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CADMIUM DETERMINATION IN LETTUCE GROWN IN CONTAMINATED SOIL BY INAA AND GFAAS

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ABSTRACT

Although Cd is not essential for the mammalian organism, it follows in body the same pathways of essential elements such as zinc and copper. There is evidence that the Cd induced reduction of Ca absorption, may lead to the development of osteoporosis. Anthropogenic activities associated with industrial activities, mining and use of fertilizers, limestone pesticides in agriculture are the main sources of Cd enrichment in soils. Due to the possibility that Cd being absorbed by plants and through them to reach the food chain, interest has increased in regard to developing techniques for remediation of contaminated sites. The addition of substances capable of immobilizing the toxic elements 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. In this study, five doses of phosphorus as triple phosphorus were used in a number of lettuce plants grown in contaminated soil. The concentration of Cd present in lettuce leaves treated with phosphate was compared with the Cd absorbed by the control plant leaves. Instrumental Neutron Activation Analysis (INAA) and Graphite-Furnace Absorption Atomic Spectrometry (GFAAS) were the analytical methods used to determine Cd contents in lettuce leaves. The objective was to evaluate the performance of the employed analytical methods: INAA and GFAAS in the assessment of the efficiency of phosphorus treatments to reduce the Cd concentrations in leaves of lettuce. Results obtained indicated that both analytical methods were efficient to discriminate the response of Cd concentration in lettuce as a function of soil treatment with phosphorus. Although INAA has shown a positive performance in this study, GFAAS seemed more appropriate because its sensitivity was much higher than that obtained by INAA, in the experimental conditions.

1. INTRODUCTION

Although Cd is not essential for the mammalian organism it follows in the body the same pathways of essential elements such as zinc and copper. The half-life of this element in humans is 16-33 years and, accumulates in the liver and kidneys that can causes kidney

dysfunctions. There is evidence that Cd induced reduction of Ca absorption may lead to the development of osteoporosis [1].

Anthropogenic activities associated with industrial activities, mining and use of fertilizers, limestone, pesticides in agriculture are the main sources of Cd enrichment in soils. Unlike organic contaminants, most metals do not undergo microbial or chemical degradation and therefore the total concentrations of metals persist in soil for long time after their appearance [2]. Due to the possibility of Cd being absorbed by plants and through them to reach the food chain, interest has increased in developing techniques for remediation of contaminated sites.

The addition of substances capable of immobilizing the toxic elements 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 [3]. For example, the anion dihydrogen phosphate ($H_2PO_4^-$) is a substance with this characteristic, due to its ability to form insoluble precipitates with a variety of metals [4].

In this study, five rates of phosphorus were used in a number of lettuce plants grown in contaminated soil. The concentration of Cd present in lettuce leaves treated with phosphate was compared with the concentration of Cd absorbed by the control plant leaves. Instrumental Neutron Activation Analysis (INAA) and Graphite-Furnace Absorption Atomic Spectrometry (GFAAS) were the analytical methods used to determine Cd contents in lettuce leaves. The objective was to evaluate the performance of employed analytical methods: INAA and GFAAS in the assessment of the efficiency of phosphorus treatments to reduce the Cd concentration in leaves of lettuce. Sensitivity, selectivity and detection limit were the parameters considered in the assessment of the analytical performance.

2. EXPERIMENTAL

2.1. Soil sampling and treatment for the experiment

The soil was collected from a site of 22,000 m^2, located in Piracicaba, SP. This site is under receivership of the Companhia de Tecnologia de Saneamento Ambiental (CETESB) because it has high level of contamination by potentially toxic elements. For this study a sample of 50 kg was collected in an area of 2 x 3 m in depth from 0-20 cm. The collected soil was passed in 4 mm mesh sieve and then homogenized. Subsamples of 2 kg soil were transferred to pots where plants were sown.

2.2. Installation of the experiment with lettuce

To assess the effect of phosphorus in reducing and 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/USP.

Experimental design was a random block, with 3 replications and 5 treatments with different rates of phosphorus: 250, 500, 1000, 2000 and 4000 mg kg^{-1} of P, and control treatment.

Thus, the total number of experimental blocks was 18 or 18 pots. The P source used was $Ca(H_2PO_4)_2$.

The P treatments were applied to respective pots, where the soils were incubated for 15 days under 60% moisture content. At the end of the incubation period six seedlings of lettuce were transplanted. 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 30 mg and 80 mg N per pot. Ten days after transplantation, 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 collected at 70 days after transplanting were rinsed with deionized water, oven dried (at 65ºC), weighed and ground in an agate mortar for the determination of Cd by methods INAA and GFAAS.

2.3. Instrumental Neutron Activation Analysis (INAA)

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.

Cadmium certified standard solution (Spex Certiprep) was used to prepare the standards. Aliquots (25 μL) were transferred to small sheets of analytical filter paper (Whatman Nº 42). After drying, these filter papers were placed into polyethylene bags.

Irradiations were carried out at the IEA-R1 nuclear research reactor of IPEN-CNEN/SP. Samples of lettuce, certified reference material (NIES-CRM -10C Rice Flour) and standard of Cd were irradiated together in an aluminum container for 8 h. The ^{115}Cd, in the samples and standard, was measured after 4 days of decay time. The photopeak of 526 keV gamma rays was used to measure the activity of ^{115}Cd. The reference material was used to monitor the process. 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.

2.4. Graphite- Furnace Absorption Atomic Spectrometry (GFAAS)

Graphite-furnace atomic absorption spectroscopy (GFAAS) was employed to measure Cd, in the Perkin Elmer Analyst 800 spectrometer with Zeeman background correction. Aliquots ranging from 40 to 150 mg of lettuce samples and certified reference material (Oyster Tissue 1566b – NIST) were digested with 4 ml of concentrated HNO_3 (Merck) and left standing for a period of 8 h, after 1 ml of 30% H_2O_2 was added. The flasks were stirred and left again for about 15 h. To finalize the sample digestion, the closed flasks were placed in an aluminum block at 90ºC, for 3 h. The digest was diluted with water up to volume of 25 ml. The reference material was analyzed to control the analytical results.

During the analysis, the following parameters were kept fixed: injection volume (for samples and standards) in 20 µL, volume of modifiers in 10 µL and drying temperatures in 110 and 130ºC [5], which were used to construct the calibration curve. All solutions were prepared with water treated in Milli-Q (Millipore) purification system. The points of calibration curve were obtained for: 1.3, 2.67, 4.01, 5.34 and 6.7µg.L^{-1}. Equipment parameters: wavelength of the Cd lamp (228.8 nm), Slit (0.7 nm), lamp current (230 mA). The solution of chemical modifier (NH_4HPO_4 0.5% and $Mg(NO_3)_2$ of Perkin Elmer) was prepared according to recommendation of the spectrometer manufacturer.

3. RESULTS AND DISCUSSION

3.1. Selectivity, Sensitivity and Detection Limit

Cadmium sensitivity and detection limit of INAA and GFAAS obtained, under the experimental conditions applied in this study, are shown in Table 1.

Table 1. Cadmium Sensitivity and Detection Limit of INAA and GFAAS

Methods	Sensitivity	Detection Limit
	(Signal*.µg^{-1})	(µ$g.kg^{-1}$)
INAA	0.2	2.14 [6]
GFAAS	2810	0.37 [7]
*Signal=counts per second (cps) for INAA; Signal= Integrated absorbance for GFAAS		

It was observed that the sensitivity for Cd determination INAA is much lower than the sensitivity via GFAAS. In the case of INAA, the Cd photopeak (526 keV) is in a region of the gamma spectrum subject to interference from other radioisotopes that make the INAA not very selective for the Cd determination in several matrices. The use of chemical modifiers for the determination of Cd by GFAAS avoids problems of matrix interference, which makes this method highly sensitive and selective for the determination of Cd in a wide variety of matrices [7].

3.2. Cadmium concentrations in lettuce as function of phosphorus treatment

Cadmium concentrations determined in lettuce leaves, by INAA and GFAAS, are shown in Table 2. Each value is the result of one determination with its uncertainty in parentheses. In the case of INAA the uncertainty was evaluated by counting statistics and, for GFAAS are expanded uncertainties calculated using K=2 [8]. As the experimental design was made in

triplicates for each treatment, some results were not considered because in these cases, the amount of sample was insufficient for analysis by both methods.

The results obtained for the two methods were mostly concordant. The variations in concentrations within each treatment may be assigned by the uncertainty of the analytical method and biological variations of plant due to cadmium uptake and the soil composition variations among the different pots.

Table 2. Cadmium concentration in lettuce as affected by phosphorus treatment, with its uncertainty in parenthesis, by the INAA and GFAAS

Treatment	Cd (INAA)	Cd (GFAAS)
P (mg kg^{-1})	µg g^{-1}	µg g^{-1}
0	13.7 (1.3) 11.6 (1.1)	11.7 (0.4) 10.7 (0.4)
250	3.4 (1.2) 7.1 (1.8)	4.8 (0.2) 5.5 (0.2)
500	6.3 (0.7) 10.4 (0.7)	5.2 (0.2) 9.5 (0.3)
1000	9.9 (0.9) 9.5 (0.9) 9.2 (0.9	8.9 (0.3) 10.7 (0.4) 10.2 (0.4)
2000	19.4 (0.9) 14.2 (1.1)	14.5 (0.5) 12.2 (0.4)
4000	14.6 (0.8) 11.8 (0.7) 18.5 (0.8)	14.5 (0.5) 15.0 (0.5) 20.5 (0.7)

Variance analysis was applied to the values of Table 2, using Tukey test, $p<0.05$ [9], to verify if there is a difference among Cd concentrations as affected by treatment with phosphorus on the absorption of Cd by lettuce leaves. Table 3 shows the results of statistical tests and, by consequence the effect of phosphorus. Each result is the arithmetic mean of the values presented in Table 2, for each treatment. Statistical t-test, $p<0.05$, was also applied to check whether the average concentrations for Cd obtained by the two methods are statistically equal. The results in Table 3 showed that the dose of 250 mg kg^{-1} (P) was the most suitable to immobilize Cd in soil.

The results, regardless of the method used in the analysis of Cd, demonstrated a reduction the Cd levels due to some of levels of P added. However this reduction in concentration was not statistically significant as observed in Table 3, therefore cannot consider the effect of P on the reduced availability of Cd.

The results are contrary opposite to those observed by Chen et al.[10] where the addition of P was shown to be effective in reducing the availability of Cd to *Brassica campestri* L. Other

results demonstrating the reduction of availability can be found in Bolan et al. [2] for *Brassica juncea* L. and Zwonitzer et al. [11] for the *Sorghum bicolor* L. Moench crop.

The Cd contents found in the lettuce leaves are above the range of values considered normal, from 0.66 to 3 mg kg^{-1} [12]. The toxic level for lettuce crop is 10 to 95 mg kg^{-1} [13].

Table 3. Results of Tukey test and t-test on the values of Table 2

Treatment P (mg kg^{-1})	Cd (INAA) µg g^{-1}	Cd GFAAS) µg g^{-1}
0	12.7 acA	11.2 acA
250	5.3 aA	5.1 aA
500	8.4 acA	7.3 acA
1000	9.5 acA	10.0 acA
2000	16.8 bcA	13.4 bcdA
4000	15.0 bcA	16.7 bdA

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

For each phosphorus treatment, mean values followed by same capital letter in horizontal indicate no difference by t-test ($p<0.05$).

3. CONCLUSIONS

The two analytical methods INAA and GFAAS used in obtaining the results achieved the same values of the amounts of Cd contents. GFAAS has shown a smaller detection limit. The addition of P did not reduce the lettuce Cd content.

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