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Influence of lead on growth and nutrient accumulation in canola (*Brassica napus* L.) cultivars

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Abstract

Canola (*Brassica napus* L.) is commonly used as a hyper-accumulator for phytoextraction of heavy metals from soil and water. Like many other heavy metals, lead (Pb) contaminates soil, water and air and thus it is a great problem. This study was conducted to investigate toxic effects of Pb on growth and nutrient uptake in four canola cultivars. Each of four cultivars of canola (Con-II, Con-III, Legend and Shiralee) was subjected to four levels of Pb (0, 30, 60 and 90 mg Pb kg^{-1} of soil) from lead chloride [$PbCl_2$]. Due to Pb toxicity, plant growth was adversely affected and relatively a severe reduction in root biomass (45.7%) was recorded. The Pb accumulation increased both in shoot and root, the highest being in root. The uptake of different nutrients, *i.e.*, N, P, K, Ca, Mg, Zn, Cu and Mn was reduced (38.4, 32.8, 33.1, 49.6, 7.78, 52.0, 42.6 and 45.9%, respectively) in the shoots and that of N, Fe, Zn, and Cu in the roots (48.5, 33.2, 24.3 and 44.8%, respectively) of all canola cultivars. The root K, P, Zn and Mn and shoot P, Mg and Fe contents were less affected, the concentration of Pb, Ca and Mg in roots of all cultivars. Among canola cultivars Con-II and Con-III performed better than Legend and Shiralee in terms of growth (26.03%) and nutrient accumulation. Overall, plant growth and nutrient accumulation in the canola cultivars was hampered due to the presence of Pb.

Key words

Lead toxicity, Growth analysis, Nutrient uptake, Canola

Introduction

Although the major causes of accumulation of high levels of heavy metals in soils are a variety of man-made activities including manufacturing, agricultural, mining, and waste removal practices (Salgare, 1991; Birke and Rauch, 2000; Hussain *et al.,* 2006; Uwah *et al.*, 2009), they are also brought in due to the use of metal-enriched fertilizers and pesticides (Shaukat *et al.*, 1999; Anjana *et al.,* 2006; Nouri *et al.,* 2008; Uwah *et al.,* 2009). These metal ions dissolved in irrigation water contaminate the cultivated soils and can have toxic impact on living system, if present in excessive amounts (Nriago, 1990). Lead (Pb) is one of the most important heavy metals frequently available in the environment and its most common sources are vehicles and automobiles (Wierzbicka and Antosiewiez, 1993; Nicholson *et al.,* 2003; Sezgin *et al.,* 2003). Its exposure can occur through many pathways, *i.e.*, through inhalation of air, water, soil or dust. However, excessive Pb exposure can cause mental retardation and behavioral disorder in humans. In plants, its accumulation has been reported in stem, leaves, roots and seeds, which increases with increase in Pb levels in the growth medium (Singh *et al.*, 1998; Sekara *et al.,* 2005; Yilmaz *et al.,* 2009). It detrimentally influences plant growth (Lopez *et al.,* 2005; Wang *et al.*, 2007; Yilmaz *et al.,* 2009) by hampering a variety of physiological processes including nutrient uptake (Fodor *et al.*, 1998; Sinha *et al.,* 2006; Gopal and Rizvi, 2008).

Plant injuries caused by Pb are very frequent and drastic, especially on the rooting system, which results into severe reduction in plant productivity (Shaukat *et al*., 1999; Uveges *et al.,* 2002). Lead toxicity alters the normal metabolic pathways in plants including photosynthesis, respiration, and other such key metabolic processes by disrupting specific cellular enzymes (Dixit *et al.*, 2001; Erdei *et al.*, 2002; Ruley *et al.,* 2004). Crop yield is also reduced due to Pb-induced inhibition in metabolic processes in plants. Since most crops can frequently accumulate high levels of Pb, they serve as a source of heavy metal supply in the food of humans and animals (Khan *et al*., 2000), which ultimately causes health hazards in them.

The family Brassicaceae comprises a variety of species, which are known for their widespread uses. The brassicas are widely grown throughout the world for food, medicinal and industrial purposes. The most common Brassica seed crops grown for industrial purposes are oil-seed rape and mustard. Rapeseed stands third as an imperative source of vegetable oil after soybean and palm oil.

Increase in its production is due to the introduction of low erucic acid rapeseed varieties (Sovero, 1993; Barthet, 2008). The rapeseed canola is being widely cultivated in many countries of the world, because of its high quality oil. However, its productivity is severely affected due to the presence of high amounts of toxic metals received generally through automobiles and irrigation water contaminating arable lands. Like in many other crops, it is expected that different cultivars of canola may differ in Pb transport and accumulation in different organs and tissues, and this differential Pb transport may antagonistically affect the uptake and accumulation of other essential inorganic nutrients. Thus, the present studies were aimed to assess the pattern of nutrient accumulation including that of Pb from root to shoot in four elite canola cultivars and its effects on the growth of the cultivars.

Materials and Methods

The study was conducted in a net-house in the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan, in plastic pots (diameter 27 cm, depth 24 cm) containing sandy loam soil having 30% saturation, 1.25 dS m^{-1} electrical conductivity (EC) and 8.3 pH. The soil sample collected from the main farm of NIAB was chemically analyzed using the methods described by Jackson (1962). Basal fertilizers *i.e.* NPK applied were: 50 mg P_2O_5 kg^{-1} and 50 mg K_2O kg^{-1} as diammonium phosphate (DAP) and sulphate of potash (SOP), while N was applied at the rate of 100 mg N kg^{-1} of soil.

All the treatments of the experiment were set up in a completely randomized design with a factorial arrangement. During the experiment, temperature 25 ± 2°C and relative humidity (RH), 58-70% were recorded. The whole experiment comprised four Pb treatments *i.e.* 0, 30, 60 and 90 mg Pb kg^{-1} of soil applied to 30-day old seedlings replicated thrice and four canola cultivars *i.e.*, Con-II, Con-III, Legend and Shiralee. The salt as a source of lead used was $PbCl_2$. The plants were watered regularly with good quality irrigation water having EC 0.973 dS m^{-1} and pH 7.73 with no Pb in it.

The plants were allowed to grow in the net-house for further 40 days after which time they were harvested. All plants from different treatments were separated into roots and shoots. Then both shoot and root samples were washed with distilled water and placed in a forced air oven for drying at 70°C for 72 hr. The fresh and dry biomass of both roots and shoots was recorded to assess the effect of Pb on plant biomass production. Dried ground plant material (0.1 g) was digested properly using diacid (HNO_3–$HClO_4$) mixture. After proper dilution of the digests, they were processed to analyze K, Ca, Mg, P, Fe, Mn, Pb, Cu, Zn, and Cd with an atomic absorption spectrophotometer (Model NovAA-400, Analytik Jena Company, Germany) and N with micro–Kjeldhal's method (Bremner, 1965).

Statistical analysis: A two-way analysis of variance of the data for each variable was worked out using the COSTAT statistical package (MSTAT Development Team, 1989). To assess the significant differences among mean values the least significant test at $p<0.05$ was worked out following (Steel and Torrie, 1986).

Results and Discussion

Due to the application of Pb, shoot and root biomass was significantly reduced in all canola cultivars (Fig. 1). However, the most severe effect was noted on roots and shoots of plants growing under the highest level of Pb (90 mg kg^{-1} soil). The effect of Pb toxicity was more severe on roots than on shoots in all canola cultivars. In shoots, percent decrease relative to control was maximum (32%) at the highest Pb level (90 mg Pb kg^{-1} soil) followed by 21% decrease at 60 mg Pb kg^{-1} soil and 13% at 30 mg Pb kg^{-1} soil. In case of root biomass, the reduction was 62% at 90 mg Pb kg^{-1} soil, 49% at 60 mg Pb kg^{-1} soil, and 25% at 30 mg Pb kg^{-1} soil. The comparison of cultivars showed that Con-II produced maximum shoot and root biomass (13.82 and 4.74 g, respectively) while the minimum (10.1 and 3.26 g, respectively) by cv. Shiralee. Lead accumulation both in shoots and roots increased with increase in external Pb level (Fig. 1). Lead accumulation in the roots was higher than that in the shoots of all canola cultivars. The maximum Pb contents (354 mg kg^{-1} DW) were observed in roots as well as in the shoots (288 mg kg^{-1} DW) under the highest level of Pb (90 mg Pb kg^{-1} soil) followed by 319 and 229 mg Pb kg^{-1} DW of roots and shoots, respectively at 60 mg Pb kg^{-1} of soil, and 289 and 184 mg Pb kg^{-1} DW of roots and shoots, respectively at 30 mg Pb kg^{-1} of soil. However, the canola cultivars did not differ significantly in shoot or root Pb content.

Nitrogen (N), potassium (K) and phosphorus (P) contents in the shoots and roots showed a similar decreasing trend with increasing Pb concentration in the growth medium. Nitrogen concentration decreased up to 58% (37.5 to 16.75 mg N g^{-1} DW from 0 to 90 mg Pb kg^{-1} soil) in shoots and 56% (19.5 to 8.5 mg N g^{-1} DW from 0 to 90 mg Pb kg^{-1} soil) in roots. Similarly, K content decreased up to 42% (27.5 to 16 mg K g^{-1} DW from 0 to 90 mg Pb kg^{-1} of soil) in shoots and 42% (11.25 to 6.50 mg g^{-1} DW from 0 to 90 mg Pb kg^{-1} soil) in roots. While reduction in P content was 45% (2.47 to 1.35 mg g^{-1} DW from 0 to 90 mg Pb kg^{-1} soil) in shoots and 37% in roots (4.87 to 3.03 mg g^{-1} DW from 0 to 90 mg Pb kg^{-1} soil).

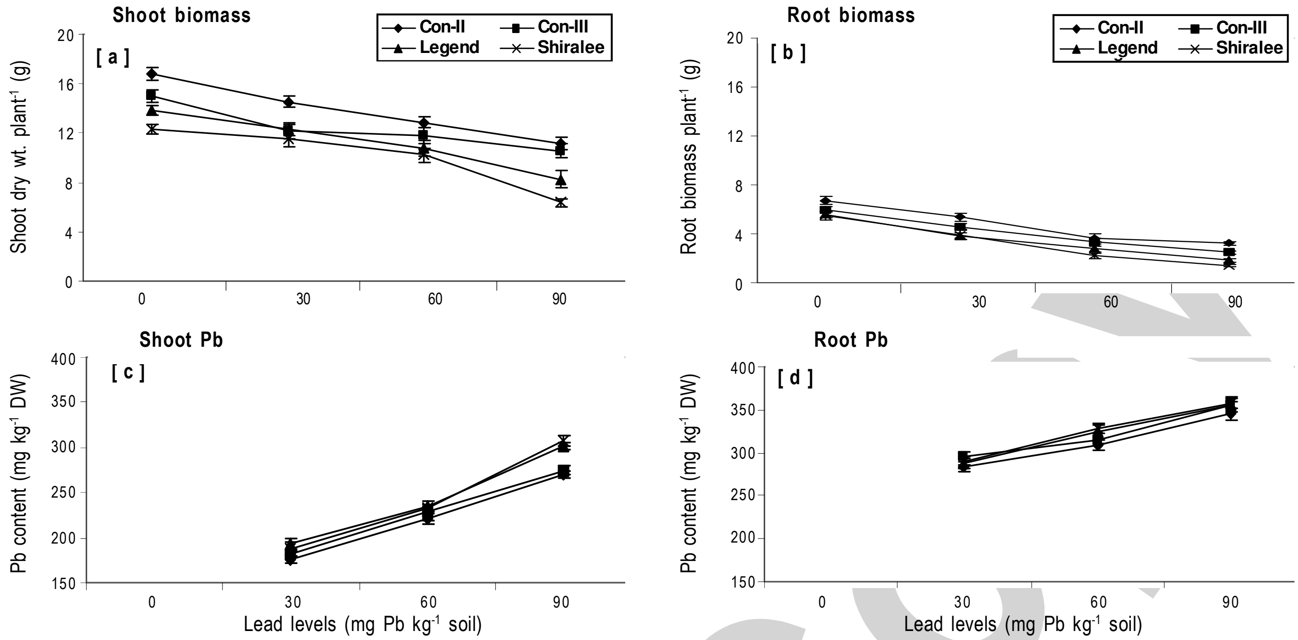

Fig. 1: Effect of Pb toxicity on shoot (a) and root (b) biomass and Pb accumulation in the shoots (c) and roots (d) of four cultivars of *Brassica napus*. Vertical bars show standard errors

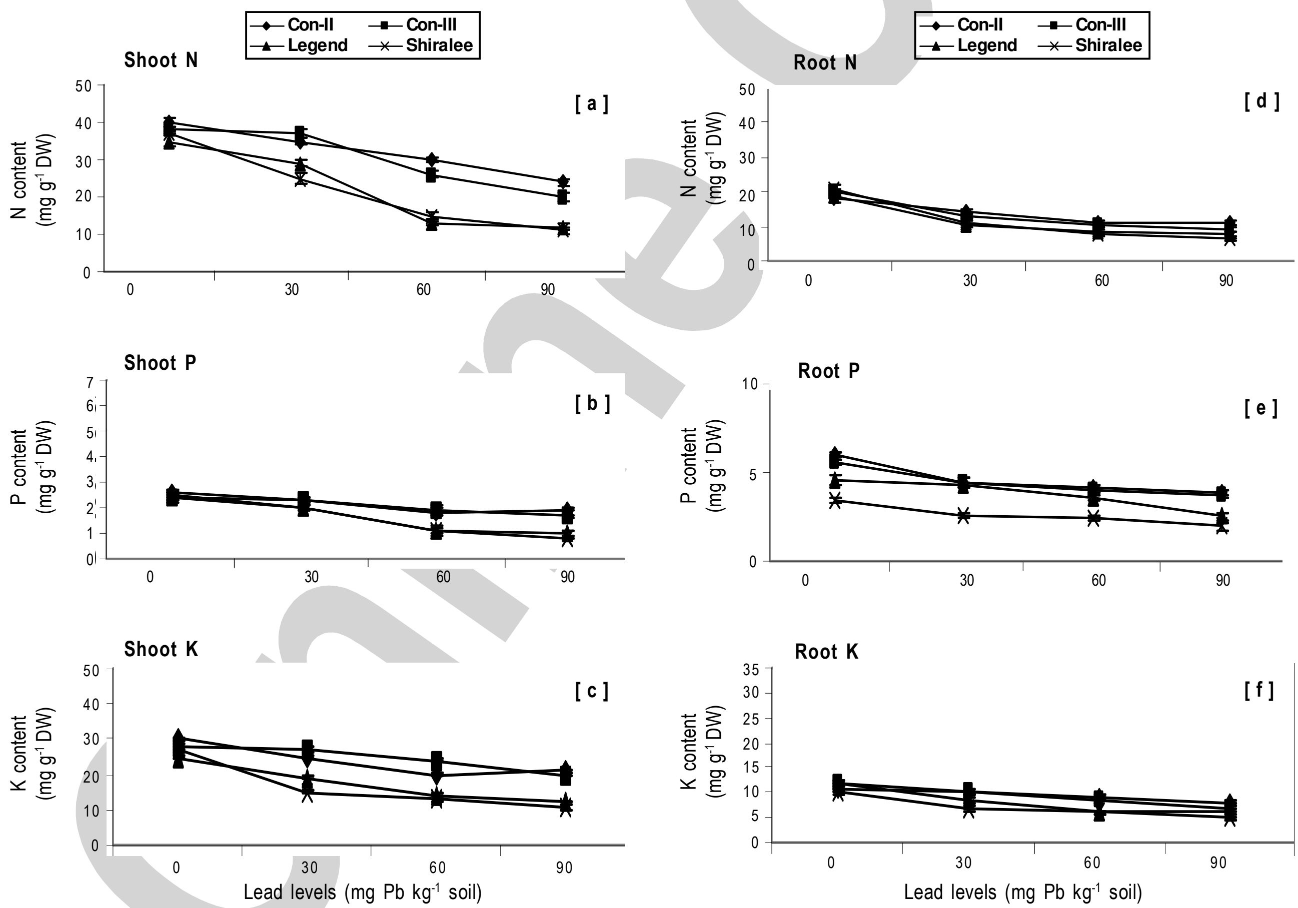

Fig. 2: Effect of Pb toxicity on N, P and K concentrations in the roots (d,e,f) and shoots (a,b,c) of four cultivars of *Brassica napus*. Vertical bars show standard errors

Phosphorus content in the roots was generally higher than that in the shoots, while N and K were higher in the shoots than those in the roots of all cultivars. All canola cultivars showed a similar response to Pb toxicity in terms of N, P and K contents that decreased with increase in Pb content in the growth medium. Overall, Con-II and Con-III maintained higher N, P and K contents than those in Legend and Shiralee under Pb toxicity.

Calcium and Mg concentrations in the roots of canola cultivars increased slightly with increase in external Pb from 0 to 90 mg Pb kg^{-1} soil (from 2.57 to 2.77 mg Ca kg^{-1} DW and 1.9 to 2.03 mg Mg kg^{-1} DW), while the reverse was true in shoots in which both Ca and Mg decreased as Pb increased from 0 to 90 mg Pb kg^{-1} of soil *i.e.* 2.29 to 0.94 and 1.67 to 1.43 mg Ca and Mg kg^{-1} DW, respectively (Fig. 3). The response of canola cultivars to external Pb levels was similar with respect to Ca and Mg in the roots and shoots. Only in case of shoot Ca, cultivars can be split into two groups, *i.e.* Con-II and Con-III maintained higher Ca content than that of Legend and Shiralee.

Iron, Zn, Cu and Mn in the shoots and roots of all canola cultivars were reduced due to varying soil Pb levels (Fig. 4, 5). Fe concentrations in roots were highly affected (42% reduction; from 155.25 to 90.25 mg Fe kg^{-1} DW) as Pb increased from 0 to 90 mg kg^{-1} soil. Decrease in Fe content of shoots was up to 16% (187.25 to 157.25 mg kg^{-1} DW) with increase in exogenous Pb from 0 to 90 mg kg^{-1} soil. However, Fe content was higher in shoots than that in the roots in all canola cultivars (Fig. 4), all canola cultivars showed a similar trend in Fe accumulation under varying Pb regimes.

Zinc concentration both in shoots and roots decreased with increase in Pb levels from 0 to 90 mg kg^{-1} soil, however, the roots contained more Zn than did the shoots (Fig. 4). The reduction in shoot Zn content was marked because it decreased from 206 to 73 mg kg^{-1} DW, while in roots from 207 to 129.5 mg kg^{-1} DW. With respect to Fe and Zn contents in shoots, canola cultivars can be categorized into two groups, *i.e.*, Con-II and Con-III with high Zn contents (138.0 and 134.5 mg kg^{-1} DW, respectively), and Legend and Shiralee with low Zn (116.75 and 114.0 mg kg^{-1} DW, respectively). However, cultivar difference with respect to root Zn content remained non- significant.

Copper levels in the roots and shoots of all cultivars decreased with increase in Pb contamination in the growth medium; more pronounced reduction was recorded in shoots where Cu contents decreased from 11.23 to 4.87 mg kg^{-1} DW than that in the roots (8.86 to 3.77 mg kg^{-1} DW) when plants were exposed from 0 to 90 mg Pb kg^{-1} soil (Fig. 5a). All canola cultivars showed a similar decreasing trend in Cu concentration under Pb toxicity and variation among them was non-significant.

A considerable reduction in Mn contents of both shoots and roots was recorded in all canola cultivars due to increased Pb level in the growth medium. Roots of all cultivars contained higher amount of Mn and showed less reduction in Mn content than that in the shoots. The shoot Mn contents decreased from 140.25 to 45 mg kg^{-1} DW, they were from 155.5 to 112.5 mg kg^{-1} DW in roots when subjected to 0 to 90 mg Pb kg^{-1} soil. All four cultivars showed a similar behavior to Pb toxicity in terms of Mn contents in the roots and shoots. In case of shoots, all cultivars exhibited almost similar values (90.5, 88.5 and 88.25 mg kg^{-1} DW in Con-III, Shiralee and Legend, respectively) for Mn except Con-II in which Mn contents (102.25 mg kg^{-1} DW) were found maximum. With respect to root Mn contents, the cultivars varied non-significantly, however, these were higher than those of shoots.

In the present study, root and shoot biomass was reduced due to Pb toxicity in all four canola cultivars. However, the decrease in root biomass was more marked than that in shoot biomass. It is now widely known that heavy metals in soil or water can adversely affect the growth and ionic concentration in different tissues of plants (Sanchez *et al.,* 1999; Sharma and Sharma, 2003; Ali *et al.*, 2009; John *et al*., 2009). The severe reduction in root growth in the canola cultivars might have been due to Pb-induced impaired nutrient uptake and metabolism in plants (Wensheng *et al*., 1997; Panda and Choudhary, 2005). As the roots contained higher amount of Pb than that of shoots, which might have antagonistically disturbed the uptake of essential nutrients and water absorption resulting into reduced root growth, as observed in the present investigation.

The nutrient contents in the shoots and roots were imbalanced by the high quantity of Pb present in the soil. Essential macro- nutrients like N, P and K in the shoots and roots decreased with increased levels of Pb from 0 to 90 mg Pb kg^{-1} soil, but reduction in K and N was more marked in the roots of all the cultivars. This clearly shows that Pb presence in the growth medium inhibited the absorption of N and K as already observed in sugar beet (Larbi *et al.,* 2002), and rice (Yang *et al.,* 2004). As these both elements are mobile so whatever quantity of K and N is absorbed by the roots, it is ultimately translocated to the shoot, because these nutrients are required in large amount to maintain the plant metabolic activities (Kalita and Sharma, 1995; Mamta *et al.*, 1997; Kaya *et al.,* 2003; Akram *et al.*, 2009a;b). Although P content decreased significantly in both shoots and roots, the roots accumulated more amount of P than that of shoots. This clearly shows that P translocation from the roots to the shoots was severely affected as previous had been observed in Rhodes grass (*Chloris gayana*) and signal grass (*Brachiaria decumbens*) (Kopittke *et al.*, 2007). It has been earlier reported that nutrient uptake by roots also depends on the plasma membrane selective properties (Gussarsson, 1994). Thus Pb may perturb the normal uptake of nutrients by changing the permeability of the plasma membrane as well as by affecting all the processes involved in nutrient transport across the membrane (Gussarsson, 1994). From the results of the present investigation it can be suggested that Pb toxicity might have damaged the plasma membrane of root cells thereby altering its permeability which might have affected P uptake by roots. The results also show that P concentrations in the shoots were less

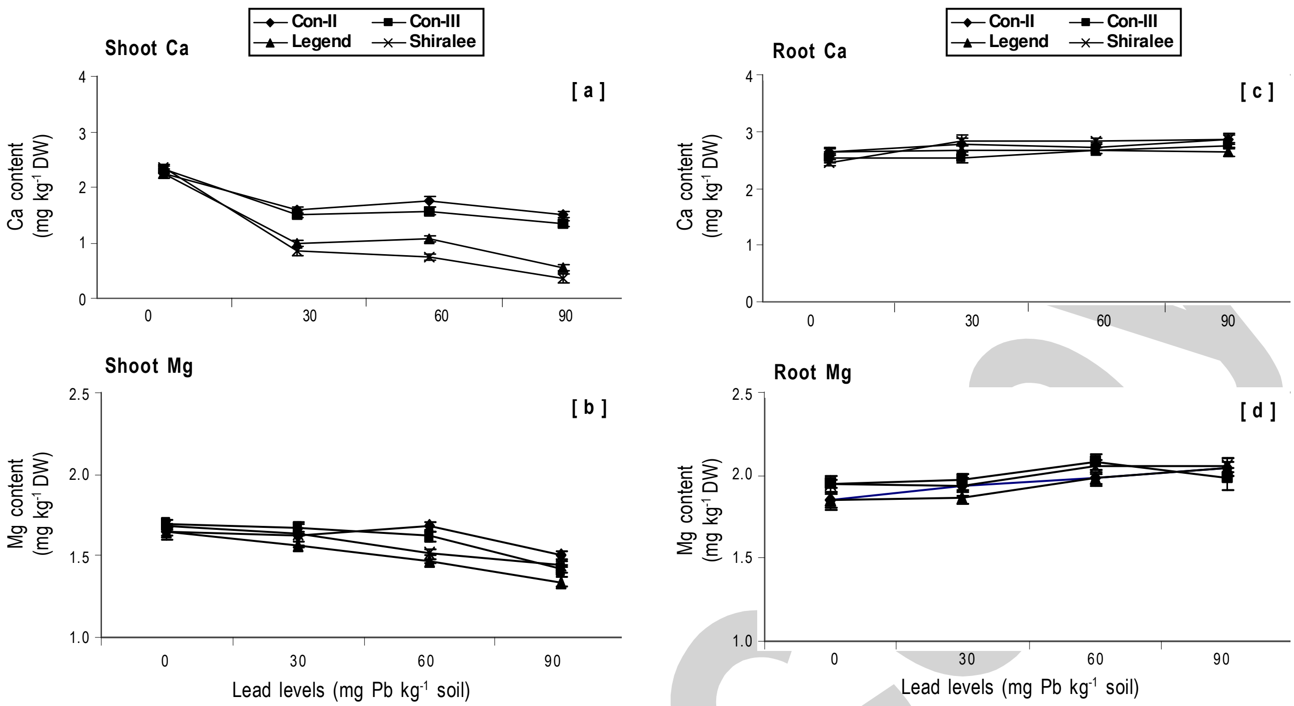

Fig. 3: Effect of Pb toxicity on Ca and Mg concentration in the roots (c, d) and shoots (a, b) of four cultivars of *Brassica napus*. Vertical bars show standard errors

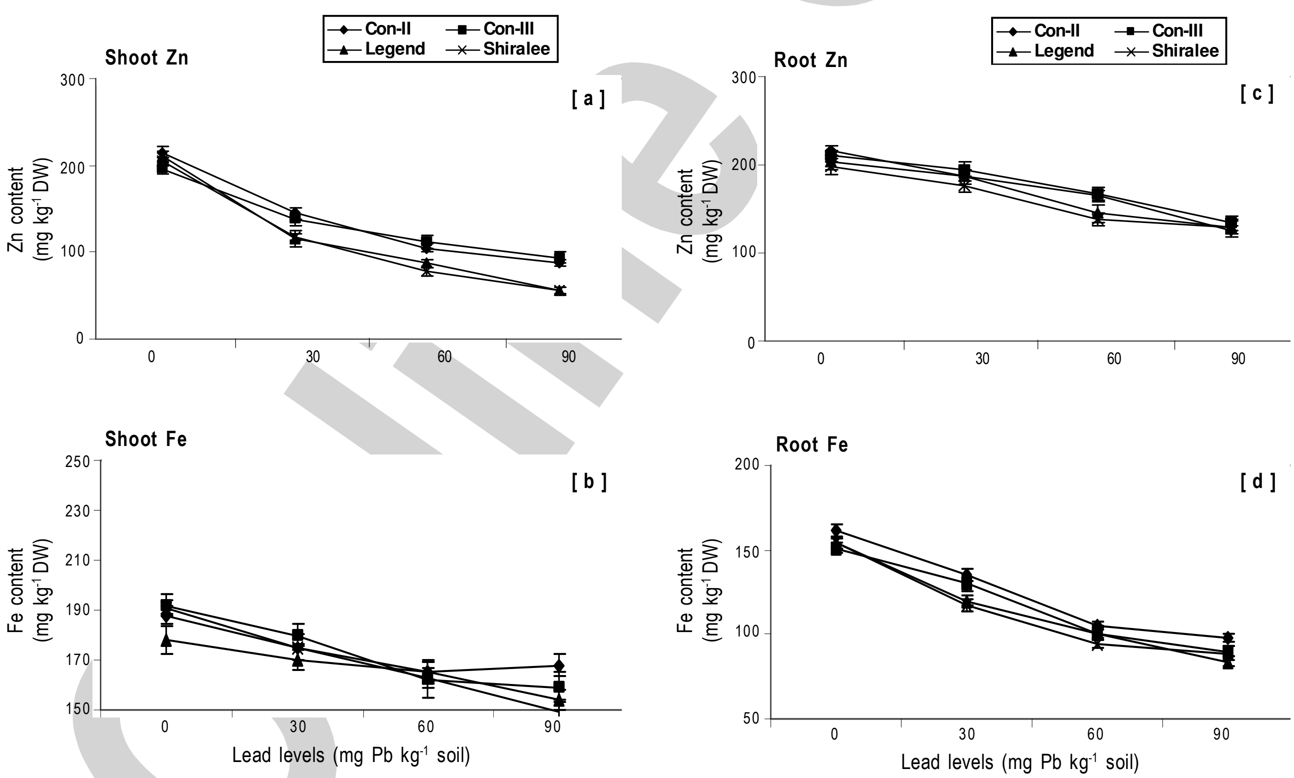

Fig. 4: Effect of Pb toxicity on Zn and Fe concentrations in the roots (c,d)and shoots (a,b) of four cultivars of *Brassica napus*. Vertical bars show standard errors

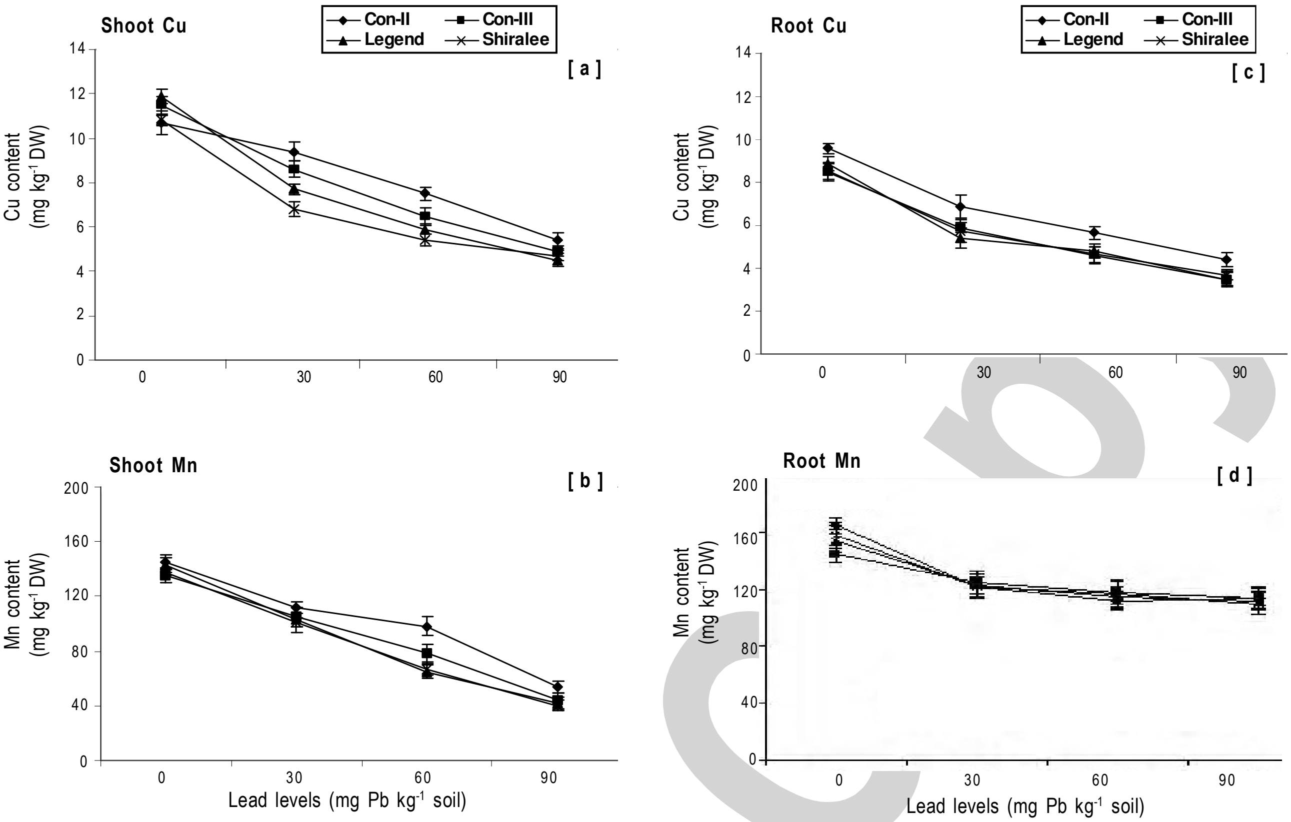

Fig. 5: Effect of Pb toxicity on Cu and Mn concentration in the roots (c, d) and shoots (a, b) of four cultivars of *Brassica napus*. Vertical bars show standard errors

affected than those in the roots, which could have been one of the reasons less effect of Pb on shoot growth than on root growth in the canola plants.

Calcium (Ca) and Mg concentrations in the roots were increased slightly due to increase in external Pb from 0 to 90 mg kg^{-1} of soil, while their amount was significantly decreased in the shoots of all canola cultivars (Fig. 3). Since Ca is an integral component of cell wall as well as it is usually bound to the exterior surface of the plasma membrane, it plays a vital role in maintaining the integrity of both cell wall and membrane (Ashraf *et al.*, 1992; Wensheng *et al*., 1997). Sanchez *et al.* (1999) and Kopittke *et al.* (2007) were of the view that presence of high amount of Ca in the cell wall is important so as to supply the plasma membrane with a reasonable amount of Ca to keep its structure intact. An increase in Ca concentration under Pb contamination in the roots could be a putatitve mechanism of minimizing the toxic effects of Pb and a decrease in Ca concentration in the shoots under Pb toxicity may have been an indication of a damaged intercellular defense system. Similar results have been reported by Rengel (1997) which showed increased Ca contents in the roots and decreased in the shoots due to high levels of Pb in the growth medium. Magnesium contents decreased in the shoots while they increased in the roots due to Pb application. This suggests that uptake of Mg was not affected due to Pb present in the growth medium, however, its translocation to the shoots was inhibited. The reduction in shoot Mg might have been one of the factors for causing reduction in chlorophyll content in almost all the canola cultivars (Jayaganesh and Venkatesan, 2010).

Iron (Fe) was less affected in the shoots than that in the roots in all canola cultivars which suggests that the Fe absorption by the roots was severely inhibited by the Pb toxicity and whatever amount of Fe was absorbed by the roots it was translocated to the shoots. Although Cu and Mn contents were affected by Pb toxicity both in shoots and roots, they were less affected in the roots, which means that Pb contamination in the growth medium inhibited their absorption more severely than their translocation to the shoots. These findings are in agreement with those of Sayyed *et al.* (2009) in safflower (*Carthamus tinctorious*) and wheat (*Triticum aestivum* L.) plants subjected to Pb, Cd, Cu, and Zn contamination and showed that heavy metal treatment decreased metal translocation from the roots to the shoots of the plants of these two crops. Furthermore Vandecasteele *et al.* (2005) reported that high levels of metal in aerial plant parts could be due to restricted root growth.

Zinc concentrations in the roots and shoots of canola cultivars decreased significantly as Pb increased from 0 to 90 mg kg^{-1} soil. However, Zn levels in the roots seemed less affected by Pb toxicity (Fig. 4), which indicates that although Zn absorption in the roots was adversely affected by Pb, its translocation to the shoots was severely inhibited. Such differential patterns of Zn uptake and translocation have been reported in different plant species such as maize and *Thlaspi caerulescens* (Lombi *et al.,* 2001).

In the present investigation, growth of all canola cultivars and nutrient uptake and the translocation into shoots was adversely perturbed in all the four cultivars when exposed to varying levels of Pb.

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