3rd-INCC

ID:58

INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS APPLIED TO MULTIELEMENT DETERMINATION IN A VARIETY OF LETTUCE GROWN IN A CONTAMINATED SOIL AND TREATED WITH PHOSPHATE

M.J.A.Armelin[1], A.R. Trevizam[2], T. Muraoka[3], M.L.S. Silva[2], M. Saiki[1], V. A. Maihara[1]

[1]Instituto de Pesquisas Energéticas e Nucleares, (IPEN/CNEN-SP), Av. Prof. Lineu Prestes 2242, CEP 05508-000, São Paulo/SP, Brasil

[2] Universidade Estadual do Centro Oeste, (UNICENTRO) Rua Camargo Vilela de Sá 03, CEP 85040-080 Guarapuava/PR, Brasil

[3] Centro de Energia Nuclear na Agricultura, (CENA/USP) Avenida Centenário 303, CP96, CEP 13400-970 Piracicaba/SP, Brasil

Corresponding Author**:** Maria José Aguirre Armelin

Fax: (11) 3133-9960

e-mail: marmelin@ipen.br

Instrumental Neutron Activation Analysis Applied to Multielement Determination in a Variety of Lettuce Grown in a Contaminated Soil and Treated with Phosphate

M.J.A.Armelin[1*], A.R. Trevizam[2], T. Muraoka[3], M.L.S. Silva[2], M. Saiki[1], V.A. Maihara[1]

[1]Instituto de Pesquisas Energéticas e Nucleares, (IPEN/CNEN), Av. Prof. Lineu Prestes 2242, CEP 05508-000, São Paulo/SP, Brasil
[*] E-mail: marmelin@ipen.br

[2] Universidade Estadual do Centro Oeste, (UNICENTRO) Rua Camargo Vilela de Sá 03, CEP 85040-080 Guarapuava/PR, Brasil

[3] Centro de Energia Nuclear na Agricultura, (CENA/USP) Avenida Centenário 303, CP96, CEP 13400-970 Piracicaba/SP, Brasil

Abstract

In recent decades, anthropogenic activities, particularly those associated with industrial processes and mining, have been the major source of inorganic elements enrichment in soils. Unlike organic contaminants, most inorganic elements do not undergo microbial or chemical degradation and therefore the total concentrations of them persist in soils for a long time after their introduction. In this case, due to the possibility of elements to be at toxic levels to plants and reaching the food chain through the plants, the interest by scientific community in developing technologies for remediating contaminated sites has increased. The addition of de substances capable of immobilizing the toxic element from the soil is a procedure that has been used for remediating contaminated sites. The purpose of this study was to evaluate the efficiency of superphosphate fertilizer in remediating a soil contaminated with elements that can be potentially toxic. For this, different rates of phosphorus (250, 500, 1000, 2000 and 4000 mg kg^{-1} of P) were used in a number of lettuce plants and, the concentrations of elements in the leaves of lettuce treated with phosphate were compared with those in the control plant leaves. Instrumental neutron activation analysis (INAA) followed by gamma-ray spectrometry was the analytical method used to determine element contents in lettuce leaves. The application of 250 mg kg^{-1}(P) was the most effective treatment to reduce the concentrations of Br, Ca, Cd, Cl, Co, Fe, K, Mg, Mn, Sb and Zn in lettuce leaves.

Keywords: Neutron activation analysis, inorganic elements, contaminated soil, lettuce.

Introduction

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

The elements boron, chlorine, cooper, iron, manganese, molybdenum, nickel and zinc are micronutrients for plants, but in high concentration in soil solution can reach toxic level to plants and microorganisms. From 92 natural existing elements, 22 are known as essential for human and animals. Besides these, some 25 others are detected in human and animal bodies. Most minerals found in the body, essential and nonessential, has high chemical and biological reactivity, in particularly when they are as 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 the total concentrations of them persist in soils for a long time after their introduction[4]. In this case, due to the possibility of elements to be at toxic levels to plants and reaching the food chain through the plants, the scientific community interest in developing technologies for remediating contaminated sites has increased.

The addition of substances capable of immobilizing the toxic element from 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 case of revegetation for example, the anion dihydrogen phosphate ($H_2PO_4^-$), due to its ability to form insoluble precipitates with a variety of metals, reduces the availability of these metals in the soil and provide phosphorus for these sites usually with low concentration of this element and several other nutrients for plants.

The objective of this study was to evaluate the efficiency of phosphorus in reducing the availability of different elements in plant grown in contaminated soil treated with phosphorus. Five rates of phosphorus were applied in contaminated soil, growing lettuce plants and the concentrations of different elements absorbed by the lettuce were compared with those of control lettuce plants (no addition of phosphorus). Instrumental neutron activation analysis (INAA)[8] followed by gamma-ray spectrometry was the analytical method used to determine element contents in lettuce leaves. In literature there are many studies on the use of phosphate to reduce availability of Cd, Cu, Zn and Pb in areas with contaminated soils. In the case of this work, INAA is a useful tool because it allows the determination of some elements that are not routinely measured in this kind of study by other analytical methods.

Experimental

Soil sampling and treatment for the experiment

The soil was collected from a site of 22.000 m^2, located in Piracicaba, SP-Brazil. This site is under receivership of the Companhia de Tecnologia e Saneamento Ambiental (CETESB) because it has high level of contamination by potentially toxic elements.

A sample of 50 kg soil was collected for this study in an area of 2 x 3 m from 0-20 cm depth, passed through 4 mm mesh sieve and then homogenized. Subsamples of 2 kg soil were transferred to pots where plants were sown. A 1 kg subsample soil was taken for chemical and physical characterizations (pH; available P, Ca, Mg and K; total acidity and organic matter)[9]. The granulometric analysis was performed according to Camargo et al.[10]. The trace elements in sample soil were determined by Inductively coupled plasma-mass spectrometry (ICP-MS). Table 1 and 2 show the chemical and physical attributes of soil.

Table 1and 2

Installation of the experiment with lettuce

To assess the effect of phosphorus in reducing and the availability of potentially toxic elements in soil, lettuce plants (*Lactuca sativa* L.) were grown in pots containing 2 kg of soil. The trial was performed at the green house with ventilation and humidification system at the CENA.

The experimental design was a randomized block, with 6 treatments (rates of phosphorus): 0, 250, 500, 1000, 2000 and 4000 mg kg^{-1} of P. The P source used was $Ca(H_2PO_4)_2$.

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 rates 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 pots.

The lettuce leaves were collected at 70 days after transplanting, rinsed with deionized water, oven dried (at 65ºC), weighed and ground in agate mortar for elemental analysis by INAA.

Multielemental Analysis by Instrumental Neutron Activation

Aliquots of approximately 150 mg of lettuce leaves were transferred to polyethylene bags, which had been cleaned by leaching with a diluted HNO_3 (1:5) 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 the synthetic standards. Aliquots (50-100 µL) were transferred to small sheets of analytical filter paper (Whatman Nº 42) for irradiation. After drying, these filter papers were placed into polyethylene bags.

Irradiation and counting

Irradiations were carried out at the IEA-R1 research nuclear reactor. The thermal neutron flux utilized ranged from 1 to 4 x 10^{12} n $cm^{-2}s^{-1}$. Two types of irradiation were carried out at the IEA-R1 nuclear research reactor. First, the sample and standards of elements Cl, Mg and Mn were irradiated together in a polyethylene container for 25 s. After a decay time of 3 min the ^{38}Cl and ^{27}Mg 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 together in a aluminum container for 6 h. The ^{82}Br, ^{47}Ca, ^{115}Cd, ^{42}K, ^{24}Na and ^{122}Sb were measured after 4 days of decay time, while ^{60}Co, ^{51}Cr, ^{59}Fe, ^{86}Rb, ^{46}Sc, ^{233}Pa ($^{233}Th \rightarrow {}^{233}Pa$) and ^{65}Zn were measured after, at least, 8 days of decay time.

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 1332 keV gamma-ray of ^{60}Co.

In addition, analyses of certified reference materials NIST 1515 Apple Leaves and IAEA Soil-7 were also carried out simultaneously and, the results obtained were within the range of certified values.

Results and discussion

Elemental concentrations determined in lettuce leaves are shown in Tables 3 and 4. Each result, presented in Tables 3 and 4, is the arithmetic mean of three pots, as the experimental design. Coefficient of variation values of these results varied from 4 up to 50% in the case of some elements. This coefficient represents the uncertainty of the analytical method and the elemental concentration variation observed within each treatment. The effect was presumably on account of the biological variations of the element uptake by vegetal tissue. Variance analysis was applied to the values of Tables 3 and 4, using Tukey test[11], $p<0.05$, to verify if there is difference among element concentrations in plants under different rates of phosphorus compared with the element concentrations in the plant control. Basically, in Table 3 are the elements considered essentials for plant and animal organisms, while various elements shown in Table 4 are considered potentially toxic, and some others have no known functions but may have influence on the environmental impact (Br, Rb, Cs, Sc).

Table 3and 4

Treatment with phosphate reduced by approximately 50% the amount of Cl present in plants in relation to the control. As Cl deficiency rarely occurs in crops, research on this aspect are scarce. This fact can be explained by the use of KCl as a source of K in agriculture and hence the addition of the Cl. According to Prado[12] the optimal range of concentration of Cl to the growth of plants is 340 to 1000 mg kg^{-1}. The levels of Cl in this study are higher but for Marchner[13], toxicity in lettuce can occur when the concentration of Cl is between 20 and 30 g kg^{-1}. Only the control plant showed concentration of Cl in this range.

The Mg content was significantly decreased when the rate of 250 mg kg^{-1} of P was applied. In general, the K and Ca contents in lettuce leaves decreased with the phosphate applied to contaminated soil. Mg, Ca and K are essential for plant growth.

The concentrations of Na in lettuce increased from 42 to 145% with P application compared to control treatment. The excess sodium in soil can affect the physical and chemical properties of soil, reducing the porosity and permeability of soil.

Leaf Mn concentration was almost 70% lower in treatment with 250 mg kg^{-1} of P compared to the control treatment. The Mn content in 4000 mg kg^{-1} treatment was similar to that obtained in control treatment.

Zn had a behavior similar to that of Mn with respect to treatments, except that the concentration of Zn in leaves obtained in treatment with 4000 mg Kg^{-1} of P was almost 50% higher compared to control treatment.

The Fe concentration in lettuce was reduced significantly (60%) compared to control treatment with application of 250 mg $kg^{-1}$of P. The excess Fe in some situations can inhibit the absorption of Mn. In this study, the Fe content was 10 to 30 times that of manganese in the leaves, although Mn deficiency in plants was not observed.

Phosphate treatments affected very little the leaf Br contents. The lowest Br concentration was found in lettuce with the applications of 250 and 4000 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} rates of P, whereas the others treatments, there was an increase in the element concentrations compared to the control treatment. Phosphate treatments increased the leaf Rb and Cs concentrations in the leaves compared to control treatment. The application of 1000 mg kg^{-1} of P increased the concentrations of Sc and Th in plants, some 270 and 570%, respectively compared to control treatment.

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

Results on reducing the availability of Cd, Pb and Zn have been demonstrated by several authors [14-17] that used P as amendment. 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 levels heavy metals in soil is pH. In general, the decrease in pH increases the availability of elements and consequently the heavy metal contents in plants. Decrease in soil pH after phosphorus application was verified by Maenpaa et al. [17], where in control treatment pH of 7.0 decreased to 6.0 after application of triple superphosphate rate of 5000 mg kg^{-1}.

Conclusion

In general, the application of 250 mg kg^{-1} of P was the most effective treatment to reduce the concentrations of Cl, Mg, K, Ca, Mn, Zn, Fe, Br, Cd, Sb, Cr and Co in lettuce. However the Na, Rb, Cs and Th increased with phosphorus treatment.

The technique revealed that other elements must be studied beyond those commonly found in the literature when dealing with the phosphate application for soil remediation.

Acknowledgements

The authors wish to thank CNEN, CENA, CNPq and FAPESP from Brazil.

References

1. Alloway JB (1990) Heavy metals in soils, In: Alloway JB (ed) New York, John Wiley & Sons.
2. Underwood E (1977) Trace elements in human and animal nutrition. New York, Academic Press.
3. McBride MB (1994) Environmental chemistry of soils. New York, Oxford.

4. Bolan NS, Adriano DC, Duraisamy P, Mani A, Arulmozhiselvan K (2003) Immobilization and phytoavailability of cadmium in variable charge soils. 1. Effect of phosphate addition. Plant and Soil 250: 83-94.
5. Andrade JCM, Tavares SRL, Mahler CF (2007) Fitoremediação, Oficina de Textos: São Paulo.
6. Trevizam AR, Villanueva FCA, Muraoka T, Nascimento Filho VF, Abreu Junior CH (2010) Aplicação de fósforo para imobilização química do cádmio em solo contaminado. Quim. Nova, 33(6): 1235-1241.
7. Basta NT, Gradwohl R, Snethen KL, Schroder JL (2001) Chemical immobilization of lead, zinc and cadmium in smelter-contaminated soils using biosolids and rock phosphate. J. Environ. Qual., 30: 1222-1230.
8. De Soete D, Gijbels R, Hoste J (1972) Neutron Activation Analysis. New York, John Wiley & Sons.
9. Raij B, Andrade JC, Cantarela H, Quaggio JA (2001) Análise química para avaliação da fertilidade de solos tropicais. IAC: Campinas.
10. Camargo OA, Moniz AC, Jorge JA, Valadares JMAS (1986) Métodos de análise química mineralógica e física de solos do Instituto Agronômico de Campinas - IAC: Campinas.
11. SAS Institute, SAS system for Windows. Release 8.02 (2001) SAS Institute: Cary, NC.
12. Prado RM (2008) Nutrição de Plantas. São Paulo, Editora Unesp.
13. Marschner (1995) Mineral nutrition of higher plants. 2ed. London, Academic Press.
14. Chen S, Xu M, Ma Y, Yang J (2007) Evaluation of different phosphate amendments on availability of metals in contaminated soil. Ecotoxicol. Environ. Saf., 67: 278-285, 2007.
15. Chen SB, Zhu YG, Ma YB (2006) The effect of grain size of rock phosphate amendment on immobilization in contaminated soils. J. Hazard. Mater., B(134): 74-79.
16. McGowen SL, Basta NT, Brown GO (2001) Use of diammonium phosphate to reduce heavy metal solubility and transport in smelter-contaminated soil. J. Environ. Qual., 30: 493-500.
17. Maenpaa KA, Kukkonen JVK, Lydy MJ (2002) Remediation of heavy metal-contaminated soils using phosphorus: evaluation of bioavailability using an earthworm bioassay. Arch. Environ. Contam. Toxicol., 43: 389-398.

Table 1. Physical and chemical characteristics of soil used in the experiment collected

Characteristics	Soil
pH ($CaCl_2$)	7.06
Organic Matter (g dm^{-3})	28
P resin (mg dm^{-3})	75
K resin ($mmol_c$ dm^{-3})	7.0
Ca resin ($mmol_c$ dm^{-3})	400
Mg resin ($mmol_c$ dm^{-3})	92.0
H + Al ($mmol_c$ dm^{-3})	9.0
SB ($mmol_c$ dm^{-3})	499
CEC ($mmol_c$ dm^{-3})	508
V (%)	98
Sand (%)	65
Silt (%)	8
Clay (%)	27

Results of analysis for fertility, according to Raij et al[9]

Table 2. Total content of elements in soil

Element	Total content mg kg^{-1}
aluminum	46808
arsenic	21.4
barium	1119
calcium	15661
cadmium	43.1
cobalt	19.7
chromium	383
copper	715
iron	154978
potassium	16329
magnesium	9677
manganese	1885
molybdenum	30.3
sodium	3050
nickel	207
lead	891
antimony	27.8
strontium	1.9
vanadium	90.5
zinc	7574

Table 3. The concentration of Cl, Mg, K, Ca, Na, Mn, Zn and Fe in lettuce leaves as a function of treatment with superphosphate

Treatment	Cl		Mg		K		Ca		Na		Mn		Zn		Fe	
mg kg^{-1} of P	mg g^{-1}		mg g^{-1}		mg g^{-1}		mg g^{-1}		mg g^{-1}		µg kg^{-1}		µg kg^{-1}		µg kg^{-1}	
0	22.8	a	5.2	c	96.1	a	30.3	a	4.2	d	42.7	a	836	b	423	cd
250	8.5	d	3.6	d	52.7	c	14.9	d	6.0	cd	13.9	d	346	d	168	e
500	10.3	cd	5.6	bc	71.6	b	16.5	cd	6.9	bc	19.9	cd	497	cd	338	de
1000	12.4	c	5.4	bc	74.9	b	20.4	bcd	10.3	a	31.8	b	537	c	970	a
2000	10.2	cd	6.4	ab	70.7	b	22.5	bc	8.8	ab	21.1	c	778	b	613	b
4000	15.1	b	6.8	a	68.5	b	22.9	b	7.4	bc	37.6	ab	1228	a	564	bc

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

Table 4. The concentration of Br, Cd, Rb, Sb, Cr, Cs, Co, Sc and Th in lettuce leaves as a function of treatment with superphosphate

Treatment	Br		Cd		Rb		Sb		Cr		Cs		Co		Sc		Th	
mg kg^{-1} of P	μg g^{-1}		μg g^{-1}		μg g^{-1}		μg kg^{-1}		μg g^{-1}		μg g^{-1}		μg kg^{-1}		μg kg^{-1}		μg kg^{-1}	
0	87.5	a	12.4	bc	52.3	c	85.0	a	1.01	b	0.05	c	302	b	42.0	d	25.0	b
250	62.0	b	4.0	e	81.0	b	30.3	b	0.58	d	2.36	a	76	c	85.0	b	24.0	b
500	77.0	ab	6.1	de	66.0	bc	44.3	b	0.84	c	0.97	b	252	bc	71.3	bc	44.3	b
1000	80.0	ab	9.5	cd	68.7	bc	71.7	a	2.45	a	0.34	c	980	a	112.3	a	143.0	a
2000	75.3	ab	16.9	a	105.3	a	82.3	a	1.13	b	0.27	c	1026	a	48.0	cd	35.7	b
4000	62.0	b	13.2	b	59.3	c	79.0	a	0.77	c	0.17	c	369	b	44.0	d	55.0	b

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