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Effects of soil-applied lead on growth and partitioning of ion concentration in *Spinacea oleracea* L. tissues

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Abstract

A pot experiment was conducted to study the effects of lead (Pb) on growth and nutrient concentration in Spinacea oleracea L. The levels of Pb used in the study were 0, 20, 40, 60, 80, 100 mg kg^{-1}. Lead application in soil caused a gradual decrease in dry weight of both the shoot and root of S. oleracea with increasing Pb level. Potassium concentration in both shoots and roots, and magnesium, zinc and iron concentration in roots of S. oleracea decreased with Pb application. On the contrary, Pb application significantly increased nitrogen, magnesium and manganese concentration in shoots as well as calcium and manganese concentration in roots. Phosphorus concentration neither in shoots nor in roots was affected by Pb application.

Key words: Lead, growth, ion concentration, *Spinacea oleracea*

Introduction

Lead pollution in Bangladesh soils has increased because of increased disposal of municipal and industrial solid and liquid wastes, vehicle exhausts to the soils (Kibria, 2008; Kashem and Singh, 1999). Lead is a toxic element that can be harmful to plants, but plants usually show the ability to accumulate large amounts of Pb without visible changes in their appearance or yield (Kibria *et al*., 2006, 2007). Heavy metals when present at an elevated level in soil are absorbed by the root system, accumulate in different parts of plants, reduce their growth and impair metabolism (Seregin and Ivanov, 2001). Among heavy metals, Pb is the major contaminant of soil (Gratão *et al*., 2005) posing significant environmental problems (Shen *et al*., 2002), including the risk of poisoning for humans and especially children (Lasat, 2002). Lead absorption by roots from soil occurs via the plasma membrane, probably involving cationic channels such as calcium channels (Sharma and Dubey, 2005). Lead absorption is regulated by pH, cation exchange capacity of soil, organic matter contents, type of plant species as well as by exudation and physicochemical parameters (Salim *et al*., 1993; Lasat, 2002). At high Pb contents in soils, photosynthesis can also decrease due to both a lower carboxylase activity and the effects on metabolites of the carbon reduction cycle (Stiborova *et al*., 1987). As a result, the effect of Pb is to decrease plant growth and development. *Spinacea oleracea* L. (spinach) is a popular leafy vegetable in Bangladesh. It has a high nutritional value and is extremely rich in antioxidants. It is also a rich source of vitamin A, vitamin C, vitamin E, vitamin K, magnesium, manganese, folate, iron, vitamin B2, calcium, potassium, vitamin B6, folic acid, copper, protein, phosphorus, zinc, niacin and omega-3 fatty acids (Wikipedia, 2010). Peoples Republic of China is the world's largest producer of spinach with 85 % of the world out put. Per capita spinach use is strongest among Asians, highest among women 40 and older, and weakest among teenage girls (USDA, 2004). The introduction of Pb into the food chain may affect human health, and thus, studies on Pb accumulation in vegetables have gained much attention. However, research on Pb effects on macro- and micro- element content in plants is relatively scarce. The present study was undertaken to determine the effects of Pb contamination of soil on the contents of some macro- and micro- elements in *Spinacea oleracea* L.

Materials and Methods

Pot experiment

A pot experiment was carried out in net house of the Department of Soil Science, University of Chittagong in November-December, 2009 in order to study the effects of Pb on nutrient concentration in *Spinacea oleracea*. Soils were collected from agricultural field near Shahid Minar of Chittagong University from a depth of 0-15 cm. Dry roots, grasses and other particulate materials were discarded from the air dry soil and processed for pot filling. A portion of the soil passed through 2 mm sieve was retained for laboratory analyses. The soil contained 57% sand, 22% silt, 23 % clay, pH, 5.5; organic carbon, 0.83%; cation exchange capacity, 6.93 cmol kg^{-1} soils; total nitrogen, 0.086%; total phosphorus, 0.029%; total potassium, 0.24 %; and aqua regia digestible Pb, 7.8 mg kg^{-1} and Zn, 58.96 mg kg^{-1}.

Eight kilograms soil was filled in each earthen pot of 30 cm diameter and 28 cm height. Fertilizers as per BARC

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(1997) recommendations were applied: 100 kg ha^{-1} N as urea, 20 kg P as TSP (Tripple Super Phosphate), 80 kg K as MP (Muriate of Potash) and 1 kg Zn as zinc sulfate. All the phosphorus, potassium and zinc and one-half nitrogen were applied and incorporated into soil during preparation of pots. Remaining N was applied in two equal installments at 10 and 25 days after sowing (DAS). Lead as lead nitrate [$Pb(NO_3)_2$] at the rate of 0, 20, 40, 60, 80 and 100 mg kg^{-1} soil were added in pots in solution form and equilibrated for 15 days. Each treatment was replicated three times. The pots were arranged according to randomized complete block design. Healthy seeds of *S. oleracea* were sown after equilibration. After 15 days of sowing, plants were thinned to 5 plants in each pot. Care was taken to keep uniform seedlings in pots. Water was applied regularly to pots as to maintain the field capacity in soil. Plants were harvested at 45 DAS. The shoots and roots were collected separately. The roots were collected carefully and washed thoroughly first with tap water to remove adhering soil particles and then with distilled water.

Soil analysis

The particle size distribution was determined by hydrometer method of Day (1965). Soil pH was measured in a 1:2.5 soil/water suspension with glass electrode pH meter (Hanna pH 209). The potassium dichromate wet-oxidation method as described by Imamul Huq and Alam (2005) was used for the determination of organic carbon followed by multiplying the values with 1.724 to calculate the organic matter contents. The cation exchange capacity was determined by saturation with 1N NH_4OAc at pH 7.0 (Ryan *et al.*, 2001). The soil samples were digested with aqua regia (Imamul Huq and Alam, 2005) on a sand bath for the determination of total Cd, Pb, Zn, Fe, Mn, P and K. Total nitrogen was determined by micro-Kjeldahl method as described by Ryan *et al.* (2001). Phosphorus was determined by vanadomolybdo phosphoric yellow color method in nitric acid system according to Imamul Huq and Alam (2005). Potassium was measured by flame photometer (Helios γ) and Pb and Zn were determined by atomic absorption spectrophotometer (Varian spectra AA-220).

Plant analysis

Oven dried (65^{o} C to constant weight) and ground plant samples were digested with a mixture of H_2SO_4, H_2O_2 and lithium sulfate for the determination of N, P, K, Ca, Mg, Zn, Fe and Mn (Allen *et al.*, 1986). The concentration of Ca, Mg, Zn, Fe and Mn in the digest was measured by atomic absorption spectrophotometer (Varian Spectra AA 220). Micro-Kjeldahl method as described by Ryan *et al.* (2001) was used for the determination of nitrogen. Phosphorus was determined by vanadomolybdo phosphoric yellow color method in nitric acid system according to Ryan *et al.* (2001). Potassium was measured by flame photometer (Helios γ).

Data analysis

The significance of differences among the treatments was evaluated by one way analysis of variance followed by Duncan's Multiple Range Test at the significance level of 5%. The statistical software Excel (Excel Inc., 2003) and SPSS version 12 (SPSS Inc., 2003) were used for this analyses.

Results and Discussion

Growth of S. oleracea

Soil applied lead significantly affected the dry weight of shoots ($P \leq 0.05$) and roots ($P \leq 0.05$) of *S. oleracea* (Figure 1A). A gradual decrease in shoots and roots weight was recorded with increasing Pb application. However, Pb application at only 100 mg kg^{-1} soil significantly decreased both shoots and roots weight compared to that with control treatment. At the highest dose of Pb application (100 mg kg^{-1}), shoots and roots weight decreased by 41 and 37 %, respectively, compared with that of the control. The results of the present study corroborate those of Kibria *et al.* (2009) who reported that shoot and root weight of *Amaranthus gangeticus* significantly decreased when Pb was applied above 40 and 60 mg kg^{-1}, respectively, from that of the control. The decreased shoot and root biomass of *S. oleracea* might be due to interference of Pb with physiological processes of the plant. Lead phytoxicity involves inhibition of enzyme activities, disturbed mineral nutrition, water imbalance, and change in hormonal status and alteration in membrane permeability (Sharma and Dubey, 2005). These disorders upset normal physiological activities of the plant resulting in low productivity. The reduction of biomass by Pb toxicity could be the direct consequences of the inhibition of chlorophyll synthesis and photosynthesis (Chatterjee *et al.,* 2004).

Effects of Pb on ion concentration

Soil applied Pb significantly increased nitrogen concentration in shoots ($P \leq 0.05$) of *S. oleracea*, being the highest with Pb @ 100 mg kg^{-1} soil which was 14 % higher compared to that of control plant (Figure 1B). This is in agreement with Kibria *et al.* (2009) who reported that Pb application in soil significantly increased nitrogen concentration @ 26 % with Pb @ 100 mg $kg^{-1}$of soil in shoots of *Amaranthus oleracea*. But it is contrary to the results found by them with *A. gangeticus*. Orhue and Inneh (2010) also reported that Pb decreased nitrogen concentration in *Celosia argentea.* The decline in nitrogen concentration due to Pb may be as a result of moisture stress created by Pb (Burzynisky and Grabowski, 1984). However, root nitrogen content in the present study was not affected by Pb.

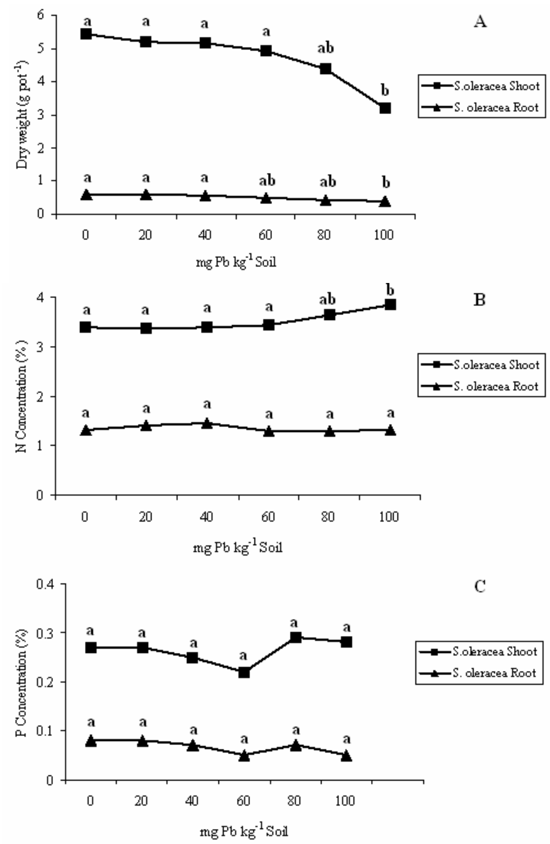

Figure 1. Effects of lead on growth (A), nitrogen (B) and phosphorus (C) concentrations in shoot and root of *S. oleracea*

Phosphorus concentration in both shoots and roots of *S. oleracea* were not significantly affected by Pb application (Figure 1C). There was also no definite trend of variation in phosphorus concentration in both shoots and roots of *S. oleracea*. Kibria *et al*. (2009) reported that phosphorus concentrations in root of *A. gangeticus* increased with higher rate of Pb (100 mg kg^{-1} soil) application in soil. On the contrary, they found that phosphorus concentrations in shoots of *A. gangeticus* and *A. oleracea* decreased with increasing rates of Pb application. Orhue and Inneh (2010) also reported that Pb decreased the uptake of phosphorus in *C. argentea*. These differences seem probably due to difference in plant species.

Lead application significantly decreased potassium concentration in both the shoots ($P \leq 0.001$) and roots ($P \leq 0.001$) of *S. oleracea* i.e. an inverse relationship between Pb and K was present (Figure 2A). The effects of Pb on K concentration were more pronounced in roots than in shoots. Potassium in shoots and roots were decreased by 27 and 54 %, respectively with the highest rate of Pb application compared to that with the control. The results of the present study are contrary to the findings of Kibria *et al.* (2009) with *A. oleracea* that might be due to difference in plant species. However, results are in conformity to those of Orhue and Inneh (2010) who reported that Pb application significantly decreased potassium concentration in *C. argentea.*

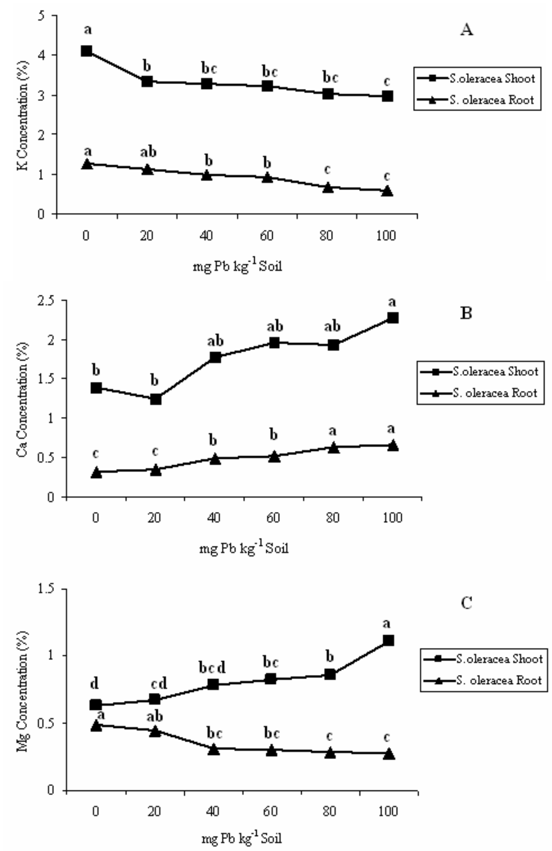

Figure 2. Effects of lead on potassium (A), calcium (B) and magnesium (C) concentrations in shoot and root of *S. oleracea*

Calcium concentration in shoots and roots significantly increased by Pb application at $P \leq 0.05$ and $P \leq 0.001$, respectively (Figure 2B). However, Ca in shoots with Pb application up to 80 mg kg^{-1} was statistically similar to that with control. A gradual increase in root Ca was recorded with increasing Pb application. At the highest rate of Pb application (100 mg kg^{-1}), Ca concentration in shoots and roots increased by 64 and 106 %, respectively over that with control. On the contrary, Kibria *et al*. (2009) reported that calcium concentration in shoots and roots of *A. gangeticus* and roots of *A. oleracea* were significantly decreased by Pb application. The decrease in Ca concentration in the roots in response to higher concentration of Pb was probably a result of osmotic adjustment (Azmat and Haider, 2007).

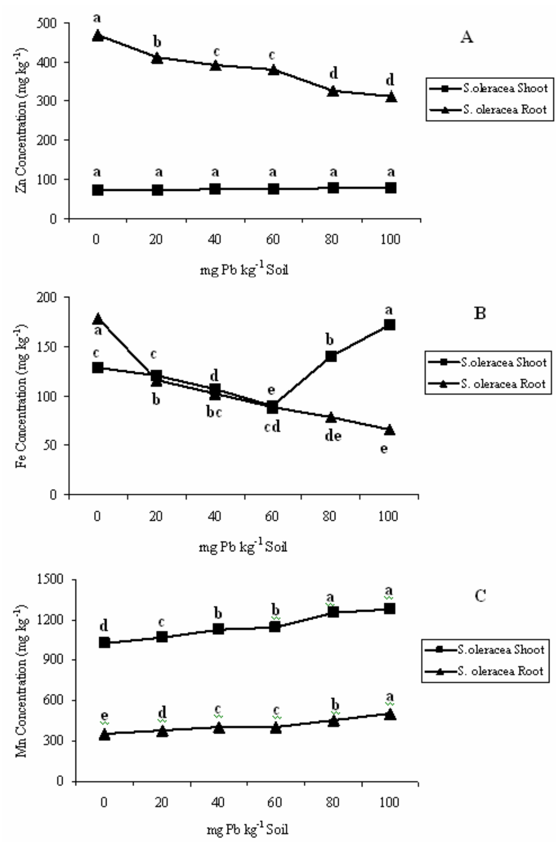

Figure 3. Effects of lead on zinc (A), iron (B) and manganese (C) concentrations in shoot and root of *S. oleracea*

Magnesium concentration in shoots of *S. oleracea* significantly increased ($P \leq 0.001$) with higher rates of Pb application (Figure 2C). Lead application up to 40 mg kg^{-1} did not affect Mg in shoots. In earlier studies, Pb application at 80 and 100 mg kg^{-1} significantly increased Mg concentration in shoots of *A. gangeticus* and *A. oleracea,* respectively (Kibria *et al*., 2009). Contrary to the shoots, Mg concentration in roots decreased significantly by Pb application ($P \leq 0.05$) in the present study. Magnesium concentration increased by 76 % in the shoots and decreased by 42 % in the roots at the highest rate of Pb application (100 mg kg^{-1} soil) over that with the control.

Lead application in soil neither affected nor showed any trend of variation in zinc content in shoots of *S. oleracea* (Figure 3A). But root Zn decreased significantly ($P \leq 0.001$) with increasing rates of Pb application showing a negative relation between Pb and Zn. Other workers also found negative effects of Pb on Zn concentration in plant parts. For example, Kibria *et al*. (2009) showed that Zn in shoots and roots of *A. gangeticus* and roots of *A. oleracea* decreased significantly with increasing rates of Pb application in soil. Zinc concentration in roots of *S. oleracea* decreased by 33% at the highest rate of Pb application (100 mg kg^{-1} soil) compared to that with the control in the present study.

Iron concentration in shoots of *S. oleracea* significantly decreased ($P \leq 0.001$) by Pb application up to 60 mg kg^{-1}of soil and then increased over control (Figure 3B). But Fe in roots gradually decreased over control with all the rates of Pb application and the lowest value was obtained with the highest rate of Pb. In contrast with the results of the present study, an increase in Fe concentration in roots of *A. gangeticus* and *A. oleracea* with increasing rates of Pb was reported by Kibria *et al*. (2009).

Lead application in soil significantly increased manganese concentration in both the shoots and roots ($P \leq 0.001$) of *S. oleracea* (Figure 3C). A gradual increase in Mn concentration in both the plant parts was observed with increasing rates of Pb application. At the highest rate of Pb application (100 mg kg^{-1} soil), Mn in shoots and roots increased by 24 and 42 %, respectively compared with that of the control. Our results are contrary to Kibria *et al*. (2009) who reported that Mn in shoots and roots of *A. oleracea* and roots of *A. gangeticus* decreased significantly with an increase in Pb application (up to 100 mg kg^{-1}) with the lowest values being obtained with the highest rate.

Significant changes in nutrient contents occurred in *S. oleracea* tissues under Pb treatments. The decrease in the levels of mineral elements in the Pb treated plant tissues may be due to the physical blocking of mineral ions from absorption sites of roots as earlier reported by Godbold and Kettner (1991). The Pb may also have caused direct damage to the tissue cells of vascular bundles (Azmat *et al*., 2006; Pahlsson, 1989) resulting in the inhibition of conduction of water molecules from root (Eun *et al*., 2002) to aerial part of *S. oleracea* hence reduction of the plant nutrients. Two mechanisms for decreased absorption of macro and micronutrients under Pb toxicity have been suggested. The first mechanism, termed physical, relies on the metal ion radii, whereas the second mechanism, which is a second one, relies on the metal-induced disorder in the cell metabolism leading to changes in membrane enzyme activities and membrane structure. It is presumed that the increase of some nutrient ions in plant tissues might be due to synergistic effects of Pb with those ions via diverse mechanisms that could be understood. Further research should be done for the understanding. However, the observed action of Pb appear to be indirect as a result of mineral imbalance within the tissues of *S. oleracea* bringing significant changes in nutrients in the plant under Pb treatments.

Conclusions

Lead application in soil decreased both shoot and root biomass of *S. oleracea*. Lead application also depressed some mineral nutrients especially K in *S. oleracea*. Protein synthesis and its content in *S. oleracea* would be decreased by reduction in absorption of K ions in shoots and roots in Pb contaminated soil.

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