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Brassica napus Growth in Lead-Polluted Soil: Bioaccumulation in Plant Organs at Different Ontogenetic Stages and Lead Fractionation in Soil

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Abstract Lead is known to be a highly toxic metal; it is often found in soils with the potential to be incorporated by plants. Here, the bioaccumulation of lead by rapeseed (*Brassica napus*) from a soil with Pb(II) added just before sowing is studied. The effect on plant organs is also studied at the ontogenetic stages of flowering and physiological maturity. Moreover, the chemical fractionation of Pb in the rhizosphere and bulk soil portions is investigated and related to Pb accumulation in plant organs. *B. napus* are found to accumulate Pb in its organs: 1.5–19.6 mg kg^{-1} in roots, 3.3–15.6 mg kg^{-1} in stems, 0.5–8.6 mg kg^{-1} in leaves in all treatments, and in grains 1.45 mg kg^{-1} at physiological maturity and only for the highest Pb dose (200 mg kg^{-1}). Plant biomass reduction was observed to be about 20% at the flowering stage and only for the highest Pb dose. The analysis of metal fractionation in soil shows Pb migration from the bulk soil to the rhizosphere, attributed to concentration gradients created by root intake. Along the time period studied, lead chemical fractionation in soil evolved toward the most stable fractions, which coupled to plant uptake depleted the soluble/exchangeable one (assumed bioavailable).

Keywords Lead bioaccumulation · Flowering · Physiological maturity · Rhizosphere · Plant lead translocation

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1 Introduction

The presence of potentially toxic metallic elements in the environment is a topic which has aroused high interest for long times. These metals often come from anthropogenic activities and can accumulate and remain in different environmental compartments (such as hydrosphere or lithosphere), exposing the biota to its toxic effects for long time periods. Some metals are toxic only in high concentrations, whereas others, like lead (Pb), can be harmful even at trace levels (Wong et al. 2006). Lead present in the environment has mostly anthropogenic origin, such as mining (Arrouays et al. 1996), paints (Needleman 2004), Pb tubing (Cartier et al. 2013), hunting pellets (Ferreyra et al. 2009), car battery residues (Rodriguez et al. 2014), and former fuel

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additives (Lavado et al. 1998). This pollutant can remain in the environment, especially in the soil, for long periods of time. In the case of contaminated agricultural soil, Pb could be uptaken by crops, reach grains or other edible organs, be consumed, and consequently incorporated by animals and humans (Shahid et al. 2012; McBride et al. 2015). Soils are composed of several organic and mineral constituents (Sparks 2002; Molina 2013). The distribution or fractionation of metals among these components (also known as speciation (Hlavay et al. 2004) or partition (Shrivastava and Banerjee 2004)) is highly relevant in the study of availability and mobility of metal cations. Consequently, the Pb fractionation in soil is a relevant aspect; usually, the soluble and exchangeable forms are bioavailable, whereas those more stable (chemisorbed, precipitated) are not (Magrisso et al. 2009; Shahid et al. 2012). Therefore, the study of Pb fractionation and bioavailability in the soil, and its relationship with the Pb uptake by plants is of prime concern.

One soil portion of interest is the rhizosphere, which is the soil ecosystem extending up to a few millimeters away from the roots. It has physical and chemical properties different from the bulk soil, as several biological functions of roots (such as nutrient sorption, breathing, deposition of chemical compounds and structures, and exudation) strongly affect the soil properties in that zone. The rhizosphere shows different properties compared with bulk soil, including nutrient and pollutant concentrations, pH, redox potential, and metal complexation capability (Hinsinger et al. 2005). The bioavailable metal concentration is also affected in the rhizosphere; the amount of metal in the exchangeable fraction (as a measure of the bioavailable amount) is important in the analysis of potential plant metal bioaccumulation (Lavado et al. 2007). It should be also taken into account the metal-plant interactions in the rhizosphere portion: on barley culture, a decrease of Cu and Zn solubility in the rhizosphere compared to bulk soil has been observed, attributed to an effect of pH variations. On the other hand, the bioavailable concentrations of Mn and Fe were found increased due to root exudation (Youssef and Chino 1989). Other studies have shown changes in the fractionation and bioavailability of Pb and other heavy metals in wheat and *Brassica juncea* (Wang et al. 2002).

In the assessment of metal fractionation, sequential selective extraction (SSE) methods constitute an important tool. These methods are based on the exposure of a sample to a sequence of wet chemistry treatments; at each step of the sequence, the sample is extracted with reactants of increasing strength to selectively remove a specific solid component. It is assumed that the chemical component of interest is freed in solution (Tessier et al. 1979; Ma and Uren 1998; Hass and Fine 2010). The fractionation of Pb(II) in the soil sample employed here was investigated previously in the absence of plants (Ferreyroa et al. 2014). It was found that Pb(II) incorporated into the soil as soluble Pb^{2+} evolves in a relatively short time (about 60 days) from the soluble fraction to the most stable, namely mineral fractions, leaving only a small amount in those soluble and exchangeable. Consequently, it is interesting to study the same system in the presence of plants; *B. napus* is chosen in the present work.

Lead fractionation in the rhizosphere has been studied for several plant species: for *Elsholtzia splendens*, a potential increase of bioavailable Pb in the long-term was reported (Yang et al. 2010). Similar results were found in the rhizosphere of wheat irrigated with water having high Pb concentration (Khan et al. 2006). Azimzadeh et al. (2014) studied the fractionation of Pb in the rhizosphere of *B. napus* and *Zea mays* separately and with both species associated. It was found that the addition of organic fertilizers increased Pb concentration in the exchangeable, carbonate-associated, and organic matter-bound fractions. In turn, bioavailability to plants increased, as the first two fractions are generally the most available.

Some species of the genus *Brassica*, such as *B. nigra L.* and *B. juncea*, have been extensively studied as metal bioaccumulators in polluted soils. They were reported as tolerant to high concentrations of Pb (among other metals), accumulating in roots, stems, and leaves (Liu et al. 2000; Bharagava et al. 2008; Karak et al. 2013). *B. napus* have been less studied regarding metal bioaccumulation (Yu et al. 2012; Azimzadeh et al. 2014; Bilal Shakoor et al. 2014). It is an important oily crop in the world and has the possibility to expand its cropping area in temperate zones (Zanetti et al. 2013). Like all plants, the growth of rapeseed is mediated by their genetic characteristics and the environment. To quantify the ontogenetic stages of crops, standardized scales provide a common reference for describing the crop's development. The growth scales are based on main growth stages, starting from germination, and ending in grain or fruit ripening. Particularly, abiotic stress, like Pb in soils, affects the normal growth and development of

plants consequently affecting the normal succession of stages (Slafer 1993).

In this work, the growth of *Brassica napus* in the presence of lead in soil, incorporated at the sowing time, is analyzed. The main objective is to analyze the Pb accumulation in the plant organs at the onset of flowering and at physiological maturity ontogenetic stages, as well as pollutant effect on biomass production at both stages. Also, to relate those aspects to Pb fractionation in the rhizospheric and non-rhizospheric soil portions. Those stages were chosen because the first is the turning point between the growth stage and the reproductive stage, being also the maximum point in the growth of the plant. The second stage is when the crop completes the reproductive phase and is ready to be harvested. An additional objective is to assess potential agricultural risks.

2 Materials and Methods

2.1 Soil and Crop

The soil sample used in the present study was taken from the A horizon of a Vertic Argiudoll (U.S.D.A. Soil Taxonomy), from the Solis area in Buenos Aires Province, Argentina (34°18′ S, 59°20′ W); sampling and soil characterization details have been given in a previous investigation (Ferreyroa et al. 2014). This area is known to be a non-polluted one (Lavado et al. 2004). This soil has a silty loam texture, high organic matter (OM) content, low cation exchange capacity, and is moderately acid (Gonzalez et al. 2013). Main soil features are presented in Table S1 (Electronic Supplementary Material, ESM).

The spring rapeseed variety Legacy was employed. The seeds have not received any treatment prior to sowing. The phenological development and ontogenetic stages were described following the scale of Miralles et al. (2003).

2.2 Shelter Experiment Setup

The experiment was carried out in a shelter between late autumn and early spring, under environmental conditions of temperature, humidity, and light (Table S2, ESM, shows the temperature minimum, maximum, and mean values); the shelter isolated the plants from rainfall but allowed exposition to ambient temperature. It was performed in 3-L plastic pots. The pots were fitted with a rhizopot device to separate the rhizosphere (Rhi) soil portion from the non-rhizosphere (bulk, NRhi) part (Silva Gonzaga et al. 2006). Soil portions containing different lead concentrations were used to fill the pots and their corresponding rhizopots (up to 2 kg total soil mass) (Fig. S1, ESM). The soil was air dried at room temperature, and appropriate amounts of $Pb(NO_3)_2$ solutions were added immediately before assembly of the pots, in order to obtain the desired concentrations. The treatments were 0, 50, 100, and 200 mg Pb kg^{-1} soil, namely Pb-0 (control), Pb-50, Pb-100, and Pb-200, respectively, which lie in the range commonly found in polluted soils. No fertilizer was added. Seven pots per treatment were prepared; the experiment had a completely random design.

Four seeds of *Brassica napus* were sown per pot at 1-cm depth approximately. All pots were watered with distilled water according to the water demand of the plants. Soil water content was maintained below 80% of soil Field Capacity, avoiding possible metal losses by leaching. When the seedlings reached four leaves were thinned, leaving one plant per pot.

Two harvests were performed. The first harvest took place around 100 days after sowing, at flowering (FL) stage, harvesting three replicas. The second harvest was performed around 140 days after sowing, at physiological maturity (PM) stage. Four replicas were harvested at this stage. Rhi and NRhi soil portions and plant organs were collected. The soil samples were dried in an oven at 70 °C for 72 h.

2.3 Soil Extracts and Determinations

Soil pH was measured on three replicas after harvesting, at the two stages considered, for both Pb-0 and Pb-200 treatments, in water (pH_w) and 1.0 M KCl (pH_{KCl}) following the usual procedure (Thomas 1996). Total lead concentration was determined for Pb-200 treatment in Rhi and NRhi soil, on three replicas at FL stage and four replicas at PM stage. The metal distribution in the soil fractions for the Pb-0 and Pb-200 treatments was analyzed by SSE according to a modified Ma and Uren procedure (Ma and Uren 1998; Ferreyroa et al. 2014). Three replicas were analyzed for the FL and PM ontogenetic stages. For the purposes of the present work, the steps of sequential extraction were limited to three, operationally defined as presented in Table 1. The extraction was conducted in sixfold for each replica. Lead

Table 1 Selective sequential extraction method employed

Fraction	Description	Treatment
I	Soluble and exchangeable Pb(II) (Ex)	$MgCl_2$, 1.0 M, pH 7 1 h shaken
II	Pb(II) bound to oxidisable soil components (mainly organic matter) (MO)	Digestion with 30% H_2O_2 in 0.5 M CH_3COONa/CH_3COOH buffer at 80 °C. Five milliliters H_2O_2 portions until end of reaction, then 1 h shaking
III	Pb(II) associated to mineral fraction and residual (MI)	Microwave digestion with H_2SO_4 (conc.) + $HClO_4$ (conc.) + HNO_3 (conc.) + HF (conc.)

concentration was determined by atomic absorption spectrometry (AAS) with air/acetylene flame using a Shimadzu AA 6800 (Shimadzu, Japan) after microwave acid digestion (Wright and Stuczynski 1996), DL 0.1 mg kg^{-1}. A closed microwave oven CEM MDS 2000 (CEM, USA) was employed for mineralization when required. The instrumental conditions for AAS and the programs employed for soil digestion were those provided by the respective manufacturers.

2.4 Measurements of Plant Biomass and Analysis of Lead Concentration

Plant biomass was analyzed in all treatments. At FL ontogenetic stage, three plants of *Brassica napus* were sorted into roots, stems, leaves, flowers, and pods. At PM stage, four samples were sorted as before except for grains instead of pods. All samples were washed with ultrapure water, and roots, particularly, were vigorously washed with tap water and then extensively rinsed with ultrapure water; care was exercised to remove all soil particles attached to the roots. The samples were oven-dried at 70 °C to constant dry weight, smashed, homogenized, and analyzed.

Lead concentrations in roots, stems, and leaves (at the two ontogenetic stages), and pods (at FL stage) or grains (at PM stage) were determined as milligrams Pb per kilogram biomass. Three replicas were performed (as long as biomass was available) for each organ at FL stage, and four replicas at PM stage. One gram of each sample was digested with 20 mL HNO_3(c) and 10 mL 30% H_2O_2 on a plate heater at 100 °C, adding more reactants as needed until no more reaction was observed; the resultant solutions were filtered with 1-μm pore PTFE membranes (USEPA 3050B method). The analysis was performed by duplicate, and the Pb concentration was determined using a Perkin-Elmer 3110 atomic absorption spectrometer with air/acetylene flame, DL 0.3 mg kg^{-1}. From these data, the bioconcentration factor was calculated as described in the following section.

2.5 Bioconcentration and Translocation Factors

In this study, the bioconcentration factor (*BCF*) (Tu et al. 2002) is defined as a measure of the absorption capability of the organism (*B. napus*) and was calculated as the ratio of Pb concentration in plant tissue to that in soil, both for total plant biomass and for the studied organs

$$BCF = \frac{C_B}{C_{TS}} \quad (1)$$

where C is Pb concentration (mg kg^{-1}), B stands for biomass and TS for total soil.

Also, the translocation factor (*TF*) was calculated for the aerial organs as the ratio of Pb concentration in an organ (C_{organ}) to the concentration of Pb in the root (C_{root}):

$$TF = \frac{C_{organ}}{C_{root}} \quad (2)$$

2.6 Statistical Analyses

Descriptive statistics was performed using GraphPad Prism (version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). ANOVA, TW-ANOVA, and General Linear Mixed Models (GLMM) were employed for statistical analysis. The analysis of variance (ANOVA) assumptions was previously verified graphically (residual vs. fitted values). Heteroscedasticity was encountered in almost all cases, and for cases of lack of heteroscedasticity, it was modulated with VarPower method on R (The R Foundation for statistical computing, https://www.r-project.

org/foundation/). To analyze the rhizosphere effect on total lead concentration in soils GLMM using InfoStat/E (Universidad Nacional de Córdoba/Córdoba Argentina) coupled to R, and DGL post hoc test was performed. Statistical differences on fractions of SSE were analyzed with TW-ANOVA test, using InfoStat/E coupled to R. Whenever the analysis indicated significant differences, DGL post hoc test was performed. Effects of lead concentration on soils over biomass were analyzed with GLMM using InfoStat/E coupled to R, and DGL post hoc test. Lead concentration in organs of *B. napus* was compared between ontogenetic stages and between treatments, using TW-ANOVA and Bonferroni post hoc test. Moreover, *BCFs* (total and for each organ) and *TF*s (for each organ) were analyzed with GLMM, comparing treatments at the two ontogenetic stages studied with DGL post hoc test.

3 Results

3.1 Soil Pb Concentrations

For the Pb-200 treatment, at FL stage, the lead average concentration in the Rhi soil portion (236 ± 47 mg kg^{-1}, $N = 3$) was significantly higher than in the NRhi portion (149 ± 50 mg kg^{-1}, $N = 3$); similar differences were found at PM stage, wherein the Rhi portion, the lead average concentration (206 ± 38 mg kg^{-1}, $N = 4$), was higher than in the NRhi one (153 ± 8 mg kg^{-1}, $N = 4$) ($p < 0.05$). No significant differences were found between the two ontogenetic stages for both Rhi and NRhi portions.

Soil pH results showed only a few changes, as presented in Table S3 (ESM). Considering pH_w, in the absence of Pb, significantly lower values are found in the NRhi part compared with the Rhi one in both ontogenetic stages, albeit these differences are relatively low: 0.5–0.7 pH units; in the Pb-200 treatment, there are no significant differences, showing results similar to the Rhi portion in the Pb-0 case. For pH_{KCl}, the values found are lower than pH_w, as expected, but no significant differences are found in the FL stage, only a small difference for Pb-0 in the PM stage. These results suggest that the presence of Pb has a low if any effect on soil pH behavior in *B. napus* crops.

The results of Selective Sequential Extraction at both ontogenetic stages on Pb-200 soil are shown in Fig. 1 and presented in Table S4 (ESM). At the FL stage (Fig. 1a), the Pb chemical fractionation presented significant differences, with distribution in the order Ex < OM < MI for Rhi portion, and Ex < OM~MI for NRhi portion ($p < 0.01$). Pb was detected in the Ex fraction in Rhi soil. Also, Pb concentration in the MI fraction in Rhi portion was higher than in NRhi portion ($p < 0.01$). Results for the PM stage are shown in Fig. 1b; globally, the distribution of Pb on soil fractions follows the same trend than for Rhi soil on FL stage ($p < 0.01$). However, no significant differences were found between Rhi and NRhi portions at each fraction, and Pb was not detectable in the Ex fraction.

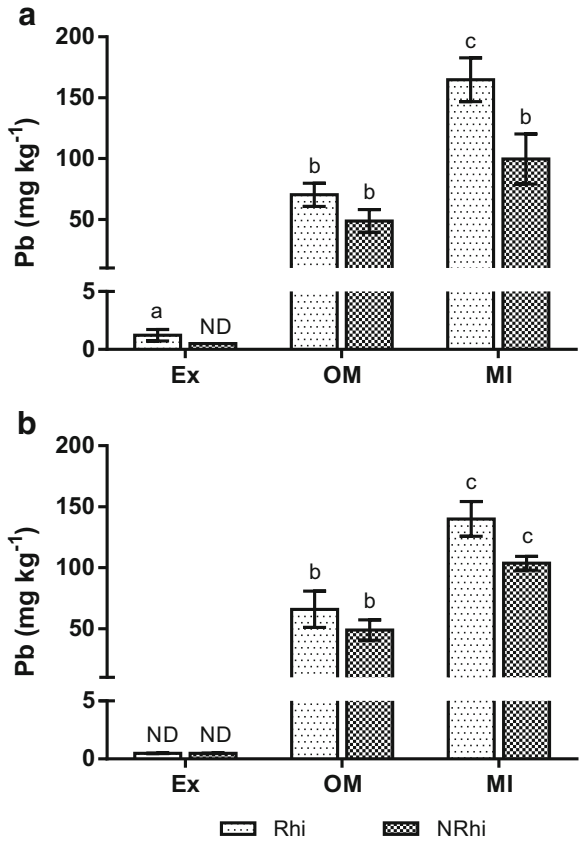

Fig. 1 Pb concentrations in soil fractions for Pb-200 treatment (mean ± SE) for rhizospheric (Rhi) and non-rhizospheric (NRhi) portions. **a** Flowering stage ($N = 18$). **b** Physiological maturity stage ($N = 18$). ND not detected. Different letters indicate statistically significant differences ($p < 0.01$). Ex soluble/exchangeable fraction, OM organic matter bound fractions, MI mineral bound fraction

3.2 Plant Biomass

Dry biomass weight of organs of harvested plants at FL ontogenetic stage is shown in Fig. 2. Treatment effects

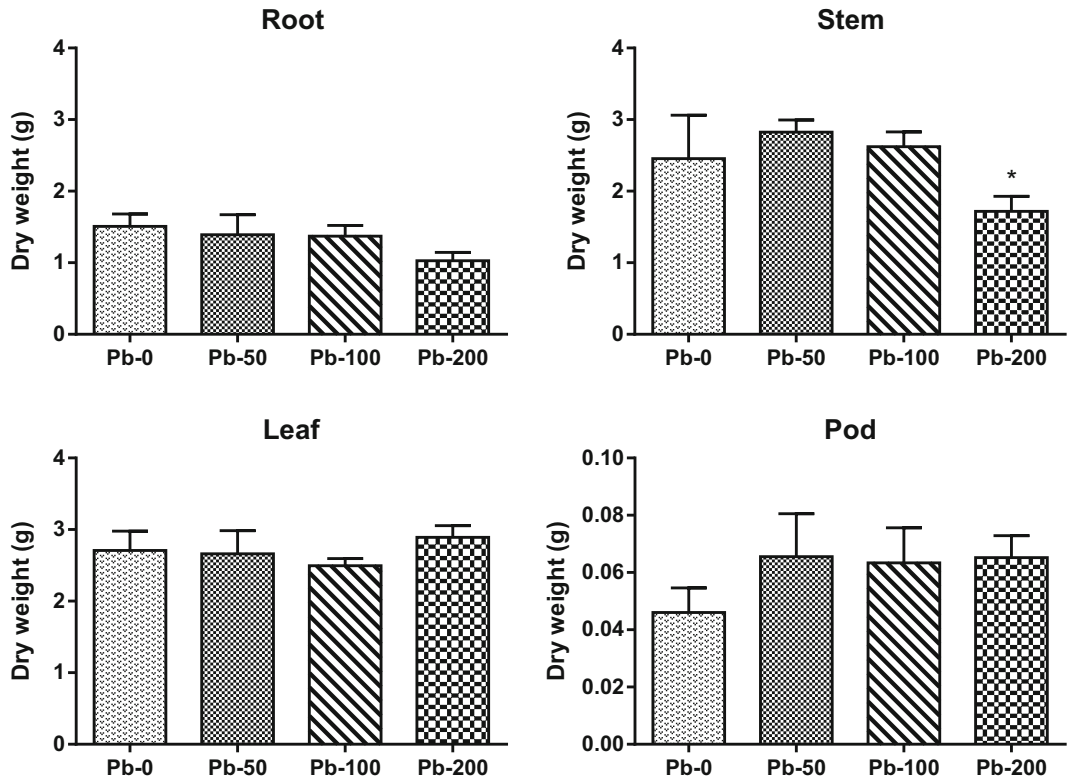

Fig. 2 Dry weight (mean ± SE) of organs of *B. napus* grown on soils with different Pb treatments, flowering ontogenetic stage: Pb-0 (control soil); Pb-50, Pb-100, and Pb-200 stand for 50, 100, and 200 mg Pb(II) kg^{-1} soil. Asterisk indicates significant differences ($p < 0.05$, $N = 12$)

were compared for each organ. Stems were the only affected organs, as the lowest weight was found on Pb-200 treatment ($p < 0.05$). For roots, leaves, and pods, no significant differences were found.

Dry biomass weights of organs of harvested plants at PM ontogenetic stage are shown in Fig. 3. No significant differences were found between treatments for roots, stems, leaves, and grains. Leaves and grains biomass values showed high variability between plants within the treatments.

3.3 Lead Concentration in Organs of *Brassica napus*

In Fig. 4, different treatments are statistically compared for each organ, at flowering and physiological maturity stages. At FL, roots of Pb-200 treatment have a higher Pb concentration than Pb-0, Pb-50, and Pb-100 treatments ($p < 0.001$). At PM stage, a higher accumulation of lead on roots was observed for Pb-100 treatment compared with control ($p < 0.05$). No significant differences were found between treatments for stems and leaves at any ontogenetic stage.

Comparing lead concentration in roots, stems, and leaves of *B. napus*, the only significant difference was found for stems in the Pb-100 treatment: A lower concentration was detected at FL compared with PM ($p < 0.05$); for roots in that treatment, the results suggest the same difference, but the lack of replicas precluded statistical comparison. In leaves, no significant differences were found between stages.

In addition, for Pb-200 treatment, it was found that lead concentration in organs significantly increases as leaves ≈ stems < roots, at the ontogenetic stage of FL ($p < 0.0001$). At PM stage, the results suggest a similar trend for roots and stems. Also, at PM stage, lead content in grains was found (Table S5, ESM).

3.4 Lead Concentration on Total Biomass, Bioconcentration, and Translocation Factors

Mean values of lead concentrations on total biomass and of bioconcentration factors are shown in Table 2. All *BCF* results are below 1.0, especially for aerial organs (Table S5, ESM); these values reveal a low

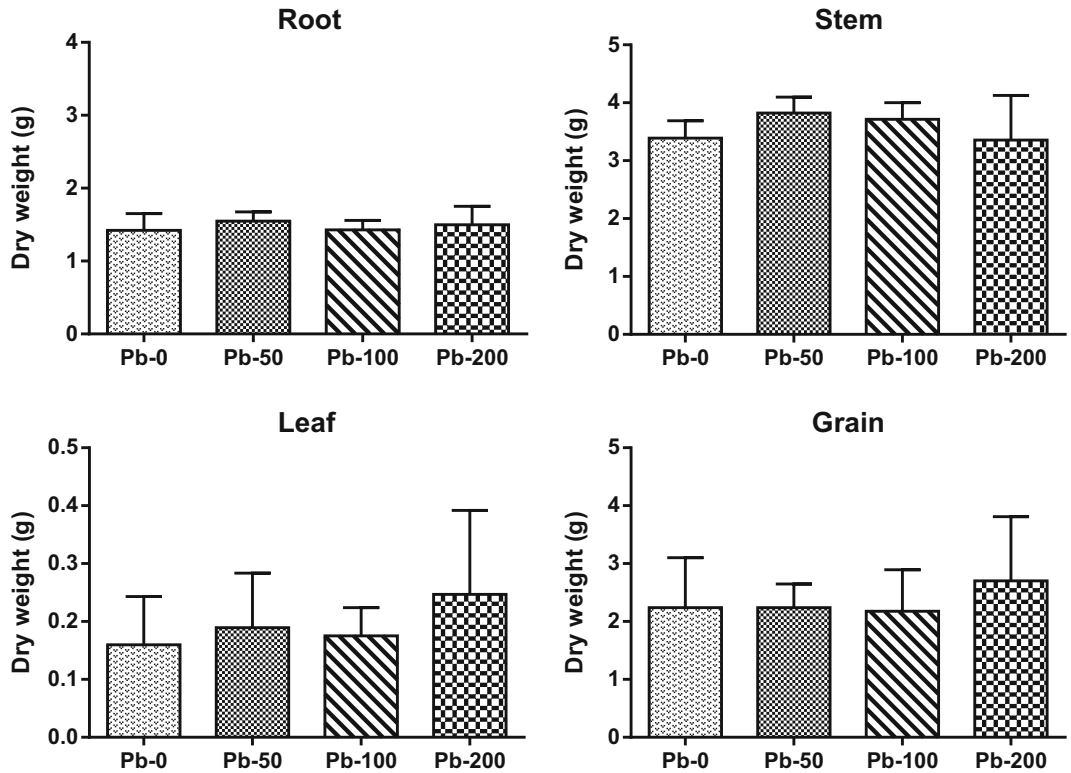

Fig. 3 Dry weight (mean ± SE) of organs of *B. napus* grown on soils with different Pb treatments, physiological maturity ontogenetic stage: Pb-0 (control soil); Pb-50, Pb-100, and Pb-200 stand for 50, 100, and 200 mg Pb(II) kg^{-1} soil. No significant differences were found ($p > 0.05$, $N = 16$)

accumulation capability of *B. napus*. Pb accumulation on total biomass at PM on Pb-100 was significantly higher than for other treatments ($p < 0.05$). It is observed that at FL stage, Pb concentration in biomass tends to increase with Pb in the soil. On the other hand, at FL stage, total *BCF* was significantly higher for Pb-50 treatment over Pb-100 and Pb-200 treatments. At PM in Pb-100, *B. napus* incorporate more lead into its biomass respect to lead in soil ($p < 0.05$), while for Pb-50 and Pb-200, the results did not differ significantly. Similar *BCF* values were reported by Yu et al. (2012).

BCFs calculated for each organ are shown in Table S5 (ESM). At FL stage, mean values for roots and leaves present statistical differences between treatments. For roots, the highest *BCF* was on Pb-200 treatment; meanwhile, for leaves, it was higher in Pb-50 treatment, whereas the lowest one was in the Pb-200 treatment. These results would suggest a differential translocation of lead from roots to aerial biomass depending on lead concentration in soils. Nevertheless, according to total *BCF*, all the *BCFs* calculated by organ were less than 1, in some cases much lower, indicating a low accumulation capacity as stated above.

There were no statistical differences between *TF* analyzed for stems, leaves, and grains at the ontogenetic stages studied (Table S5, ESM). At FL stage, the highest mean value of *TF* was 2.41 for stems on Pb-50 treatment, and 3.91 for leaves on Pb-100 treatment. At PM stage, *TF* values did not exceed 1.05 for stems on Pb-100 treatment, but they reach a mean value of 5.72 for leaves on Pb-50 treatment. For grains, *TF* value was 0.27 on Pb-200 treatment.

4 Discussion

This study shows differences in lead bioavailability between rhizosphere and bulk soil, on the culture of *Brassica napus*. Also, Pb accumulation in the plant organs at the onset of flowering and at physiological maturity ontogenetic stages is observed; particularly, lead accumulation in grains was observed in plants

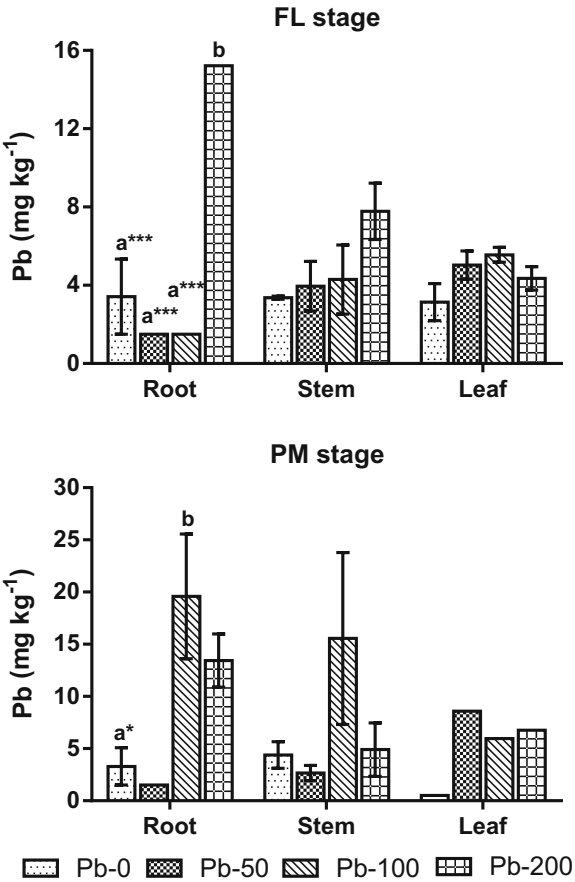

Fig. 4 Pb content (mean ± SE) in roots, stems, and leaves of *B. napus* grown at flowering (FL) and physiological maturity (PM) stages for the different treatments: Pb-0 (control soil); Pb-50, Pb-100, and Pb-200 stand for 50, 100, and 200 mg Pb(II) kg^{-1} soil. Asterisk indicates significant differences: $p < 0.05$; ***$p < 0.001$. $N = 12$. Bars without error bars indicate no replicas

grown in Pb-200 soil. Regarding metal fractionation, Pb was detected in the exchangeable fraction only at FL stage in Rhi portion.

In the previous study (Ferreyroa et al. 2014), it was found that Pb(II) incorporated into the soil as soluble Pb^{2+} evolves in a relatively short time (about 60 days) from the soluble fraction to the mineral (most stable) fraction, leaving only a small amount in the soluble and exchangeable fractions. The combined effect of plant uptake and metal evolution in soil is proposed as the cause for the absence (within the limits of detection) of the metal in the Ex fraction at the PM stage, whereas the OM and MI fractions show low changes. The Ex fraction is expected to be of high bioavailability; thus, the available Pb is expected to decrease in the period found in the previous study. Thus, this behavior explains the results obtained here: During the first ontogenetic stages, Pb uptake by the plants occurred, and effects on plants were observed. Meanwhile, the metal in soil was evolving to more stable forms, a process which coupled with plant uptake exhausted the bioavailable pollutant. A similar effect was observed by Smolders et al. (2015) in a study with soils spiked with high levels of Pb; it was found that toxicity effects decreased significantly or became absent after aging.

At both ontogenetic stages, the Pb concentration in the Rhi portion was higher than in the NRhi one. This suggests a metal migration from non-rhizospheric to rhizospheric portions, presumably due to uptake by the roots at early stages. Azimzadeh et al. (2014) reported a similar behavior on the culture of *B. napus*, finding decreased metal concentration as the distance from roots increased. This behavior was attributed to pH effects, namely a pH decrease in the rhizosphere causing an increase in Pb solubility. In the present study, increased solubility should not be the cause, since total Pb concentration in the rhizosphere is essentially equal to the initial one, even when plants incorporated Pb, and in the NRhi portion a decrease is observed. A possible

Table 2 Pb content (mean ± SE) in total biomass and bioconcentration factor (*BCF*) at the flowering (FL) and physiological maturity (PM) ontogenetic stages. Comparisons were made between treatments at the two ontogenetic stages. ND not determined. Same letter indicates no significant differences, whereas different letters indicate significant differences at $p < 0.05$

Ontogenetic stage	Treatment	*N*	Pb in total biomass (mg kg^{-1})	BCF
FL	Pb-0	3	2.64 ± 0.26 a	ND
	Pb-50	3	3.80 ± 0.70 a	0.08 ± 0.01 a
	Pb-100	3	3.88 ± 0.68 a	0.04 ± 0.01 b
	Pb-200	3	5.65 ± 0.91 a	0.03 ± 0.005 b
PM	Pb-0	4	1.04 ± 0.63 b	ND
	Pb-50	4	1.14 ± 0.21 b	0.02 ± 0.004 a
	Pb-100	3	6.37 ± 1.53 a	0.06 ± 0.02 b
	Pb-200	4	3.11 ± 0.48 b	0.02 ± 0.002 a

explanation is that in the initial growth stages, Pb(II) from the Ex fraction was complexated by root exudates, thus decreasing its free concentration and causing migration from the same fraction in the NRhi portion, in turn decreasing total Pb concentration in that fraction. Differences between both soil portions have been observed with other metals: Kim et al. (2010) found a decrease of Cd on the Rhi portion of soil with *B. juncea* crop, compared with the NRhi part, attributed to high pH and cation absorption by the plant, inducing a cation gradient through the soil.

Lead was detected at the two ontogenetic stages in roots, stems, and leaves in plants grown in control soils, with exception of leaves at PM stage. It is possible that lead was present at trace levels in the control soil but not detected with the analytical method employed. Other authors reported that even at very low concentrations of lead in agricultural soils, different species of plants (*Glycine max*, *Tagetes minuta* L., *Bidens pilosa* L.) accumulated this metal in roots, shoots, leaves, and grains (Salazar et al. 2012; Rodriguez et al. 2014; Salazar et al. 2016). Here, for the polluted soils, the root was the organ which accumulated the highest lead concentration, but the metal was also detected in leaves, stems, and grains, which indicates the translocation of the metal inside the plant. The analysis of the *BCF* values suggests that differences in lead concentrations at FL and PM stages may be due to the fact that Pb was incorporated in soil at the time of sowing, as discussed below.

Yu et al. (2012) studied the accumulation and translocation of several metals, including Pb, in rapeseed plants grown in the Yangtze River Delta, also determining the Pb concentration in the rhizosphere portion. The mean value of this concentration was 21.1 mg kg^{-1}, lower than those found here. The mean values of Pb concentration in roots, stems, and grains (leaves were not studied) at maturity (time period not reported) were 1.45, 0.416, and 0.102 mg kg^{-1}, consistently lower than the results of the present study in the PM stage; on the other hand, these results follow the order grains < stems < roots, in agreement with the present results.

During the growth of *B. napus*, a negative effect of lead in polluted soils was observed only in stems of plants grown in Pb-200 soils at flowering stage. Bilal Shakoor et al. (2014) also found a decrease in dry weight of stems and roots of *B. napus* growing in hydroponic culture with solutions of high lead concentration, after 45 days of treatment. Liu et al. (2000) investigated the growth of stems and roots in *Brassica juncea* seedlings after 15 days in solutions with increasing concentrations of Pb, obtaining evidence of a dry mass decrease in stems. These results agree with the Pb effects observed in the present study, although no previous studies have analyzed the relationship between Pb in soil and the growth of *Brassica napus* at different ontogenetic stages. The fact that at PM, the plants with Pb-200 treatment recover normal mass values could be attributed, among other causes, to detoxification via senescent leaves, as Ferreyroa et al. have described in a recent publication (Ferreyroa et al. 2017).

The present results (Table 2 and Table S5, ESM) show low lead bioaccumulation of *B. napus* in its organs in agreement with other studies (Brunetti et al. 2011; Yu et al. 2012; Azimzadeh et al. 2014). Several *Brassicaceae* species have been proposed as potential Pb accumulators, but most results reported are referred to *B. juncea* and *B. nigra* (Adams et al. 2000). Karak et al. (2013) reported, for *B. juncea* grown in soils with up to 58 mg kg^{-1} Pb and organic amendments, that after 90 days growing, the Pb concentrations in leaves were similar to those found in the present study for *B. napus* at 50–100 mg kg^{-1} Pb in the soil. Thus, *B. napus* show Pb accumulation capability comparable to that of *B. juncea*. However, the *BCF* values found here, both total and for organs, suggest a low to moderate accumulation capability, even when in some cases, the *TF* values are greater than unity, indicating effective translocation from roots to aerial organs, including grains. It has been calculated theoretically (Yu et al. 2012), based on Pb concentrations in biomass, that full removal of Pb from typical polluted soils may take hundreds of years. However, the present study strongly suggests that the removal of the bioavailable fraction can be accomplished in a much shorter time, since the major part of Pb, for soils like those analyzed here, is stabilized in the studied period of time (Ferreyroa et al. 2014); the stable Pb fractions (such as mineral forms) are expected to be not bioavailable and, in such case, would not require removal.

A further observation is the capability to translocate the pollutant to grains, as observed in this study, which raises health safety concerns, for agricultural areas contaminated with Pb. Even when no statistical differences were found, these results suggest that at low lead concentrations on soils, the translocation of the metal from root to leaves is higher and that even on small amounts, the translocation to grains is a fact. However, the present study also suggests that significant Pb uptake is to be

expected mainly in soils recently contaminated with this metal.

5 Conclusions

The following conclusions stem from the present work:

- *Brassica napus* is found to absorb lead recently incorporated in soil and translocate it to its aerial organs, including grains.
- There is a competence between plant Pb intake and metal stabilization processes in soil, leading to exhaustion of the bioavailable metal.
- Lead effects on plant growth are observed at the flowering ontogenetic stage, with recovery at the physiological maturity stage.

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