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ABSTRACT

Soil compaction is known to drastically modify soil properties and hence to affect both plants growth and metals distribution in the soil. Phosphate amendment is generally used to improve plants production but unfortunately it also gives rise to higher metal contamination in soils and plants. In this study, the effects of various parameters on the growth of *Lactuca sativa* including soil density, phosphate fertilization and cadmium contamination, were investigated. In particular, the migration of cadmium in the soil columns, its accumulation and translocation in lettuces were also examined. *Lactuca sativa* was selected as a model plant because it is widely cultivated in alkaline clay soils of eastern Mediterranean countries. Two levels of soil compaction (1.2 and 1.4 $g.cm^{-3}$), two rates of P amendment (0 and 109 mg $P.kg^{-1}$), and two levels of Cd contamination (0 and 84 mg $Cd.kg^{-1}$) were used in 24 model columns with a factorial randomized block experimental design. Soil compaction increased considerably both leaf area and dry weight of roots and shoots, whereas both chlorophyll content and NRA decreased. For the two soil bulk densities, the phosphate fertilizer improved lettuce growth characterized by plant height, dry matter, leaf number and NRA, whereas Cd contamination altered those parameters and increased the chlorophyll content. In soils contaminated with cadmium, the combination of compaction and phosphate fertilization resulted in a significant decrease in Cd migration along the soil columns. Cd uptake by plants increased in Cd treated soils; its accumulation was found to be more important than in plants grown in P-Cd treated soil where Cd uptake was clearly reduced in shoots and roots.

Keywords: Soil density, phosphate fertilizer, cadmium, *Lactuca sativa*, chlorophyll, NRA

1. INTRODUCTION

Mechanization of agriculture and addition of chemical fertilizers have been the typical responses to increase crop yields and to improve soil fertility. However, such approaches have led to significant soil compaction and soil structure deterioration. According to the literature, soil compaction is one of the main factors that influences soil physical, microbial and biochemical properties (Barzegar, et al. 2006; Rosolem et al., 2002). Soil compaction increases soil bulk density, soil mechanical resistance, and surface runoff; it also reduces soil porosity and modifies the pore size distribution in the soil profile (Kulli et al., 2003; Zhang et al., 2006). The increase in soil compaction strongly influences plant productivity and crop growth rate by reducing roots growth and their penetration into the soil, thus reducing water and air availability as well as ions transfer to roots and nutrients uptake (Barzegar et al., 2000; Chen et al., 2014; Głąb, 2014; Kuncoro et al., 2014; Miransari et al., 2009).

If porosity reduction by compaction is a common problem encountered in plough soils (Kuncoro et al., 2014; Lipiec et al., 2012), chemical fertilizers amendment has represented the main practice to improve the agriculture productivity. Thus, leaf surface area, leaf mass ratio and leaf area ratio of an Oleaceae species (*Fraxinus angustifolia* Vahl.) cultivated in a loamy soil were increased in a compacted soil as a result of increased amount of nutrients per volume unit (Alameda and Villar 2009, 2012; Arvidsson 1999). Unfortunately, intensive phosphate fertilization has conducted to the accumulation of trace metals such as zinc, cadmium, and lead in cultivated soils (Jiao et al., 2012; Lavado et al., 2001; Azzi et al. 2016). Metallic contaminants are transferred to cropland and subsequently along the food chain, which represents a critical environmental issue (Giuffré et al., 1997; Jiao et al., 2012; Nicholson et al., 2003; Luo et al., 2009). Soil compaction has been shown to inhibit nutrients transfer to plants; it thus limits the availability and the uptake of major nutrients (N, P, K, Ca, Mg and S) and micronutrients (Mn, Fe, Zn and Cu) (Barzegar et al., 2006; Lipiec and Stępniewski, 1995; Miransari et al., 2009; Zhao et al., 2007). Obviously, soil compaction also affects trace metals bioavailability (Basta et al., 2001; Qiu et al., 2011). In the case of *Trifolium alexandrimum,* soil compaction reduced both P and Zn uptakes (Barzegar et al., 2006). In addition, high levels of phosphorus in soil may also slow down the uptake of trace contaminants by plants, as illustrated by *Pteris vittata* in presence of arsenic (Bolan et al., 2003a; Huang et al., 2007; Qiu et al., 2011; Yu and Zhou, 2009)

Cadmium has been identified as the most common toxic element that readily reaches the food chains because of its great bioavailability. It accumulates in large amounts in plant tissues without showing any noticeable toxic signs (Grant and Bailey, 1997; Renella et al., 2004). Previous studies of phosphate interaction with cadmium in cultivated soils evidenced antagonistic results. Pot cultivation with various crops revealed that cadmium uptake by plants was inhibited in presence of phosphate (He and Singh, 1994; Naidu et al., 1994; Yu and Zhou, 2009). Unexpectedly, soil Cd phytoextractability and Cd uptake by *Raphanus sativa L.* were found to be increased with superphosphate use in the field (Hong et al., 2008). Cadmium bioavailability depends on soil pH, organic matter content, clays and iron oxides/hydroxides contents (François et al., 2009; Grant et al., 2010; Williams and David, 1976). However, little attention has been given to the influence of soil compaction and phosphate fertilization on the cadmium behaviour in soil and its influence on plant growth.

The main goal of this study is to investigate the effects of soil compaction on *Lactuca sativa* growth in presence of phosphate fertilizer and cadmium. *Lactuca sativa* is the main leafy vegetable in mediterranean cooking and is intensively cultivated in Eastern Mediterranean countries. In the Beaqaa valley, around 1500 hectares are annually cultivated with *Lactuca sativa*

(Karam et al., 2002). The accumulation and translocation of Cd in plants and its distribution in compacted and non-compacted soil columns are simultaneously investigated to provide evidences of physiological and morphological modifications in the plants.

2. EXPERIMENTAL SECTION

2.1 Soil sampling

A typical Mediterranean terra rosa soil was selected for this study. Soil samples were collected in the Ammik plain, a semi-arid region located in the western Bekaa valley in Lebanon (Lat. 33° 44' 17.2'' N, Long. 35° 46' 49.8'' E). No anthropogenic activity (agriculture and industry) has been reported for this sampling site. The soil was collected over an area of 50 m^2 and at a depth between 0 and 50 cm. It was then air-dried at ambient room temperature, crushed and sieved through a 7 mm mesh sieve to remove coarse fragments, and finally homogenized.

The main physical and chemical properties of the soil were determined following standard methods listed in Table 1. To determine the trace metals content, soil samples were mineralized and digested using an aqua regia digestion (HNO_3: $HCl_{v/v}$ 1:3). The concentrations of Cd, Zn, Cu, Pb, Ca, Al and Fe in the digested soil solutions were determined by Atomic Absorption Spectrometry (AAS) using a Rayleigh WXF-210 AA Spectrophotometer and WF-10A Autosampler. All reagents were of analytical grade and each value reported is the average of triplicate determinations.

2.2 Experiment design

Two soil bulk densities, 1.2 and 1.4 $g.cm^{-3}$, were selected to assess the effects of soil compaction on *Lactuca sativa* growth in the presence of phosphate fertilizer and cadmium contaminant. A 2x2x2 factorial randomized block experimental design was used with three replicate columns per treatment. Eight treatments were prepared by combining two soil bulk densities, two rates of P_2O_5 amendment and two Cd concentrations. The 1.2 $g.cm^{-3}$ bulk density is representative of the density of a clay soil. The 1.4 g.cm^{-3} bulk density is selected to evaluate the effect of an increased compaction on plant growth since a 1.39 g.cm^{-3} density affects root growth according the USDA. A single superphosphate (18% P_2O_5), graciously provided by the 'Lebanon Chemicals Company-LCC,' was used as fertilizer. It was added to provide two phosphorus levels (0 and 109 mg P.kg^{-1} of soil). Cadmium was added to the soil as cadmium chloride monohydrate ($CdCl_2.H_2O$, 99.99%; Sigma-Aldrich) to lead to 0 and 83.8 mg Cd.kg^{-1} of soil (Table 2). The content of metals identified in the superphosphate fertilizer was negligible; Pb, Cd, Zn and Cu levels were respectively 10 ± 0.2, 5.1 ± 0.8, 92.26 ± 12 and 6 ± 0.5 mg.kg^{-1} of fertilizer, which leads to very low added contents considering the amount of fertilizer added (Kratz et al., 2016). The soils without P and Cd additions were used as control samples.

Cylindrical PVC tubes of 19.5 cm internal diameter and 45 cm high were used. The soil was deposited horizontally into the PVC columns in three layers of 10 cm thick and then two layers of 5 cm thick, and compacted according to Jusoff method (1991). An increasing load was applied five times to obtain the desired bulk density. When phosphorus and cadmium were added to the soil, they were both mixed with the upper 5 cm layer of soil before being transferred to the columns. The pot experiment was conducted in a greenhouse of controlled temperature and humidity.

2.3 Plant growth and samples preparation

The seeds of lettuce (*Lactuca sativa*) were pre-germinated for 2 weeks at 20°C in a mixture of perlite and coconut husk. One seedling was then transplanted into each column. The 24 columns were each irrigated with 750 ml of mineral water per week. Irrigation water was previously characterized (electrical conductivity of 297 µS.cm^{-1} (at 25°C), pH 7.5, HCO^{3-} 195 mg.l^{-1}, Ca^{2+} 67 mg.l^{-1}, Mg^{2+} 1.45 mg.l^{-1}, Na^+ 4 mg.l^{-1}, K^+ 0.2 mg.l^{-1} and Cl^- 6.5 mg.l^{-1}). The leachates of each column were collected in separate polyethylene containers the third, the fifth and the seventh week after planting.

The lettuces were grown for 77 days under controlled conditions, i.e. temperature set to 21/19 °C day/night, 12 h of light period and 60-70% air moisture. The shoots of each column were then harvested while the roots were carefully removed from the soil and washed with distilled water. Leaf number, leaf area, plant height, maximum root length and leaf length, stems and roots fresh weight were measured. The roots and shoots were then dried at 80°C for 48 hours in a ventilated oven, and the dry mass of each part was subsequently determined.

Finally, the PVC columns were cut lengthwise and divided into 6 layers from the upper bottom, i.e. 4 layers of 5 cm thick and the last 2 layers of 10 cm thick. Once sliced, the soil layers were individually air-dried in a dark room for seven days followed by 2 days at 40°C. The samples were then sieved through a 2 mm mesh and digested to investigate the Cd distribution in the soil.

2.4 Total chlorophyll content

During the last days of the experiment, leaf discs were taken from each plant to determine the total chlorophyll content. Each leaf section was first immersed in 80% acetone for 24 h in dark room at 20°C, and the total chlorophyll content Chl (a + b) was then determined by measuring the absorbance at 645 and 663 nm (spectrophotometer Thermo Spectronic 20 Genesys). The calculation was based on the method described by Arnon (1949).

2.5 Nitrate reductase activity (NRA)

A sub-sample of fresh leaves was used to determine the nitrate reductase activity according to Jaworski (1971). The analyses were performed in triplicate. A 0.5 g sample of fresh leaf tissue was sliced into small pieces and incubated with phosphate buffer (pH 7.5), and then with potassium nitrate (0.03 M) and isopropanol solutions (5%) for 2 h at 28°C. The nitrite content

(NRA in µmole $NO_2.g^{-1}.h^{-1}$) was determined colorimetrically at 540 nm after addition of N-1 naphthylethylenediamine dihydrochloride (0.02%) and sulphanilamide (1%).

2.6 Heavy metals in soil layers and leachate water samples

For heavy metal analysis, soil sub-samples were digested with aqua regia (3:1 HCl/HNO_3 (v/v)) according to ISO 11466:1995. The Cd, Pb, and Zn concentrations of digested solutions were then analyzed by Flame Atomic Absorption Spectrometry (Rayleigh WFX-210 AA Spectrophotometer).

pH and electrical conductivity were measured in all leachates. After filtration with 0.45 µm filters, the anions concentrations (Cl^-, NO_3^- and SO_4^{2-}) of leachates were obtained by ion chromatography (Shimadzu Shim-pack IC-A3) equipped with a conductivity detector CDD-10AVP. Heavy metals in leachates (Cd, Pb and Zn) were also analyzed using AAS.

2.7 Accumulation, translocation and bioaccumulation of heavy metals

The dried soil samples were homogenized by grinding using a stainless steel blender. A sample mass between 50 to 1000 mg of each plant part, was digested in 20 ml HNO_3. The heavy metals concentrations in the various parts of the plants were then analyzed using AAS. The cadmium translocation factor from roots to shoots (F_T), was described as the ratio of Cd concentration in the shoot to the Cd concentration in the root ($F_T = [Cd]_{shoot}/[Cd]_{root}$). Bioaccumulation factors (F_B) were obtained as the ratio between the concentrations of Cd in shoot tissue and those in the soil ($F_B = [Cd]_{shoot}/[Cd]_{soil}$) (Durand et al., 2015).

2.8 Statistical analyses

All data were analyzed using SPSS statistics 17.0. Two-way ANOVA (generalized linear models) was used at $P < 0.05$ to assess the statistical significance effects of soil compaction, phosphorus, cadmium and their interactive effects. Duncan multiple range test ($\alpha = 5\%$) was performed to analyze statistical differences between different treatments for each parameter.

3. RESULTS AND DISCUSSION

The soil used in this study is a typical Mediterranean clay soil based on USDA textural diagram. It is a medium to fine textured soil of high clay content (53%) and has a CEC of about 19 meq/100g. The soil is of moderate salinity (electrical conductivity 1.157 $mS.cm^{-1}$), shows a very low content in organic matter (OM) (2.082%) and a relatively low total calcareous (about 1.4%), which is consistent with the alkaline soil pH (8.21) (Table 1). Accordingly, the amount of clay-OM complexes is expected to be low and soil compaction should easily take place affecting capillarity and water availability to roots (Kooistra et al., 1992). The levels of Zn, Cu, Pb, and Cd were under the maximum allowed limits by USEPA, 1997. The high levels in iron and aluminum were expected for a clay terra Rosa Mediterranean soil.

3.1 Crop growth

3.1.1 Leaf growth

As shown in Table 3, the leaf number was not affected by soil compaction even when cadmium was added to the soil column, whereas a significant increase in leaf number ($P < 0.05$) was observed in lettuces treated by phosphorus fertilizer (P). Moreover, in compacted soil (C) treated with P (C-P), plants showed a significant increase in leaf area. The highest leaf area (1635.1 cm^2 ± 321.2) was found for the treatments receiving P in soils of 1.4 $g.cm^{-3}$ bulk density. Several studies have shown that the application of phosphate significantly increases leaf number, leaf elongation and leaf area of *Lolium* perenne, maize (*Zea mays* L.), soybean and wheat (*Triticum aestivum* L.) (Assuero et al., 2004; Chiera et al., 2002; Kavanová et al., 2006; Rodriguez et al. 1998). In addition, compaction has been shown to enhance the contact between roots and substrate, thus determining an increase in the volume of root cells related to the higher amounts of nutrients per unit volume of soil and a greater water availability (Arvidsson, 1999; Barzegar et al., 2006). Such conditions have also been shown to enhance the development of leaf area for seven woody plants (Alameda and Villar, 2009).

3.1.2 Root development

Soil compaction resulted in a decrease in root length for all treatments with or without the presence of phosphate fertilizer and cadmium. Root length was reduced by 29.3% between plants growing in non-compacted and compacted soil treated with P (NC-P and C-P) (Table 3). These results are in line with those of Lipiec et al. (2012) showing a reduction by more than 50% of root length between compacted and non-compacted soil (Grzesiak et al., 2013; Rosolem et al., 2002). Soil compaction delays roots development and increases the resistance to roots penetration (Konopka et al., 2008). In addition, the availability of oxygen decreases and becomes a limiting factor the normal activity of roots (Arvidsson, 1999). Nevertheless, P addition leads to an increase in root length in both compacted and non-compacted soils. Thus, root length of NC-P was 1.57 times longer than that of control NC. Phosphorus as phospholipids is a constituent of plant cell membrane that it is concentrated in the fast growing parts of plants particularly in the root tips. Furthermore, it stimulates the development and elongation of root systems (Al-Niemi et al., 1998; Naeem et al., 2010).

3.1.3 Plant height

After 77 days of growth, the variation in soil bulk density was unsignificant on aboveground length for all treatments. The decrease in root length development observed in compacted soils, was compensated by a higher dry weight of roots revealing a higher nutrients uptake (Table 3). In contrast, the applications of phosphate and cadmium had a significant effect on lettuce height

($P < 0.05$). Superphosphate amendment significantly enhanced shoot length by 1.2 times higher than those cultivated in both C and NC soil. Such increase results from the stimulating effect of phosphorus on root development leading to a beneficial nutrient absorption.

Cadmium application had a strong negative impact on plant height. Thus, shoot height decreased by 21 and 30% for C-Cd and NC-Cd tests, respectively, in comparison with the corresponding blanks. The growth inhibition induced by a cadmium treatment is generally attributed to a perturbation of hormonal activity, especially that related to abscisic acid. The high affinity of cadmium for sulfahydryl proteins groups may also delay the lettuce growth. Such observations are consistent with previous studies reporting that plant height, leaf area and plant weight are reduced in the presence of cadmium (Chaffei et al., 2004; Dong et al., 2005; Greger and Örgen, 1991).

The presence of phosphorus mitigates the negative effect of cadmium since the plant heights in (C-P-Cd) and (NC-P-Cd) tests were equivalent to those of (C) and (NC) controls. Phosphorus application promotes Cd immobilization in the soil through the formation of cadmium-orthophosphate complexes, thus decreases the availability, of Cd to plants.

3.1.4 Dry weight of shoots and roots

While both soil compaction and P application enhanced lettuce growth and increased the dry weight of roots and shoots, Cd application led to a significant decrease in shoot dry weight (Table 3). Shoots and roots dry weight increased on average by 36% and 33% respectively, in compacted soil (C) compared with non-compacted (NC) soil. The highest shoot and root dry weight was recorded in compacted soil treated with P (C-P). Such increase in biomass of aerial and subterranean parts can be related to the role of phosphorus in the development of a more extensive root system, which allows an increase of nutrient and water absorption (Naeem et al., 2010). The lowest dry weight was obtained for the NC-Cd treatment (Table 3). In that case, the reduction in dry weight is attributed to the effect of Cd on the enzymatic activities regulating growth and physiological behavior: inhibition of water transport to the stem, decrease of essential elements uptake, reduced stomata openings and limited CO_2 absorption, are the principal factors that cause the reduction of biomass (Chaffei et al., 2004; Greger and Örgen, 1991; Grzesiak et al., 2013).

3.1.5 Chlorophyll content

The soil compaction was unsignificant on the chlorophyll content in both C and N-C treatments. The most pronounced physiological response of lettuce to the various treatments was observed for the soils treated with Cd, especially that of compacted soil (C-Cd) (Table 4). Plants grown in the presence of cadmium (C-Cd and NC-Cd) or in the presence of Cd and superphosphate (C-P-Cd and NC-P-Cd), had significantly higher chlorophyll contents than those of controls and plants only treated with the phosphate fertilizer (C-P, NC-P), which implies a relationship between cadmium contamination and chlorophyll production. Such result is in agreement with that of Manios et al. (2003), who reported an increase in total chlorophyll for *Typha latifolia* plants after irrigation with solutions containing various concentrations of Cd, Cu, Ni, Pb and Zn. In that case, the increase in chlorophyll content was attributed to a change in the activity of hormones involved in chlorophyll synthesis . Furthermore, the reduction of leaf surface was correlated with the accumulation of chlorophyll pigments. Treating bean plants with Cd, Skórzyńska-Polit and Baszyński (1997) observed a disruption of thylakoid membranes in comparison with those of control. Nonetheless, soil compaction had significantly decreased the chlorophyll concentration for plants grown in the presence of superphosphate (C-P) and superphosphate with cadmium (C-P-Cd) in comparison with the same treatments for non-compacted soil (the last four treatments).

Kozlowski (1999) mentioned a reduction of the rate of photosynthesis, i.e. a reduction in chlorophyll content, for two trees species (*Rubus* sp. and *Pinus contorta*) grown in compacted soils, but such observation had never been previously reported for Compositae species (*Lactuca sativa*). A significant increase in chlorophyll concentration was also observed for lettuces grown in non-compacted soil treated with superphosphate (NC-P). Such result is consistent with those of Jiang et al. (2007) and Castillo-Michel et al. (2009) who showed that phosphate increases the chlorophyll content in leaves, and hence improves the nutritional quality and the plant photosynthesis ability. Similar increases in chlorophyll contents resulting from P applications, have also been reported by Naeem et al (2010).

3.1.6 Nitrate reductase Activity

Soil compaction led to a significant decrease in nitrate reductase activity (NRA) for all applied treatments except for the C and NC controls. A significant reduction of 17.8% was observed between NC-P and C-P (Table 4). Soil compaction is involved in nitrate leaching which promotes denitrification. In addition, it contributes to root shortening and hence to a decrase of nutrients uptake, especially nitrates. All these factors contribute to the reduction of the nitrate reductase activity in compacted soils. The observed results are in accordance with those of Goupil et al. (1998) who observed a decrease in NRA in the presence of mechanical stress. As previously shown for the compacted soils, cadmium significantly reduced the NRA of plants grown in presence of Cd (C-Cd, NC-Cd, C-P-Cd and NC-P-Cd). NRA decreased from 105.45 to 63.93 mol NO_2^- g^{-1} h^{-1} between C and C-Cd. The cadmium toxicity to plants influenced both nitrate absorption and transport from the roots to the leaves, and then led to a reduction in nitric oxide assimilation (Chaffei et al., 2004; DalCorso et al., 2008). This also affects the activity of various enzymes involved in the nitrogen metabolism within the leaf such as glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase (Chaffei et al., 2004). In comparison with other treatments, plants treated with superphosphate (C-P and NC-P) showed a significant incrase in NRA (35% and 38% greater than that of C and NC controls, respectively). Such increase is due to the positive effect of P in plant metabolism. Indeed, it has been shown that NRA is enhanced by the application of mineral nutrients, especially phosphorus (Lillo, 1994a, b; Campbell, 1999). Mineral nutrients were also found to improve nitrate assimilation in castor beans and soybean (Jeschke et al., 1997; Rufty Jr. et al., 1993).

3.2 Cd distribution along the soil columns

After 77 days, the Cd concentration in the soil columns was determined as a function of depth for all treatments. A surprising effect of soil compaction was observed below the 0-5 cm layer (C-Cd and NC-Cd tests). The Cd content in the compacted soil was only half (17.7±0.9 $mg.kg^{-1}$) of that of the non-compacted soil (35.6±3.4 $mg.kg^{-1}$) in the 5-10 cm layer (Fig. 1a). Therefore, soil compaction delayed the migration of Cd from the 5-10 cm layer to the 10-15 cm layer. Similarly, but to a lesser extent, in the soil treated with the phosphate fertilizer, the migration of Cd from the 0-5 cm layer to the 5-10 cm layer was less in compacted (C-P-Cd) than in non-compacted soil (NC-P-Cd) by about 7% and 21%, respectively (Fig. 1b). In all cases, the geochemical background level for Cd is reached below the 10-15 cm layer. The Cd behaviour in the soil is mainly related to the soil density as well as to the phosphate content. Hence, it can be inferred that phosphate fertilizer and compaction are key factors in reducing cadmium migration since P-Cd complexes can be formed (Bolan et al., 2003a; Bolan et al., 2003b; Hong et al., 2008, Jiang et al., 2007). Nevertheless, soil compaction, with or without phosphate treatment, can be considered as the main physical barrier that increases the metal retention in the upper soil layers.

3.3 Leachates composition

Leachates were characterized at the 3rd, the 5th and the 7th week. The pH of first leachates sampled varied between 8 and 8.2, the values of second leachates varied between 8.3 and 8.45 and those of third leachates were between 8.38 and 8.7 (Fig. 2a). However, such pH increase remains moderate and may simply be attributed to a CO_2 decrease in the soil (Summerfelt et al., 2003).

In contrast, the electrical conductivity (EC) decreased between the first and the third leachate collection (Fig. 2b). The EC of first samples varied between 1220 and 1715 $\mu S.cm^{-1}$ for the various treatments, whereas those of the third leachate decreased from 963.33 to 656 $\mu S.cm^{-1}$. Under such conditions, the formation of metal hydroxides may occur thus decreasing the leachate conductivity (Rich et al., 2008).

The nitrate concentrations in the three leachates were greater for compacted soils compared with those of non-compacted soils (Fig. 3a). In particular, a decrease in nitrate concentration from 499 to 342 $mg.l^{-1}$ can be observed between C-Cd and NC-Cd. Previous studies by Barzegar et al. (2006) has suggested that compaction may facilitate nitrate migration. The observed decrease in nitrate concentration between consecutive leachates may be the result of combined factors, such as the denitrification in anaerobic conditions (deep soil columns), soil biomass metabolisms, nitrate leaching and nitrate assimilation by plant as a function of time (Lipiec and Stępniewski, 1995; Arvidsson, 1999).

In contrast, chloride and sulfate concentrations were higher in leachates from non-compacted soils (Fig. 3b, 3c). In the first leachate, soil compaction determined a 68% and 12% decrease in chloride and sulfate, respectively. Indeed, soil compaction alters soil water retention and decreases infiltration ability, thus leading to a decrease in the concentrations of those ions in the leachate (Kulli et al., 2003; Zhang et al., 2006). In both compacted and non-compacted soil, chloride leaching decreased with time and sulfate concentration was below the detection limit in the third leachates for C, NC, C-Cd and NC-Cd. When the phosphate fertilizer was added, both chloride and sulfate concentrations in the leachate of NC-P were more twice greater than those of C-P. That sulfate concentration is higher in leachates of soil columns treated with the phosphate fertilizer is expected since the latter contains 41%wt SO_4 (Azzi et al. 2016) (Fig. 3c). Moreover, no soluble cadmium was found in those leachates because of the immobilization of available Cd by sulfate and/or phosphate.

Both Cd availability and Cd uptake by plant are significantly influenced by soil pH (Kirkham, 2006). Previous studies indicated that low pH values enhance Cd accumulation in plant tissues (Tsadilas et al., 2005; Waisberg et al., 2004; Yanai et al., 2006). A linear relationship between soil pH and cadmium absorption has even been reported (Christensen 1989, Tudoreanu and Phillips, 2004). Therefore, a low pH increases the concentration of cadmium ions available to root uptake.

3.4 Cd transfer to lettuce

The Cd concentration in shoots and roots of lettuce for all soil treatments is shown in Fig. 4. The cadmium content in plant tissues grown in soil without Cd addition reflects the geochemical background level. Cd contents were similar between roots and shoots (Fig. 4). In contrast, in soils where Cd was added, root tissues and plant shoots accumulated the highest amount of Cd of all soil treatments. Furthermore, the increase in cadmium in shoots and roots was found to be associated with the increase in chlorophyll content (Table 4, Fig. 4). Similar results were for tumbleweed, wheat, cucumber, sorghum and corn (Castillo-Michel et al., 2009; De la Rosa et al., 2004; De la Rosa et al., 2005; Youn-Joo, 2004).

Cd concentration in shoots and roots increased when Cd was added to soils with or without fertilizer amendment. Phosphate addition to the soil contaminated by Cd led to a decrease in the Cd concentration of shoots and roots (Fig. 4). Unexpectedly, the phosphate fertilizer did not show significant effects on cadmium retention into the soil whatever the soil density. Previous studies revealed that the total Cd accumulation in Mirabilis jalapa, Chinese flowering cabbage (*Brassica parachinensis* L.), cauliflowers (*Brassica oleracea* L.) and spinach (*Spinacia oleracea* L.) significantly decreased after phosphate amendment (Chen et al., 2006; Dheri et al., 2007; Qiu et al., 2011; Yu and Zhou 2009), Cd being retained in soil as $Cd_3(PO_4)_2$ deposits (Bolan et al., 2003a; Bolan et al., 2003b; Hong et al., 2008; Hong et al., 2010). The soil compaction enhanced Cd accumulation in shoots by 12% for soils treated with Cd, and by 25% for soils treated with Cd and P. Such accumulations might be attributed to the increase of root cells volume that improves nutrient uptake per root unit length (Rosolem et al., 2002).

The behaviours of Zn and Pb differ to that of cadmium in roots and shoots. The levels of Zn and Pb in shoot and root tissue of lettuce are shown in Fig. 4c - 4f. Pb levels varied between 3 and 33 $mg.kg^{-1}$ and Zn levels between 21 and 115 $mg.kg^{-1}$ of dry weight tissue. On the whole, the Zn content in shoot was significantly reduced for the soils treated with phosphate and cadmium. Unexpectedly, the Zn concentration in roots increased for soils treated with Cd (C-Cd and NC-Cd) and decreased in roots grown for fertilized soils (C-P, NC-P, C-P-Cd and NC-P-Cd). The concentration of Pb in shoot for P treated soils clearly decreased without being necessary correlated to P addition. Nevertheless, in the presence of both cadmium and phosphate (C-P-Cd and NC-P-Cd), it seems that the cadmium phosphate complexation in the soil competes with the Pb-phosphate interaction, and hence the Pb uptake by shoots was improved. On the other hand, Pb concentration was higher in roots of lettuce grown in P treated soil. Such result is in agreement with that of Cao et al. (2002) who observed a decrease in Pb concentration in St. Augustine grass (*Stenotaphrum secundatum*) tissue after P application to Pb contaminated soils. Pb-P precipitates may form either on the root surface, within the root rhizosphere or in the bulk soil. However, soluble P decreases the concentrations of Pb, Zn and Cd in plant tissue due to the formation of mixed-metal phosphates (Hettiarachchi and Pierzynski 2002). The same authors also reported that P addition decreased Zn concentration in cabbage shoots because of the formation of mixed-metal phosphates in soil.

Both Translocation factor (F_T) and bioaccumulation factor (F_B) were used to evaluate the effectiveness of lettuce in Cd translocation from roots to shoots and to evaluate its accumulation efficiency in the plants. Plants growing in compacted soils show significantly greater F_T and F_B than plants growing in non-compacted soils (Fig. 5). F_T(Cd) decreased in soils treated with Cd (C-Cd and NC-Cd) and in soils treated with both Cd and P (C-P-Cd and NC-P-Cd), whereas F_B for soils contaminated with Cd (0.81 and 0.56 for C-Cd and NC-Cd, respectively) were significantly higher when compared with soils treated with P and Cd (0.65 and 0.42 for C-P-Cd and NC-P-Cd, respectively). The relative decline in total Cd uptake and in Cd translocation factor revealed that the presence of phosphate fertilizer in soil lowers the amount of cadmium uptake by lettuce. Hence, phosphate plays a significant role in Cd translocation from root to shoot. Such process involves the immobilization of Cd^{2+} by phosphate anions especially HPO_4^{2-} onto cell walls by various mechanisms such as adsorption, complexation, precipitation, and crystallization. This leads to the formation of Cd-phosphate complexes which limits the mobility of Cd in plants (Jiang et al., 2007; Qiu et al. 2011).

4. CONCLUSIONS

Soil compaction is a key factor in agriculture production because it deeply affects plants growth, phosphate fertilizer benefits and metals transfer between soil and plants. Actually, soil compaction led to a decrease in root length, chlorophyll content and NRA in *Lactuca sativa*. Furthermore, soil compaction enhanced the cadmium transfer to roots and shoots thus inducing an increased chlorophyll production. In cadmium contaminated soils, either compacted or not, phosphate fertilization inhibited the negative effect of cadmium on all the morphological parameters of plants. The fertilization had an antagonistic role both decreasing the chlorophyll content and increasing the NRA. Soil compaction and phosphate fertilization are considered key players for limiting Cd mobility in soil. However, a decrease in both chloride and sulfate concentrations of leachates were observed for compacted soil columns, whereas a net increase in nitrate was recorded at the same time. In soils contaminated with cadmium, a phosphate fertilizer addition is recommended to inhibit Cd accumulation in *Lactuca sativa*. Soil compaction increased both Cd translocation factor (F_T) and bioaccumulation factor (F_B). On the other hand, more attention must be paid to soil density that controls both cadmium availability and uptake by *Lactuca sativa*.

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