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Phosphate effect on the content of selected elements in a lettuce variety grown at a contaminated soil

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Abstract The purpose of this study was to evaluate the efficiency of superphosphate fertilizer in remediating a contaminated soil with potentially toxic elements. For this, different phosphorus doses were used in a number of lettuce plants. The element concentrations determined in their leaves were compared with those found in control lettuce plants. Instrumental neutron activation analysis was the analytical technique used to determine element concentration in lettuce leave samples. The application of 250 mg kg^{-1} of P was the most effective treatment to reduce the concentrations of Br, Ca, Cd, Cl, Co, Cr, Fe, K, Mg, Mn, Sb and Zn in lettuce leaves.

Keywords Neutron activation analysis · Inorganic elements · Contaminated soil · Lettuce leaves

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Introduction

In recent decades, anthropogenic activities particularly those associated with industrial processes and mining have been the major sources of inorganic element enrichment in soils [1]. However, several research projects have shown that some procedures normally used to improve the physical and chemical conditions of soils such as application of fertilizers, limestone and pesticides can also increase the element concentrations in the soil.

Boron, chlorine, copper, iron, manganese, molybdenum, nickel and zinc in soil are micronutrients for plants. However, if these same micronutrients are found in high concentrations they can become toxic to plants. Of the 92 natural existing elements, 22 are known to be essential for human beings and animals. Besides these, 25 others are detected in human and animal bodies. Most minerals found in the body, essential and nonessential, present high chemical and biological reactivity, particularly when they are ions, radicals or organic complexes. Thus, an essential trace element might be a contaminant when found in foods above nutritionally desirable levels [2]. For McBride [3], the elements arsenic, beryllium, antimony, cadmium, chromium, cooper, lead, mercury, nickel, selenium, silver, thallium, and zinc are considered potentially harmful to human health depending on their concentrations. Unlike organic contaminants, most inorganic elements do not undergo microbial or chemical degradation and therefore their total concentrations persist in soils for a long time after their introduction [4]. In this case, due to the possibility of elements being found at toxic levels in plants and thus reaching the food chain research to develop technologies for remediating contaminated sites has increased.

The addition of substances capable of immobilizing toxic elements in the soil is a procedure that has been used for remediating contaminated sites. The function of these substances is to reduce the mobility and bioavailability of potentially toxic elements in the soil [5, 6]. The substances commonly used for this purpose are phosphates, limestone, Fe or Mn oxides, organic materials and zeolites [7]. In the case of revegetation for example, the anion dihydrogen phosphate ($H_2PO_4^-$), with its ability to form insoluble precipitates with a variety of metals, reduces the availability of these metals in the soil and provides phosphorus for these sites usually with low concentration of this element and several other nutrients for plants.

The aim of this study was to evaluate the efficiency of phosphorus in reducing the availability of different elements in a plant grown in a contaminated soil and treated with phosphorus. Five doses of phosphorus were applied into a contaminated soil and lettuce plants were grown in. The concentration of different elements absorbed by the lettuce was then compared to the control lettuce plants with no addition of phosphorus. Instrumental neutron activation analysis (INAA) was the analytical technique used to determine element concentrations in lettuce leaves.

Table 1 Total content of elements in contaminated soil used in this study

Element	Total content[a] (mg kg^{-1})
Al	46,808 ± 2,710
As	21.4 ± 6.7
Ba	1,119 ± 355
Ca	15,661 ± 1,687
Cd	43 ± 14
Co	19.7 ± 1.2
Cr	383 ± 82
Cu	715 ± 45
Fe	154,978 ± 12,712
K	16,329 ± 417
Mg	9,677 ± 497
Mn	1,885 ± 472
Mo	30.3 ± 2.2
Na	3,050 ± 489
Ni	207 ± 62
Pb	891 ± 216
Sb	28 ± 12
Sr	1.9 ± 1.0
V	91 ± 14
Zn	7,574 ± 1,294

[a] Arithmetic mean and confidence interval at 95 % confidence level for 3 determinations

Experimental

Soil sampling and treatment for the experiment

The soil was collected from a site of 22,000 m^2, located in the city of Piracicaba, São Paulo State, Brazil. This site is under receivership of the São Paulo Environmental Company (CETESB) because of high level of contamination by potentially toxic elements, as can be observed in Table 1. Element concentrations in soil presented in Table 1 were obtained by ICP-MS technique, and its experimental procedure was described by Trevizam et al. [6].

A sample of 50 kg soil collected for this study in an area of 2 × 3 m from 0–20 cm deep, was passed through a 4 mm mesh sieve and then homogenized. Subsamples of 2 kg of soil were transferred to pots where lettuce seeds were sown. The pH of soil was 7.06 determined in 0.01 M of $CaCl_2$.

Installation of the experiment with lettuce

To assess the effect of phosphorus in reducing the availability of potentially toxic elements in soil, lettuce plants (*Lactuca sativa* L.) were grown in pots containing 2 kg of soil. The experiment was performed in a greenhouse at the Center for Nuclear Energy in Agriculture (CENA/USP), under controlled conditions of ventilation and humidity.

The experimental design was a randomized block, with six treatments using phosphorus doses of 0, 250, 500, 1000, 2000 and 4000 mg kg^{-1} of P. The P source used was $Ca(H_2PO_4)_2$, with three replicate pots for each treatment.

The soils of the pots, after receiving the P fertilizer, were incubated for 15 days under 60 % moisture content. At the end of the incubation period six seedlings of lettuce were transplanted in each pot. After 7 days, the plants were thinned to two plants per pot. Soil moisture was maintained at 70 % by daily watering with deionized water. As additional fertilizer, nitrogen was applied as ammonium nitrate at doses of 100 mg N per pot in four applications. Ten days after transplanting, 0.2 mg of boron as boric acid and 0.25 mg of molybdenum in the form of ammonium molybdate were applied in all the pots.

Lettuce leaves were collected at 70 days after transplanting, rinsed with deionized water and oven dried (at 65 °C). In order to obtain homogeneous samples for elemental determination by INAA, the dried materials were ground in an agate mortar up to fine powder form.

Multielemental analysis by instrumental neutron activation

Approximately 150 mg of dried and ground lettuce leaves were weighted in a polyethylene bag. This bag had been cleaned by leaching with a diluted HNO_3 (1:5) solution and purified water.

Preparation of standards

Certified standard solutions (Spex Certiprep) of Br, Ca, Cd, Cl, Co, Cr, Cs, Fe, K, Mg, Mn, Na, Rb, Sb, Sc, Th and Zn were used to prepare synthetic standards. Aliquots (50–100 μL) of these solutions were transferred to small sheets of analytical filter paper (Whatman number 42). After drying, these filter papers were placed into polyethylene bags for irradiation.

Irradiation and counting

Irradiations were carried out at the IEA-R1 nuclear research reactor. The thermal neutron flux utilized ranged from 1 to 4×10^{12} n cm^{-2} s^{-1}. Two types of irradiation were carried out at the IEA-R1. First, the sample and standards of Cl, Mg and Mn elements were irradiated together in a polyethylene container for 25 s. After a decay time of 3 min, ^{38}Cl and ^{27}Mg activities were measured in sample and in standards. ^{56}Mn was measured after 90 min of decay time. In the second irradiation, the sample and standards (Br, Ca, Cd, Co, Cr, Cs, Fe, K, Na, Rb, Sb, Sc, Th and Zn) were irradiated simultaneously in an aluminium container for 6 h. The ^{82}Br, ^{47}Ca, ^{115}Cd, ^{42}K, ^{24}Na and ^{122}Sb activities were measured after 4 days of decay time, while ^{60}Co, ^{51}Cr, ^{59}Fe, ^{86}Rb, ^{46}Sc, ^{233}Pa (^{233}Th → ^{233}Pa) and ^{65}Zn were measured after, at least, 8 days of decay time. In addition, analyses of certified reference materials NIST 1515 Apple Leaves was also carried out simultaneously.

The equipment used to measure the gamma radiation was a Canberra model GX2020 hyperpure Ge detector, coupled to a model 1510 Integrated Signal Processor and MCA System 100, both from Canberra. The detector used had a resolution (FWHM) of 0.9 keV for 122 keV gamma rays of ^{57}Co and 1.9 keV for 1,332 keV gamma rays of ^{60}Co.

Results and discussion

The results obtained in the certified reference material NIST SRM 1515 Apple Leaves, used for quality control, showed good agreement with the certified values, for most of elements. Cadmium was determined in the reference material by Graphite-Furnace Absorption Atomic Spectrometry (GFAAS) (Table 2). The results obtained for Cd in lettuce leaves by GFAAS were in agreement within 4–13 %, with those obtained by INAA.

Elemental concentrations determined in lettuce leaves are shown in Tables 3 and 4. Basically, Table 3 presents the elements considered essentials for plant and animal organisms, while various elements shown in Table 4 are considered potentially toxic, and some others (Br, Rb, Cs, Sc) have no known functions but may have influence on the environmental impact. Each result is the arithmetic mean of elemental concentrations obtained in samples from three different pots, according to the experimental design.

Table 2 Concentrations of Br, Ca, Cd,Cl, Cr, Co, Fe, K, Mg, Mn, Na, Rb, Sb, Sc, Th, and Zn obtained in the by INAA and Cd by GF AAS in the certified reference material NIST SRM 1515 Apple Leaves

Element, unit	This study (mean ± SD)[a]	Values of the certificate
Br, μg g^{-1}	1.7 ± 0.1	(1.8)
Ca, mg g^{-1}	13.5 ± 1.4	15.26 ± 0.15
Cd, μg g^{-1}	0.015 ± 0.004	0.013 ± 0.002
Cl, μg g^{-1}	572 ± 76	579 ± 23
Cr, μg g^{-1}	0.4 ± 0.1	(0.3)
Co, μg g^{-1}	0.10 ± 0.01	(0.09)
Fe, μg g^{-1}	78 ± 6	83 ± 5
K, mg g^{-1}	15.1 ± 0.8	16.1 ± 0.2
Mg, mg g^{-1}	2.5 ± 0.3	2.71 ± 0.08
Mn, μg g^{-1}	53 ± 8	54 ± 3
Na, μg g^{-1}	21 ± 2	24.4 ± 1.2
Rb, μg g^{-1}	9.7 ± 0.7	10.2 ± 1.5
Sb, μg g^{-1}	0.017 ± 0.002	(0.013)
Sc, μg g^{-1}	0.031 ± 0.003	(0.03)
Th, μg g^{-1}	0.028 ± 0.003	(0.03)
Zn, μg g^{-1}	12.6 ± 0.3	12.5 ± 0.3

Numbers in parentheses are information values

[a] Arithmetic mean and standard deviation from four individual determinations

For most elements, the variation coefficient values of these results were between 4 and 15 %, but in the case of Sb and Th these values were 30 and 50 %, respectively. These values represent variation within each treatment. In this case, besides the error of the analytical method, possible differences of element uptake by vegetal tissue must be taken into account, since three different samples were considered.

Analysis of variance was applied to the values of Tables 3 and 4. Tukey test [8], $p < 0.05$, was applied to verify if there are differences among element concentrations found in plants cultivated in soils with different phosphorus doses and those determined in the control plant. Statistical analysis showed that with the exception of Na, Rb, Cs, Sc and Th, all the other elements presented reduced concentration in leaves of lettuce plants cultivated using a dose of 250 mg kg^{-1} of P when compared to results obtained in leaves of control plants. Furthermore, for other phosphorus doses, the statistical test indicated an increase of element uptake comparing with the results obtained in the control samples.

Treatment with phosphate reduced by approximately 50 % the amount of Cl present in plants in relation to the control. Since Cl deficiency rarely occurs in crops, investigations on this aspect are scarce. Results obtained in this study may be attributed to the use of KCl as a source of K

Table 3 Mean concentration of Cl, Mg, K, Ca, Na, Mn, Zn and Fe in lettuce leaves as a function of treatments with superphosphate and the results of the statistical treatment

Treatment (mg kg^{-1} of P)	Cl (mg g^{-1})	Mg (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Na (mg g^{-1})	Mn (µg kg^{-1})	Zn (µg kg^{-1})	Fe (µg kg^{-1})
0	22.8a	5.2c	96.1a	30.3a	4.2d	42.7a	836b	423cd
250	8.5d	3.6d	52.7c	14.9d	6.0cd	13.9d	346d	168e
500	10.3cd	5.6bc	71.6b	16.5cd	6.9bc	19.9cd	497cd	338de
1,000	12.4c	5.4bc	74.9b	20.4bcd	10.3a	31.8b	537c	970a
2,000	10.2cd	6.4ab	70.7b	22.5bc	8.8ab	21.1c	778b	613b
4,000	15.1b	6.8a	68.5b	22.9b	7.4bc	37.6ab	1,228a	564bc

Mean values followed by same letter in column indicate no difference by Tukey test ($p < 0.05$)

Table 4 Mean concentration of Br, Cd, Rb, Sb, Cr, Cs, Co, Sc and Th in lettuce leaves as a function of treatments with superphosphate and the results of the statistical treatment

Treatment (mg kg^{-1} of P)	Br (µg g^{-1})	Cd (µg g^{-1})	Rb (µg g^{-1})	Sb (µg kg^{-1})	Cr (µg g^{-1})	Cs (µg g^{-1})	Co (µg kg^{-1})	Sc (µg kg^{-1})	Th (µg kg^{-1})
0	87.5a	12.4bc	52.3c	85.0a	1.01b	0.05c	302b	42.0d	25.0b
250	62.0b	4.0e	81.0b	30.3b	0.58d	2.36a	76c	85.0b	24.0b
500	77.0ab	6.1de	66.0bc	44.3b	0.84c	0.97b	252bc	71.3bc	44.3b
1,000	80.0ab	9.5cd	68.7bc	71.7a	2.45a	0.34c	980a	112.3a	143.0a
2,000	75.3ab	16.9a	105.3a	82.3a	1.13b	0.27c	1,026a	48.0cd	35.7b
4,000	62.0b	13.2b	59.3c	79.0a	0.77c	0.17c	369b	44.0d	55.0b

Mean values followed by same letter in column indicate no difference by Tukey test ($p < 0.05$)

in agriculture and hence the addition of the Cl. According to Prado [9] the optimal range of Cl concentration for plant growth is from 0.34 to 1.0 mg g^{-1}. The concentration of Cl in the shoots of lettuce was more than the optimal range for plant. Furthermore, according to Marchner [10] toxicity in lettuce by Cl can occur when the concentration of Cl in the shoot of the plant is between 20 and 30 mg g^{-1}. Only the control plant of this study showed Cl toxicity concentration and hence, a phosphate Cl-withdraw effect was observed.

The Mg concentration in lettuce leaves decreased significantly when the dose of 250 mg kg^{-1} of P was applied. In general, K and Ca contents in lettuce leaves also decreased when this dose was applied to contaminated soil. Mg, Ca and K are essential for plant growth, however the deficiency of these elements reduces the crops production and excess reduces the absorption of other essential elements to plant development [11].

The Na concentrations in lettuce increased from 42 to 145 % with P application when compared to that obtained for control plant. The excess of Na in soil can affect in the physical and chemical properties of soil, reducing its porosity and permeability [12, 13].

The concentration of Mn was almost 70 % lower in treatment with 250 mg kg^{-1} of P than control plant. However, when a dose 4,000 mg kg^{-1} of P was used, both concentrations were similar.

Zn presented a similar behavior to that of Mn with respect to treatments, excepting the Zn concentration obtained in treatment with 4,000 mg kg^{-1} of P that was about 50 % higher compared to control treatment.

Fe concentration in treatment with 250 mg kg^{-1}of P in lettuces was significantly reduced (60 %) when compared to the result of control plant. The excess of Fe in some instances can inhibit the absorption of Mn. In this study, the Fe concentration was from 10 to 30 times of manganese concentration, although Mn deficiency in plants was not observed.

Phosphate treatments did not affect Br content in leaves to a great extent. The lowest Br concentration was found in lettuces with the applications of 250 and 4,000 mg kg^{-1} of P. The concentrations of Cd, Co, Cr and Sb in the leaves were reduced with applications of 250 and 500 mg kg^{-1} doses of P, whereas in the others treatments, presented increase in the element concentrations compared to the control treatment. Phosphate treatments increased Rb and Cs concentrations in the leaves compared to the control. The application of 1,000 mg kg^{-1} of P increased the concentrations of Sc and Th in comparison to control plant of about 270 and 570 %, respectively.

The positive effect of phosphate for the toxic metal amendments on lettuce may be attributed to the effect of P on decreasing metal toxicity, as estimated with tissue metal analyses. However, there are many studies obtaining results on the behavior of Pb, Cd and Zn, but other elements, such as Br, Co, Sc and Th, have not been evaluated [6, 14–18].

Results on reducing the availability of Cd, Pb and Zn have been demonstrated by several authors that used P as amendment [15–18]. These authors studied contaminated soils, although the contamination usually does not occur only with two or four elements, but with different elements. In the present study, other elements, such as Rb and Sc, increased their concentrations on lettuce leaves when P was used.

Furthermore one of the factors that may affect the toxic element levels in soil is pH. In general, the decrease in pH increases the availability of elements and consequently the toxic element contents in plants. Decrease in pH soil after phosphorus application was verified by Maenpaa et al. [18], where in control treatment pH of 7.0 decreased to 6.0 after application of triple superphosphate rate of 5,000 mg kg^{-1}.

Conclusion

The application of 250 mg kg^{-1} of P as superphosphate was effective to reduce the concentrations of Br, Ca, Cd, Cl, Co, Cr, K, Mg, Mn, Sb and Zn in lettuce plant cultivated in a contaminated soil, showing the ability of the P fertilizer as a soil amendment. However the study showed the increase of Cs, Na, Rb, Sc and Th with phosphorus treatment. The application of INAA proved to be a useful tool for the determination of some elements such as Br, Cl, Co, Cr, Cs, Rb, Sc and Th which are not routinely measured in soil amendment studies with other analytical techniques. As element concentration changes were observed in lettuce plant for less studied elements, it is concluded that these additional elements should also be analyzed when dealing with the phosphate application for soil remediation.

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