sustainable in terms of consumption of raw materials and waste production and costly for large contaminated sites (Basta et al., 2004; Raicevic et al., 2005; Pandey et al., 2009). Less invasive, low-cost phytoremediation options such as phytostabilization, singly and in combination with in situ stabilization (i.e. aided phytostabilisation) are potential technologies to restore the physical, chemical and biological properties of PTE contaminated soils based on the stabilization of PTE in the plant rhizosphere (Mench et al., 2000; Bolan et al., 2003; Raicevic et al., 2005; Kumpiene et al., 2006, 2008; Phillips et al., 2012).

Rhizospheric soil is a dynamic region where multiple interactions occur in plant roots-soil-microbe system (Darrah et al., 2006). It is characterized by high microbial activity, and is

clearly distinct from bulk soil with regard to pH, redox potential and availability of nutrients (Hinsinger et al., 2005). The fate of PTE in soils is influenced by physical and chemical reactions between the solid components of soil and the liquid phase. Soil factors such as pH, soil organic matter (SOM), texture, redox potential, and temperature (Nyamangara, 1998) and biological processes controlled by soil micro-organisms and plants are key-players in the root zone for the PTE mobility and bioavailability (Chaignon et al., 2002). Several studies investigated the effect of root activities on the speciation and bioavailability of PTE such as root-induced pH changes, exudation of organic compounds, N mineralization, soil enzyme activities, nitrification and denitrification (Priha et al., 1999; Norton and Firestone, 1996; Weintraub et al., 2007; Kaiser et al., 2010). Roots can indeed modify the PTE mobility by changing soil pH, electrochemical potentials through element sorption in apoplast and functioning of membrane transporters, and their rhizodeposition or complexation in the rhizosphere, including soluble root exudates and mucilages (Hinsinger, 2001; Lombi et al., 2001; Chaignon et al., 2002). Moreover, the PTE solubility can be increased by the methods of acidification, complexation with chelates and PTE desorption or dissolution when the soluble PTE fraction is depleted (Marschner, 1995; Monsanto et al., 2008).

With a high biomass production and a large capacity to store PTE into the woody organs, trees are good candidates for phytostabilization. Furthermore, these species are often able to explore a large volume of soil, which potentially allows a better phytostabilisation than smaller plants. Among trees under temperate latitude, *Populus* species exhibit the greatest growth rates at the expense of large water and nitrogen requirements (Barigah et al., 2014). For this reason poplars are good candidate for phytoremediation.

The dynamics of the availability of PTE in rhizospheric soils are influenced by N fertilizers. In fact PTE phytoavailability is strongly associated with the pH in the root environment. Therefore it is important to adopt a practicable field method to alter the pH. The altering of the N source is one of the suggested methods for modifying the rhizosphere pH (Nye, 1981; Marschner and Romheld, 1996). Additionally the production of root exudates is a potential source of complexing agents for PTE (Hinsinger, 2001).

The main objective of this study was therefore to investigate the effect of two nitrogen fertilizers ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) on rhizospheric soil pore water (SPW) pH, dissolved organic carbon (DOC) concentrations, PTE (Zn, Cd and Pb) concentrations in SPW and their uptake by *Populus euramericana* Dorskamp grown in contaminated soils.

## 2 Materials and methods

### 2.1 Soil sampling

Soil samples used in this study were collected from a metallophyte grassland contaminated with Zn, Pb and Cd located at Mortagne-du-Nord (MDN) in Northern France. 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 geological context is made of the Sand of Ostricourt (Paleocene/ lower Eocene). These are glauconious sand with a medium granulometry, on top of them are the clay alluvial material of the nearby River Scarpe, clays and fine sands rich in organic matter. 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) and spatial distribution of the vegetation which essentially consists of *Arabidopsis halleri* L. and *Avena sativa* L. Surface soils (0-20 cm) were sampled at each location. The main physico- chemical properties of the selected soil samples are summarized in Table 1.

### 2.2 Experimental design and plant analysis

A plant growth experiment was conducted with plastic pots: 0.5 kg of dry soil sub-sample taken after homogenizing a larger volume of composite technosol sample was used per pot for each soil location. Stems of *Populus euramericana* Dorskamp with rooting were grown on the soils for 35 days. The N treatments were  $\text{NH}_4\text{Cl}$  and  $\text{KNO}_3$  which were applied at 200 mg N  $\text{kg}^{-1}$  on days 7, 14, 21 and 28 by mixed with distilled water used to maintain 80% of the water holding capacity (WHC), whereas, only distilled water was applied for the untreated control soils. The experiment was replicated 5 times, performed in forty-five pots (3 soil samples  $\times$  3 treatments  $\times$  5 replicates for each soil).

Woody stem cuttings obtained from 1-year-old cutback stems of *Populus euramericana* Dorskamp genotype were planted into sand in order to obtain rooted cuttings. After rooting (15 days), plants were transferred into pots (one plant per pot) containing the contaminated soils. Concomitantly, the rooted cutting plants were pruned in order to make sure that the new leafy stems were entirely formed while plants were exposed to PTE.

Plants were cultivated in a controlled environmental growth chamber (20 – 22°C, 14h day /10h night length, 150  $\mu\text{E m}^{-2}\text{s}^{-1}$  of light intensity and 80% relative humidity) for the whole duration of the experiment. 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, woody stem cuttings (correspond to the organ used to obtained the rooted cuttings),

stems and leaves. The different plants organs were oven dried in 70°C for three days until constant weight. Dried plant organs were then ground with a laboratory grinder and 200mg ( $\pm 0.5$ mg) of each plant organ was 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 ( $\text{HNO}_3/\text{HCl}$ , 1:3v/v), heated for 20min at 200°C and then rinsed with double deionized water. ICP-MS (Finnigan Element XR, Thermo Electron, Germany) measurements were carried out to determine the plant PTE concentrations in the different organs.

### 2.3 Soil solution collection and analysis

Rhizospheric SPW was collected five times at regular intervals during the cultivation period using Rhizon soil moisture samplers (Rhizosphere Research Products, Wageningen, The Netherlands). Distilled water was added to the soils to maintain 80% of the WHC. The system was allowed to equilibrate for 24hr before SPW collections. The collected SWP was separated into several sub-samples for analysis. The extracted soil solutions were used for the determination of pH, DOC concentrations, and PTE total dissolved concentrations. SPW pH was determined using a combined pH-EC meter (WTW, ProfiLine 1970i, Germany). DOC was analyzed using an automatic carbon analyzer (Shimadzu© TOC 5000A). SPW were analyzed with ICP-MS (Finnigan Element XR, Thermo Electron, Germany).

### 2.4 Statistical analysis

Results were analyzed with the SPSS statistical software package (SPSS, Chicago, IL, USA). 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 Rhizospheric soil solution pH and dissolved organic carbon concentration

The temporal variation of rhizospheric SPW pH in untreated control and N treatments for all studied soil samples during the cultivation period are shown in Fig.1.

It can be seen that there was a significant difference in the SPW pH between the N treatment and the untreated control sample. For all studied samples at the end of experiment, the  $\text{NH}_4^+$  treatment resulted in the lowest pH value (6.05, 5.43 and 4.96), whereas the  $\text{NO}_3^-$  treatment

resulted in the highest pH value (7.90, 7.04 and 6.97) for MDN1, MDN2 and MDN3 samples respectively. Among them, the lowest pH value was observed for the MDN3 sample, whilst, the highest was for the MDN1 sample.

The application of N fertilizers such as  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{KNO}_3$  and urea has been considered as one of the main factors causing acidification or alkalization of agricultural soils, and its uptakes by many plants have increased or decreased rhizospheric pH related to the proton release through nitrification of  $\text{NH}_4^+$  or  $\text{OH}^-$  release through  $\text{NO}_3^-$  uptake (Bouman et al. 1995).

Monsat et al. (2008) observed a pattern of pH changes similar to our findings in a *Thlaspi caerulescens* rhizospheric soil due to N fertilization. Our results were also consistent with those of Sabir et al. (2013) which showed that nitrogen forms significantly affected the soil pH. These authors reported that the  $\text{NO}_3^-$  fed plants recorded the maximum soil pH whereas minimum soil pH was recorded where only  $\text{NH}_4^+$  was applied. Tachibana et al. (1995) also reported that soil pH decreased as low as 2.9 by application of N fertilizers at high rates in a green tea experiment. Ruan et al. (2000) showed that the application of  $\text{NH}_4^+$  to the soil of tea plants resulted in significant reduction in rhizospheric pH due to the cation-anion balance during nutrient uptake by plants.

In the case of the untreated control soils, a change in the pH values was also observed for all studied samples but remained lower from those with N fertilization. Rhizospheric SPW of the untreated controls increased by 0.2 – 0.3 pH units compared to the initial value, which can be explained as a consequence of differential rates in the uptake of cations and anions by plants in order to maintain electrical neutrality within their roots or probably related to changes in Ca concentration. Our findings are also in agreement with that of Knight et al. (1997), which used Rhizon soil moisture samplers and reported an increase in the solution pH between 0.4 and 0.9 units after *Noccaea caerulescens* growth. Tao et al. (2003) also reported an increase in pH in maize rhizospheric soil during the cultivation period.

Globally rhizospheric pH can be influenced by N source via three mechanisms: nitrification/denitrification reactions, displacement of  $\text{H}^+/\text{OH}^-$  adsorbed on the soil solid phase and release/uptake of  $\text{H}^+$  by roots in response to  $\text{NH}_4^+/\text{NO}_3^-$  uptake by plant roots (Nye, 1981; Marschner and Romheld, 1996; Tang and Rengel, 2003; Silber et al., 2004). It is well known that the uptake of  $\text{NH}_4^+$  or  $\text{NO}_3^-$  by plants depend on their concentrations in soil solution, root absorption and plant growth rate (Richardson et al., 2009). When plants take up  $\text{NH}_4^+$ , more cations than anions will be release through proton release to regulate pH and charge balance resulting in rhizosphere pH decreases, whereas uptake of  $\text{NO}_3^-$  may increase

rhizosphere pH through the release of  $\text{OH}^-$  (Haynes, 1990; Taylor and Bloom, 1998; Hinsinger et al., 2003).

The changes in DOC concentrations within the rhizosphere during the cultivation period are shown in Fig. 2. DOC concentrations in the rhizospheric SPW for the untreated control and N treated soils increased gradually with the cultivated period for all studied samples. Fig.2 showed that the N fertilizers application significantly increased the concentrations of DOC for both MDN1 and MDN3 compared to untreated control but not for MDN2. For all studied samples, the highest DOC concentrations at the end of the experiment were recorded for the  $\text{NH}_4^+$  treatment.

There are various components involved in the release of organic carbon into the rhizosphere (Rovira et al., 1979). Soil humus, plant litter and the organic compounds in root exudates are active substances which considered as the main sources of SOM in soils (Kalbitz et al., 2000) which exhibiting strong affinities with respect to PTE. In the literature, many studies have reported that application of N can influence the DOC depending on microbial activity (Park et al., 2002; Scheuner and Makeschin, 2005; Cory et al., 2004; McDowell et al., 2004). In this study, the increase in DOC concentrations due to the application of N fertilizers confirms the hypothesis that the increase in microbial activity and utilization of C substrate stimulated the release of DOC in comparison to the untreated control. These findings are in agreement with that of Lakzian et al. (2010) which reported that the application of N fertilizers increased the DOC concentration by 30% in comparison to soils without treatment. Pregitzer et al. (2004) also reported that chronic N fertilization increases the production and leaching of DOC. Similarly, Sitaula et al. (2004) and Curtis et al. (1995) reported that the application of N fertilizers had direct or indirect effects on microbial activity and the release of DOC.

### 3.2 Zn, Pb and Cd concentrations in rhizospheric SPW

The total dissolved concentrations of Zn, Pb and Cd in the rhizospheric SPW of the untreated control and N fertilized soils during the cultivation period are shown in Fig. 3, 4 and 5 respectively.

A significant difference was observed for Zn concentrations in rhizospheric SPW between untreated controls and treated fertilized soils for all studied soil samples during the cultivation period. Zn concentration in the rhizospheric SPW for MDN1 dropped markedly with the  $\text{NO}_3^-$  fertilization and increased gradually in the case of the  $\text{NH}_4^+$  fertilization, whilst in the untreated control sample, it remained almost constant at the beginning and decreased towards the end of the experiment (Fig. 3A). For MDN2 and MDN3, Zn rhizospheric SPW



concentrations did not differ among them at the beginning of the experiment. However a significant difference was observed among them at the end of the experiment and the highest Zn concentration was recorded for the  $\text{NH}_4^+$  treatment whilst the lowest was found for  $\text{NO}_3^-$  (Fig. 3B, 3C) respectively.

Pb total dissolved concentration in the rhizospheric SPW for the untreated control for MDN1 and MDN2 was relatively higher at the beginning and then dropped remarkably at the end of the experiment. On the other hand, Pb concentration gradually decreased and increased with time for the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  treatments respectively. A significant difference between the untreated control and N treatments was observed (Fig. 4A, 4B). For MDN3, Pb concentrations in the untreated control increased gradually similarly to that of the  $\text{NH}_4^+$  treatment but dropped considerably at the end of the experiment. Gradual decrease in Pb concentration with time was also observed with  $\text{NO}_3^-$  (Fig. 4C).

In the case of Cd, total dissolved concentrations in the rhizospheric SPW for MDN1 remarkably increased and decreased with time for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  respectively. In contrast for the untreated control, Cd concentration was higher at the beginning then decreased at the end of the experiment (Fig. 5A). The same pattern was also observed for MDN2 with the exception of a significant increase at the end of the experiment for the  $\text{NH}_4^+$  treatment (Fig. 5B). For MDN3, Cd concentration trend was similar to that of Pb (Fig. 5C).

The solubility of Zn showed an increase in the rhizospheric SPW for all studied samples after  $\text{NH}_4^+$  addition in comparison to the untreated control and that fertilized with  $\text{NO}_3^-$ . This can be attributed to decrease in the soil pH, but also to its ability to form selective complexes with DOC compounds due to the higher affinity of Zn for DOC (Kim et al., 2010a).

In contrast to soils amended with  $\text{NH}_4^+$ , Zn, Pb and Cd concentrations decreased in the rhizospheric SPW of untreated control and that fertilized with  $\text{NO}_3^-$ . The decrease in PTE solubility may be due to the increase in pH in spite of the increase in DOC concentrations. In fact, Zn is generally relatively insoluble at  $\text{pH} > 7$  and its solubility decreases with pH (Ross, 1994). Moreover, PTE occurring predominantly as free ion forms are more sensitive to effects arising from differences in pH value (Luo et al., 2001). In fact the decrease in Zn in the SPW in compost- and biochar-amended soils was due to the Zn presence mainly in water-soluble fractions (Beesley et al., 2010). Therefore the increase in pH values due to  $\text{NO}_3^-$  addition and the competing effect of changes in pH and DOC after plant growth on PTE chemistry might be enhanced by sorption on soil organic and inorganic particle surfaces.

In addition, despite a higher DOC concentration for MDN3, the studied PTE solubility in the rhizospheric SPW increased more for MDN1 than for MDN3 which may be attributed to the

competition between cations in the SPW for the DOC binding sites. A high portion of DOC exuded from the root can in fact react with different major cations such as Mg and Ca for the MDN3 sample, whereas, for the MDN1 sample, DOC induced by roots may interact easily with PTE because of less competition for DOC binding sites.

The Pb concentration in the rhizospheric SPW decreased in MDN3 with the lowest pH value even though in the case of  $\text{NH}_4^+$  treatment in comparison to both MDN1 and MDN2. This result is in agreement with those of Sauvé et al. (1998) which reported that Pb solubility is higher near neutrality, or which was likely due to formation of Pb organic complexes in soil solution.

In the case of untreated control, the increase of PTE in the SPW at the beginning of the experiment could be attributed to root related processes such as pH decrease and DOC increase, or might be attributed to an exchange reaction by  $\text{NH}_4^+$  originating from mineralization of organic N, whereas the decrease at the end of the experiment was attributed to the increasing in the pH value even though the increase in DOC concentrations may favor re-adsorption of PTE onto the soil particles. The addition of organic matter (OM) may influence Zn mobility and increase negatively-charged adsorption sites in the OM-treated soils (Hartley et al., 2010). Conversely, Zn was immobilized in an acid soil by humic acids isolated from organic materials, (Clemente and Bernal, 2006). In another study on PTE contaminated acidic sandy soils phytostabilised with poplars, the Zn concentration in SPW significantly decreased with the addition of dolomitic limestone in soil that increased the soil pH (Hattab et al., 2014a). The efficiency of assisted phytostabilization using organic amendments such as ramial chipped wood (RCW) and composted sewage sludge (CSS) was studied on contaminated techno-soils. Addition of sewage sludge increased the solubility of Zn due to the formation of soluble organo-metallic complexes. Moreover, Zn was abundant in the fulvic acid fraction than in the humic acid fraction which may explain its mobility (Hattab et al., 2014b).

### 3.3 Plant growth and uptake of Zn, Cd and Pb

During the growth period, several plants appeared unhealthy, with visual symptoms of studied metals toxicity. There was foliar necrosis and chlorosis with no significant differences in shoots and root biomass between the treatments and between each plant. The growth disorders and the cases of necrosis and chlorosis cannot be attributed to any direct effect of nitrogen treatments, because the control plants have the same disorders. Localized supply of nitrogen treatments had no significant influence on leaves growth, total area and biomass in

comparison to the untreated control sample in all studied samples. However, there was a little difference among the studied samples. However, the highest leaves biomass was for MDN1 and the lowest was for MDN3.

No significant difference was observed for the leaves dry weight between the untreated control and nitrogen treatments for all studied samples (Fig. 6A). The same pattern was observed for roots dry weight for both MDN1 and MDN2, whilst, in MDN3, the nitrogen treatments were significantly decreased the root biomass in comparison to untreated control (Fig. 6C). Contrary to leaves and roots biomass in MDN2, the stem biomass on the untreated control varied significantly, represented the minimum dry weight values in comparison to those of nitrogen treatments, but not for MDN1 and MDN2 (Fig. 6B). As mentioned above, the shoot biomass did not respond to pH changes, whereas only the root biomass for MDN3 was affected by the pH changes due to  $\text{NH}_4^+$  supplied which caused a dramatic yield reduction.

In the literature, many researchers confirmed the effect of N forms on plants shoot and root biomass. Kraus and Staurt (2002) reported that each of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and ( $\text{NH}_4^+/\text{NO}_3^-$ ) forms were increased the plant dry matter. Also, Ruan et al. (2004, 2007) showed that the root, shoot and whole tea plant (*Camellia sinensis* (L.) O. Kuntze) dry matter productions were significantly increased by nitrogen fertilization. Similarly, Zhou et al. (2011) reported that a higher dry matter production of cucumber plant resulted from plant fed with  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ . However, our results were not in line with their observations but partially similar to that of Monsanto et al. (2008) which observed that no significant difference in *Thlaspi caerulescens* plant dry weight caused by nitrogen treatments compared to the control sample.

The pots experiment of *Populus euramericana* Dorskamp grown in the studied contaminated soils showed an accumulation of varying amounts of studied PTE among the plant organs, the roots presenting the highest accumulation of Zn, Cd and Pb. Nitrogen fertilization did not affected the plant leaves Zn concentration in all studied samples (Fig. 7A). Zn concentrations in plant leaves for the untreated control and that fed with  $\text{NO}_3^-$  and  $\text{NH}_4^+$  was similar (no significant difference was observed) and were statistically at par with each other for all studied samples. The same pattern was also observed for Zn concentration in the plant cuttings and stems (Fig. 7B, D). Zn concentration in poplar roots was similar between the untreated control and that fed with nitrogen treatments in MDN2, whereas for both MDN1 and MDN3 it was significantly affected by N forms supplied to plants (Fig. 7C). The addition of N resulted in the highest Zn concentration in plant roots, whereas the untreated control had

the minimum. In general, the poplar roots contained much higher concentrations of Zn than leaves in all studied samples, probably due to the preferential distribution of Zn in the roots.

Fig. 8A showed that N fertilization also did not affect the Pb concentration in plant leaves, which showed that for all studied samples, Pb concentrations were statistically at par with each other. In the other hand, N forms significantly affected Pb concentrations in both poplar cuttings and roots for MDN1 compared to the untreated control but not for MDN2 and MDN3 (Fig. 8B, 8C). In roots and shoots, the plants amended with  $\text{NO}_3^-$  and  $\text{NH}_4^+$  in MDN1 had the highest Pb concentration compared to the untreated control. However, the Pb distribution between roots and leaves for MDN1 was significantly affected by N forms. In MDN1, the Pb distribution in the roots was maximum in the plants fed with  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , whilst it was minimum for the untreated control. On the other hand, in both MDN2 and MDN3, Pb concentrations were statistically at par with each other.

Addition of N fertilizers significantly affected the plant leaves Cd concentration in comparison to untreated control in MDN1. Only  $\text{NH}_4^+$  exhibited the highest Cd concentration in MDN2, and no significant difference was observed for MDN3 (Fig. 9A). These findings are in consistent with that of Fageria and Baligar (2005), Diatta and Grzebisz (2006) which reported that acidification of the rhizosphere with  $\text{NH}_4^+$  can enhance the plant metals uptake such as cadmium in soils. The same picture was observed in plant cuttings (Fig. 9B). Contrary to the Cd concentration in plant leaves and cuttings in MDN2, Cd concentration in roots was non-significantly affected by N forms (Fig. 9C). However, only  $\text{NH}_4^+$  affected the plant roots Cd concentration in comparison to  $\text{NO}_3^-$  and untreated control. The concentration of both Pb and Cd in plant stems was not detected (under detection limit).

Little studies have been published on the PTE accumulation of poplars grown in real contaminated soils. Among different poplar species, the cultivar Dorskamp has demonstrate the largest metal concentrations in leaves when growing in a field containing a polymetallic pollution characterized by Pb, Cu, Zn and Cd concentrations 10 times higher than in a reference non contaminated soil (Pottier et al., 2015). It is well known that physicochemical properties of the soil such as pH and COD and the plant species are able to affect the PTE transfer to plant organs. Among them, pH is the most important factor which plays an important role in PTE mobility and availability. In the case of rhizospheric soil studies, several authors showed that under both field and glasshouse conditions, N supply could effectively affect plant metals uptake and metal translocation from root to shoot via the increasing or decreasing of pH value (Shi et al., 2010; Erenoglu et al., 2011).

#### **4. Conclusions**

When N fertilizers respectively  $\text{NH}_4\text{Cl}$  and  $\text{KNO}_3$  were supplied to *Populus euramericana* Dorskamp grown in soils contaminated with Zn, Pb, and Cd, rhizospheric SPW pH decreased and increased respectively for ammonium and nitrate significantly compared to the untreated control with time for all studied soils. DOC concentrations increased gradually and a significant difference between both nitrogen forms and untreated control was observed. PTE total concentrations in the rhizospheric SPW increased with the addition of  $\text{NH}_4^+$ , whilst it decreased with the addition of  $\text{NO}_3^-$ . No significant difference was observed in the shoot biomass between the two N forms and also the untreated control for all studied samples.

Despite the difference in SPW pH value caused by the addition of N fertilizers in comparison to the untreated control, metals uptake by *Populus euramericana* Dorskamp shoots is less dependent on enhanced metal solubility caused by rhizosphere acidification. PTE stabilization by *Populus euramericana* Dorskamp rhizosphere was found with nitrogen fertilization and the potential of this species for the phytoremediation of contaminated technosol was shown.

#### **Acknowledgements**

The authors wish to acknowledge the Ministry of Higher Education and Scientific Research (Baghdad, Iraq) for financial support, and are grateful to Florie Miard (Laboratoire de Biologie des Ligneux et des Grandes Cultures) for her excellent technical assistance.

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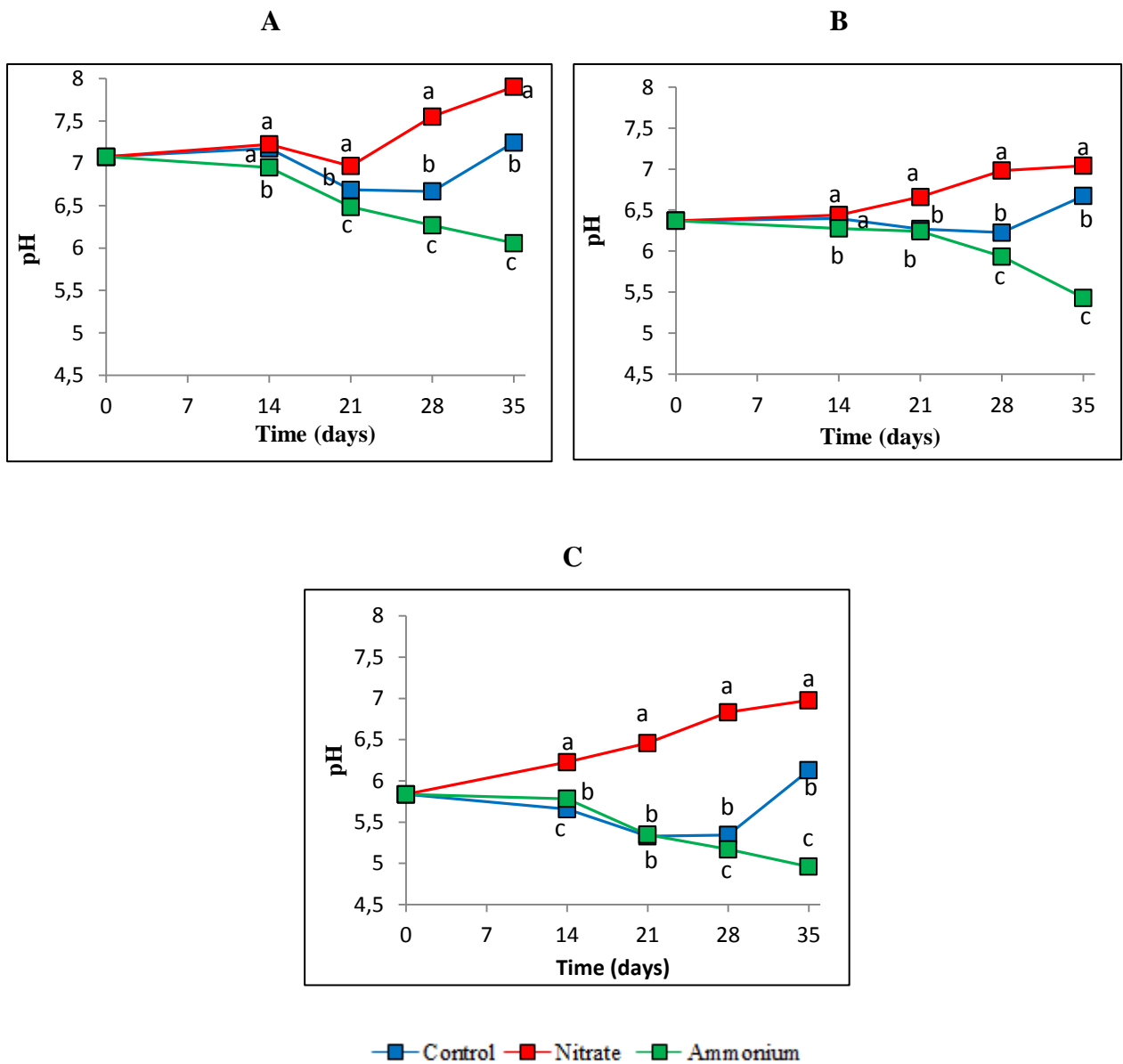
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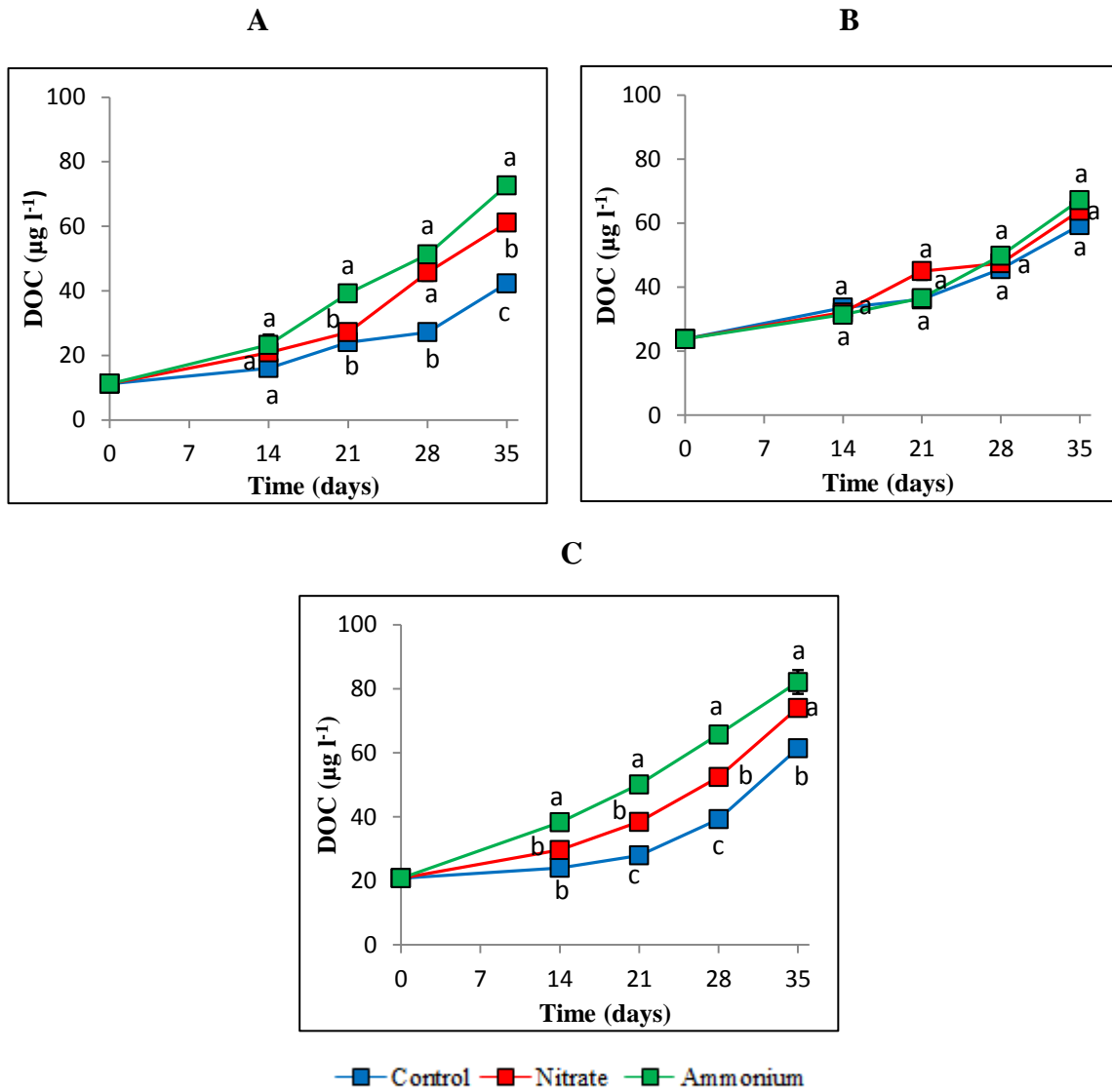
**Table 1:** Physico-chemical characteristics of the selected samples from Mortagne-du-Nord (MDN) (n=3;  $\pm$  standard deviation)

<b>Parameters</b>	<b>MDN1</b>	<b>MDN2</b>	<b>MDN3</b>
pH-H <sub>2</sub> O	6.92 $\pm$ 0.12	6.35 $\pm$ 0.34	6.14 $\pm$ 0.17
EC ( $\mu$ s.cm <sup>-1</sup> )	112.27 $\pm$ 3.85	112.64 $\pm$ 7.41	113.71 $\pm$ 1.93
CEC (c mol(+) kg <sup>-1</sup> )	7.21 $\pm$ 0.70	8.53 $\pm$ 0.25	6.74 $\pm$ 1.21
TOC %	3.35 $\pm$ 0.94	4.39 $\pm$ 0.14	6.45 $\pm$ 0.10
Clay %	2.2 $\pm$ 0.24	1 $\pm$ 0.01	1.2 $\pm$ 0.24
Silt %	22.34 $\pm$ 0.35	22.16 $\pm$ 0.41	22.13 $\pm$ 0.34
Sand %	75.45 $\pm$ 0.12	76.84 $\pm$ 1.03	76.66 $\pm$ 0.28
Tot. Zn (mg kg <sup>-1</sup> )	7726 $\pm$ 12	3114 $\pm$ 11	3127 $\pm$ 9
Tot. Pb (mg kg <sup>-1</sup> )	3551 $\pm$ 10	881 $\pm$ 8	874 $\pm$ 5
Tot. Cd (mg kg <sup>-1</sup> )	72 $\pm$ 11	64 $\pm$ 5	51 $\pm$ 6

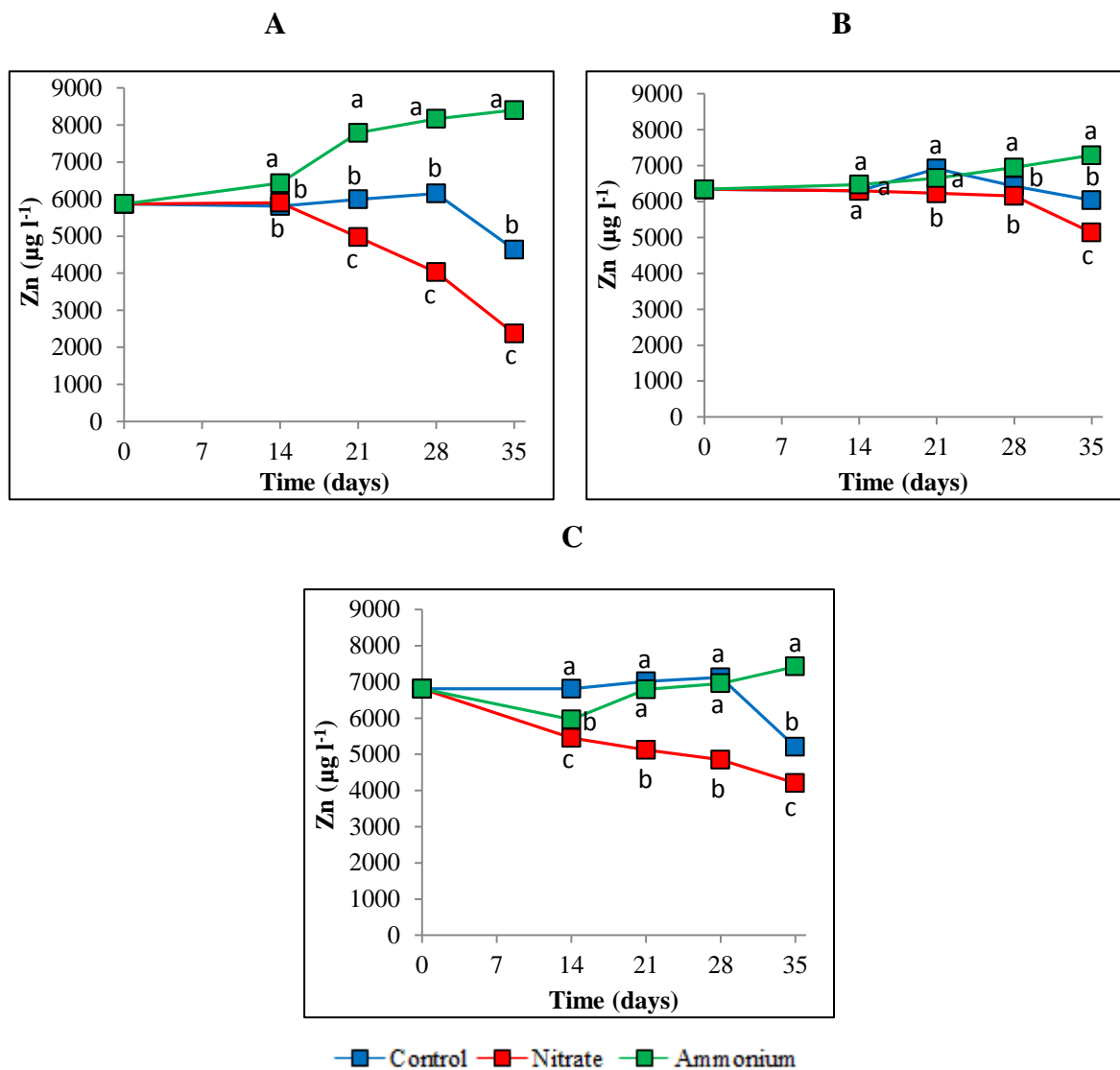
EC: electrical conductivity, CEC: cation exchange capacity, TOC: total organic carbon



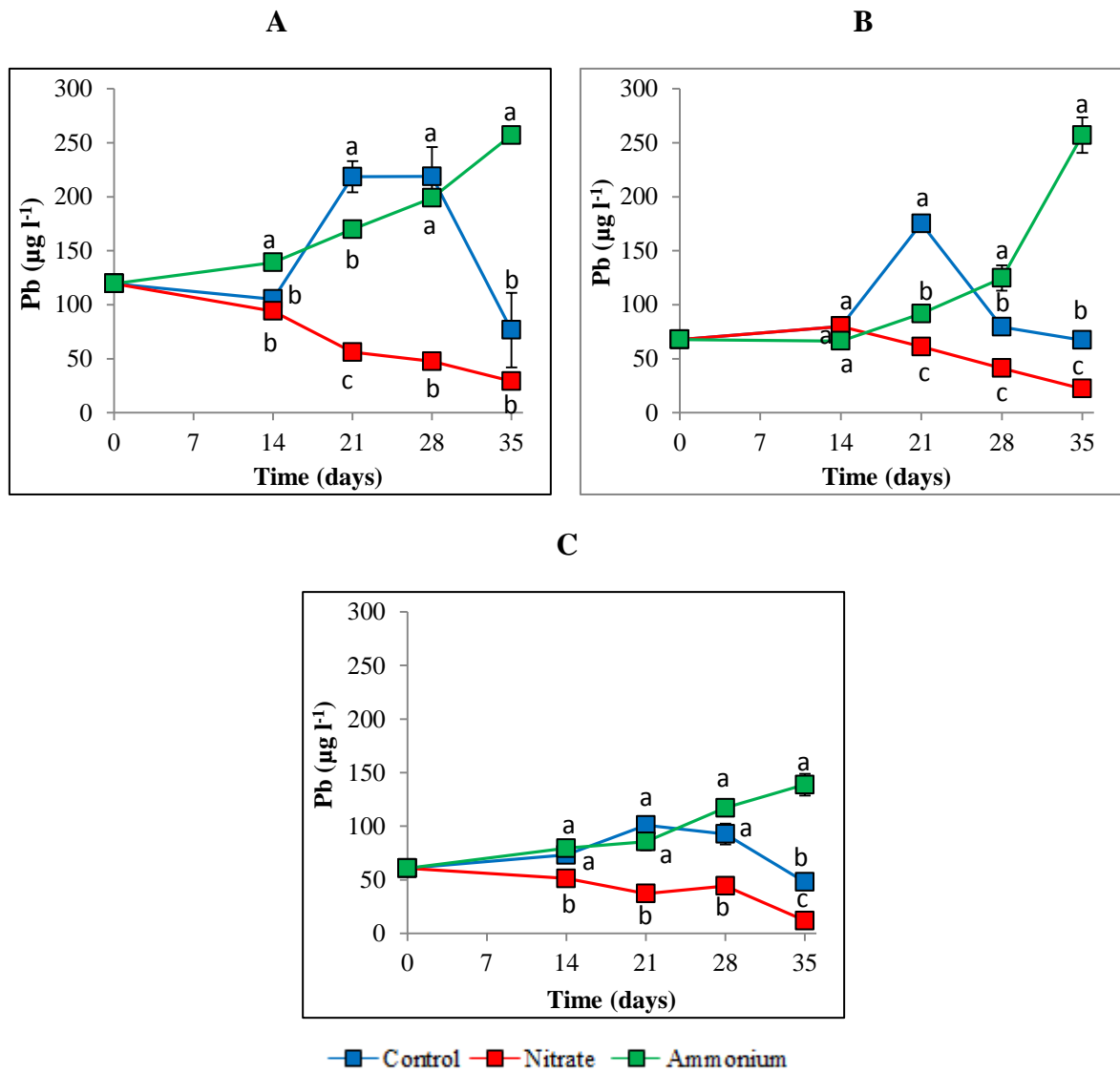
**Fig. 1** Effects of nitrogen nutrition on rhizospheric pH of *Populus euramericana* Dorskamp grown in a contaminated technosol, MDN1 (A), MDN2 (B) and MDN3 (C), during 35 days (n = 5), bars refer to standard error. For each day measurement, letters indicate differences between control and treated soils.



**Fig. 2** Effects of nitrogen nutrition on rhizospheric DOC concentrations of *Populus euramericana* Dorskamp grown in a contaminated technosol, MDN1 (A), MDN2 (B) and MDN3 (C), during 35 days (n = 5), bars refer to standard error. For each day measurement, letters indicate differences between control and treated soils.

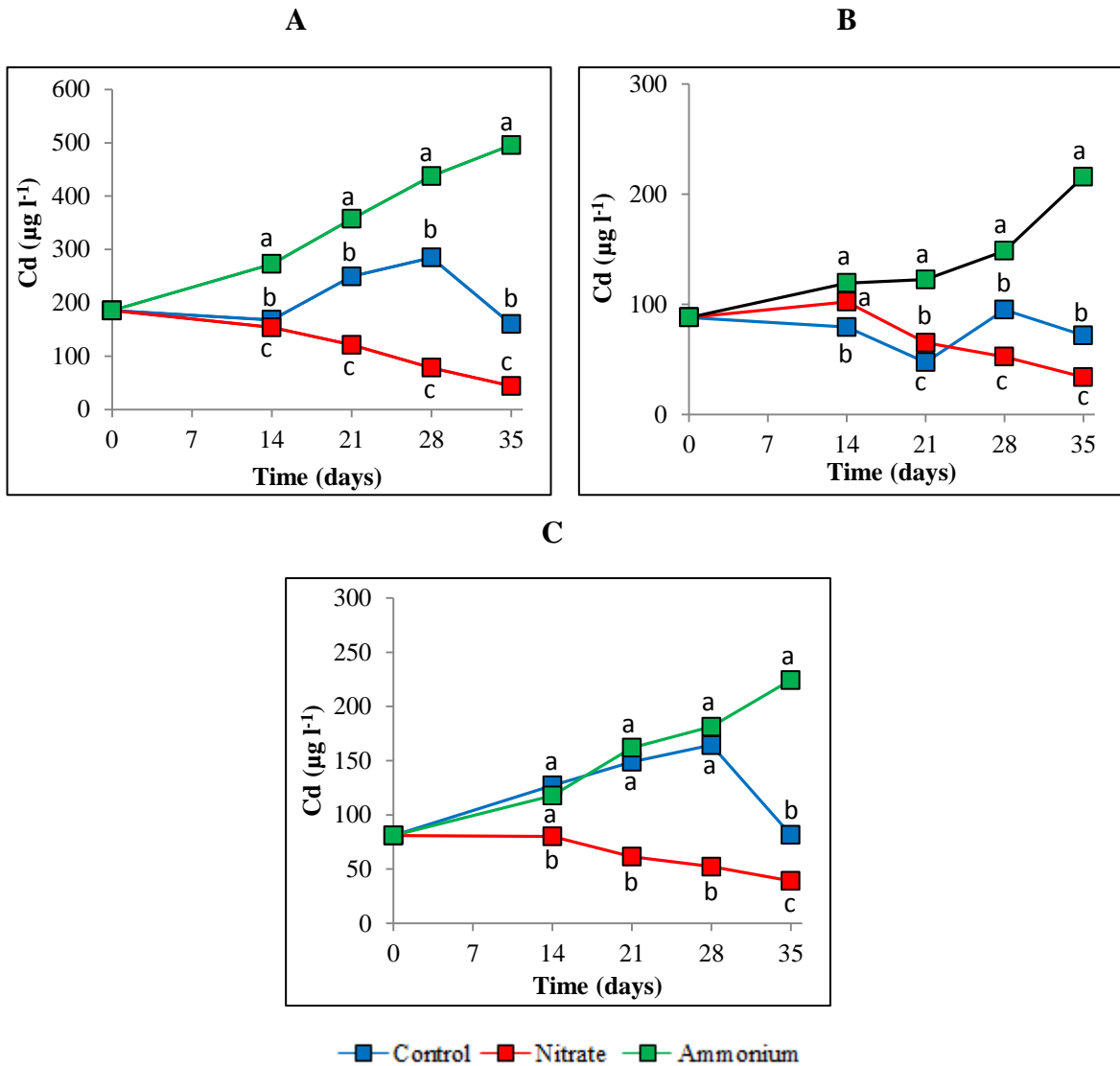


**Fig. 3** Effects of nitrogen nutrition on Zn soil pore water of *Populus euramericana Dorskamp* grown in a contaminated technosol, MDN1 (A), MDN2 (B) and MDN3 (C), during 35 days (n = 5), bars refer to standard error. For each day measurement, letters indicate differences between control and treated soils.

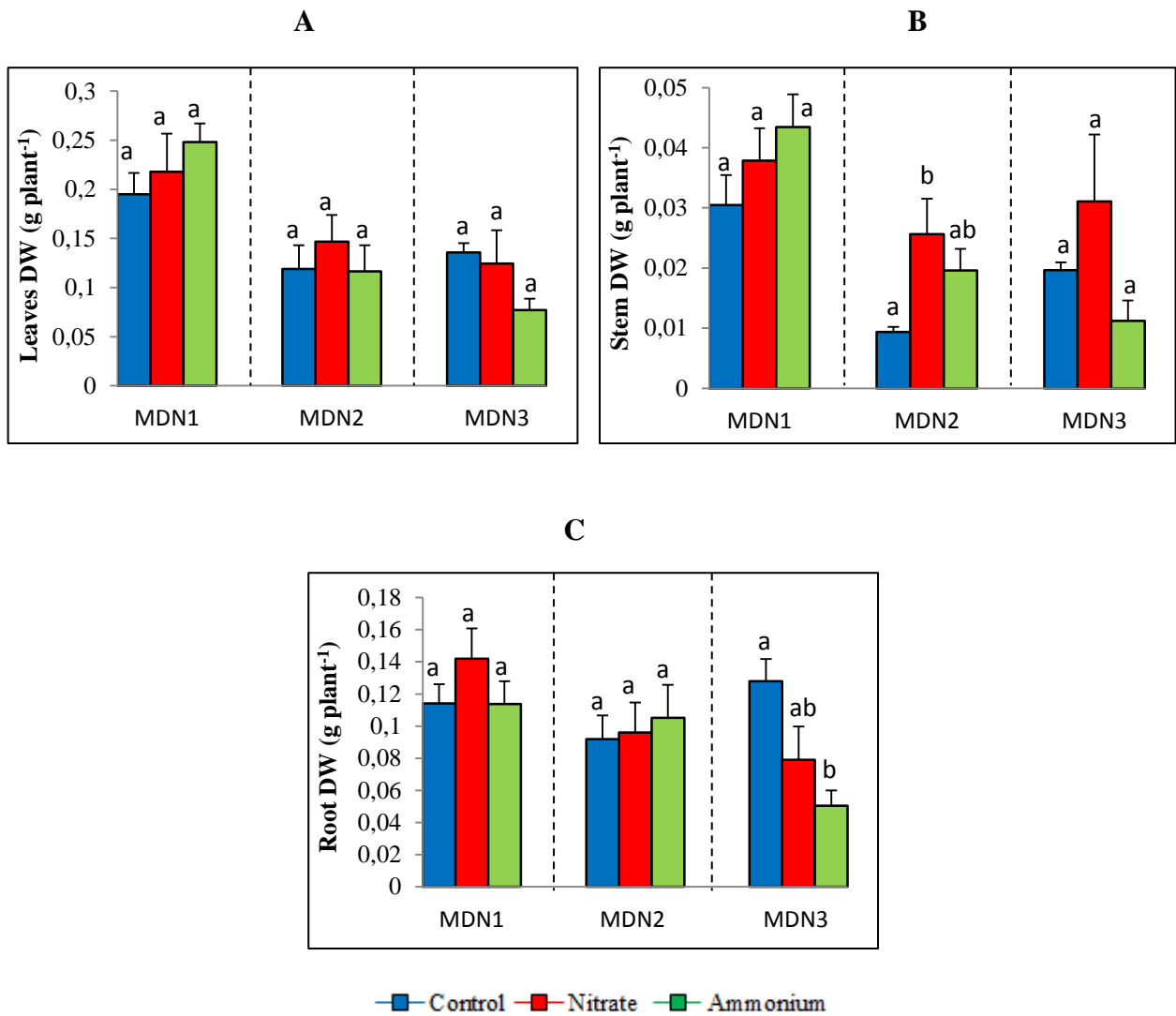


**Fig. 4** Effects of nitrogen nutrition on Pb soil pore water of *Populus euramericana* Dorskamp grown in a contaminated technosol, MDN1 (A), MDN2 (B) and MDN3 (C), during 35 days (n = 5), bars refer to standard error. For each day measurement, letters indicate differences between control and treated soils.

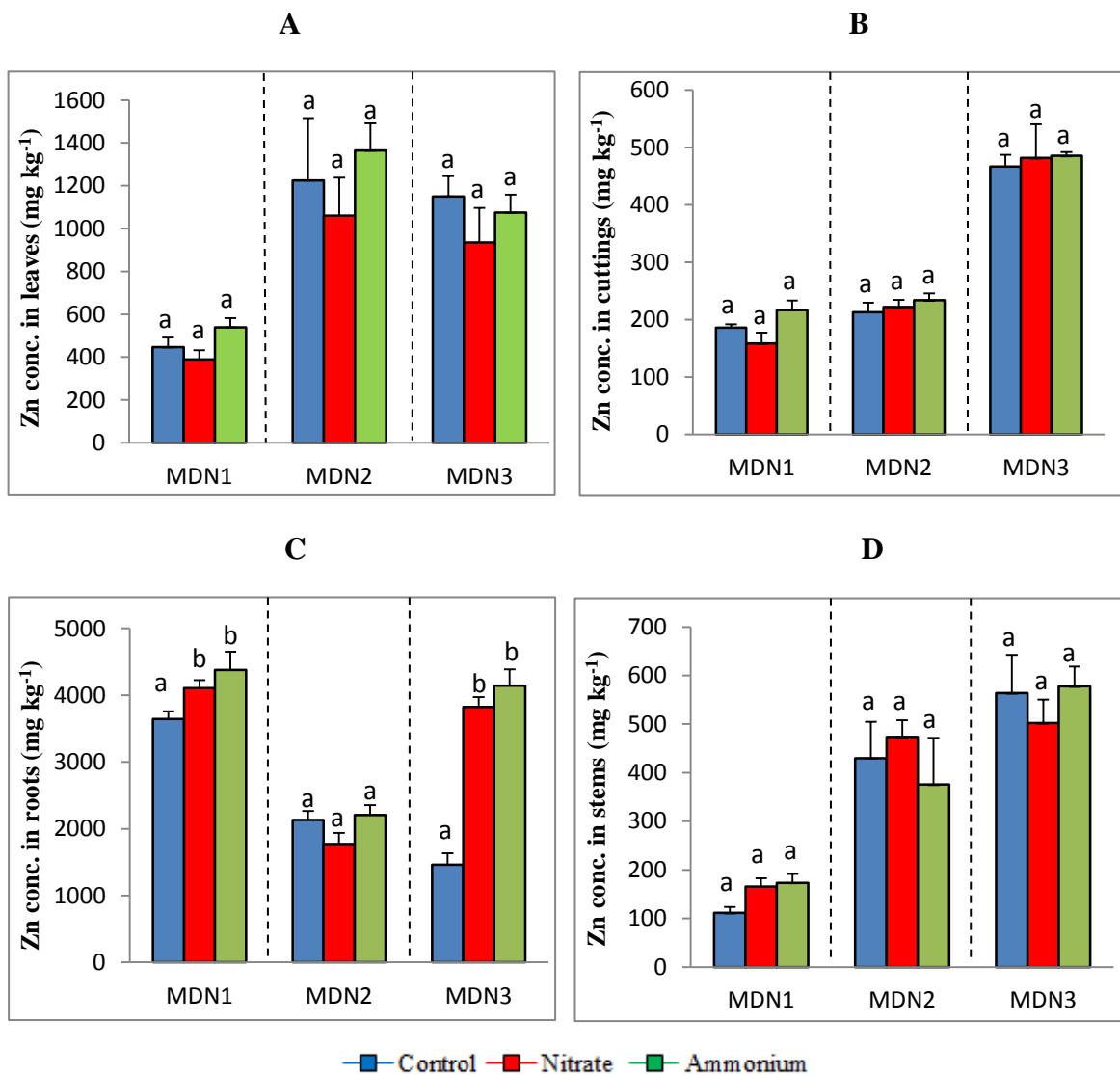




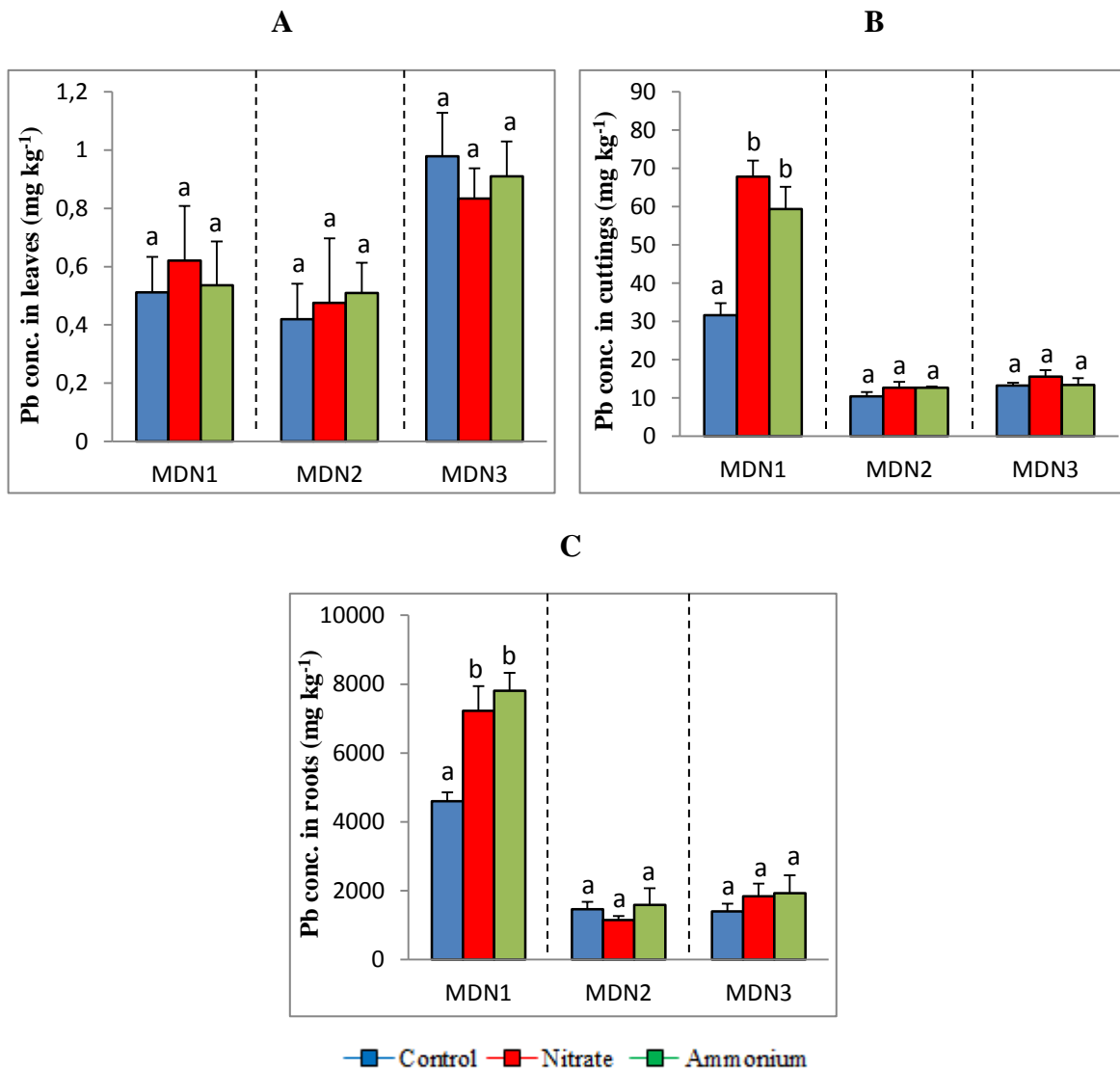
**Fig. 5** Effects of nitrogen nutrition on Cd soil pore water of *Populus euramericana* Dorskamp grown in a contaminated technosol, MDN1 (A), MDN2 (B) and MDN3 (C), during 35 days (n = 5), bars refer to standard error. For each day measurement, letters indicate differences between control and treated soils.



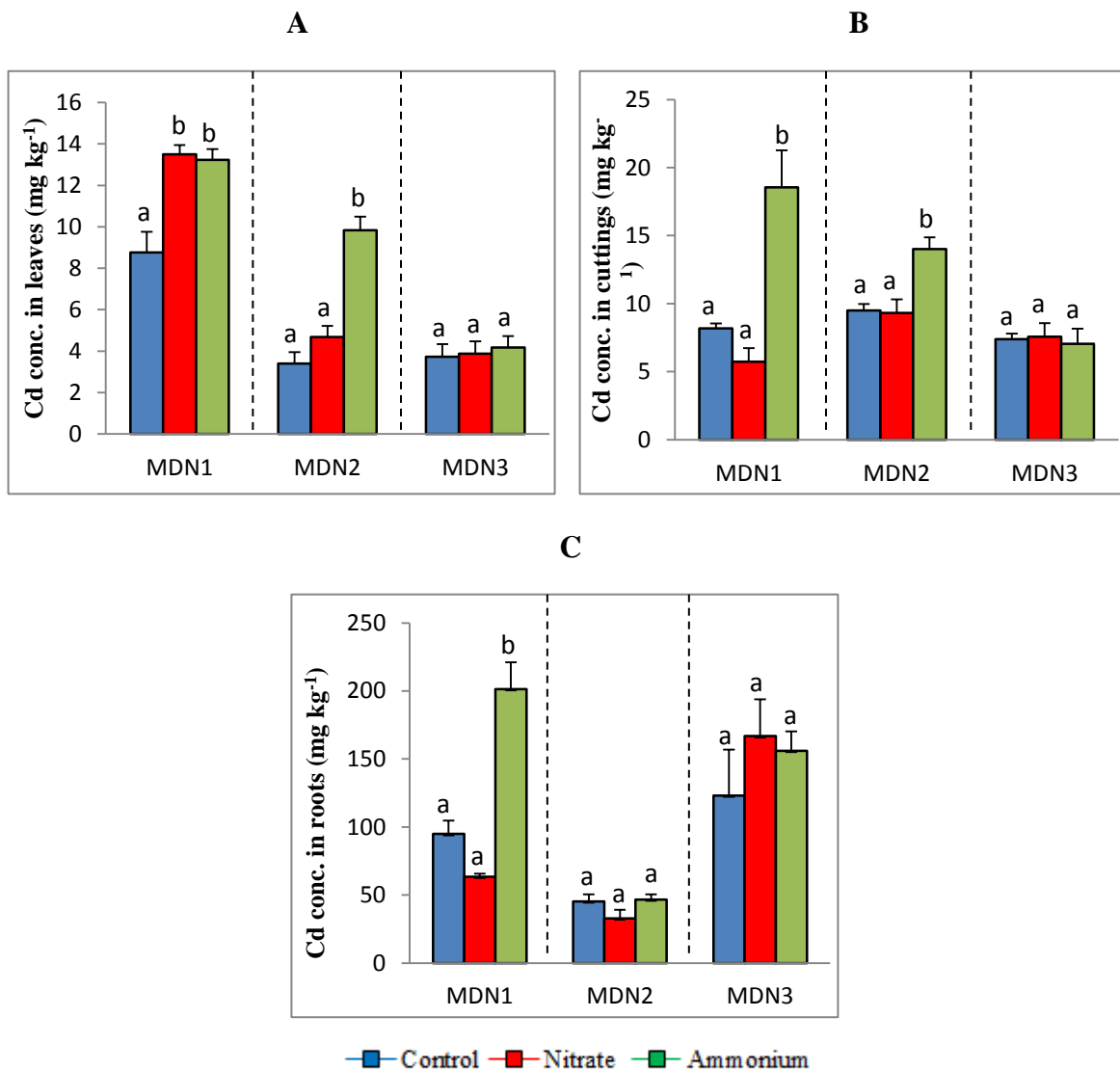
**Fig. 6** Effects of nitrogen nutrition on dry weights of leaves (A), stems (B) and roots (C) of *Populus euramericana Dorskamp* grown in a contaminated technosol during 35 days (n = 5), bars refer to standard error, letters indicate differences between treatment for a given soil.



**Fig. 7** Effects of nitrogen nutrition on Zn concentrations in leaves (A), woody stem cuttings (B), roots (C) and stems (D) of *Populus euramericana* Dorskamp grown in a contaminated technosol during 35 days (n = 5), bars refer to standard error, letters indicate differences between treatment for a given soil.



**Fig. 8** Effects of nitrogen nutrition on Pb concentrations in leaves (A), woody stem cuttings (B) and roots (C) of *Populus euramericana* Dorskamp grown in a contaminated technosol during 35 days (n = 5), bars refer to standard error, letters indicate differences between treatment for a given soil.



**Fig. 9** Effects of nitrogen nutrition on Cd concentrations in leaves (A), woody stem cuttings (B) and roots (C) of *Populus euramericana* Dorskamp grown in a contaminated technosol during 35 days (n = 5), bars refer to standard error, letters indicate differences between treatment for a given soil.