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UPTAKE OF LEAD IN THE LEAVES OF *VITEX NEGUNDO* LINN. (LAGUNDI)

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Abstract - The uptake of lead in the leaves *Vitex negundo,* Linn., (lagundi) was determined. Seedlings were planted in sand which was acid-leached and treated with known concentrations of lead nitrate. The presence of lead in lagundi leaves was determined by Atomic Absorption Spectrophotometry using the acid-digestion and dry-ashing methods. Using the single-classification ANOVA, it was found that lagundi took up lead from the medium where it is planted. The lead uptake was evident from the higher concentrations found in lagundi grown in the treated pots versus that of the control. Lagundi leaves from the control gave an average concentration of 5.14 μg Pb/g while the leaves from pots treated with 5 ppm and 10 ppm lead nitrate solutions gave average concentrations of 8.42 and 10.78 μg Pb/g, respectively. Of the total lead present in the pots, the lead in leaves account for only 0.03 to 0.07 %. Both sample preparation methods were found to be efficient as shown by the high percentage recovery of the lead added.

INTRODUCTION

The recent trend in the use of herbal medicines has given rise to various studies particularly on the leading medicinal plants in the country such as *Vitex negundo,* Linn. locally known as lagundi. The leaves of *V. negundo* are used in the manufacture of lagundi tablet which is antitussive, antipyretic, and anti-asthma (Quisumbing 1978). This study is an offshoot of a paper which reported that the lead content in lagundi leaves was 9.32 μg/g (Dayrit 1989). The presence of lead in the plant leaves is undesirable as lead acts directly on the central nervous system and can even cause mental retardation in children (Casarett 1975). This study sought to determine the tendency of lagundi to take up lead and translocate the heavy metal to the leaves. This study determines the extent of lead uptake in leaves based on the known amount added to each pot. The two methods of sample preparation, namely acid-digestion and dry-ashing methods were compared regarding their percentage recovery.

EXPERIMENTAL METHODS

Planting

Forty-five seedlings of lagundi were planted in plastic pots using acid-leached sand. A sand sample was taken for lead analysis to serve as baseline. The sand was then transferred to individual plastic pots where the lagundi seedlings were planted. Fertilizer was formulated according to Quintana (1989) and this was also analyzed for lead content. One batch was composed of fifteen pots, which was divided into three sets of five pots each: the control, the 5 ppm, and the 10 ppm treatments. There were three batches hence a total of forty five pots were planted with lagundi. These were placed under a garden net. A laminated sack was used to protect the pots from rain.

Treatment with Lead Nitrate Solutions

The first set of five pots was used as the control group and was treated with tap water. The tap water was also analyzed for lead. The remaining two sets were treated with known concentrations of $Pb(NO_3)_2$ solutions - 5 ppm and 10 ppm - after

three weeks from the time of planting. The plants except the first set were treated with 200 to 250 mL (every volume added was recorded and counted at the end of the experiment) each of lead solution everyday during dry conditions, or every other day during humid conditions.
respectively, after three weeks from the time of planting. The plants except the first set were treated with 200 to 250 mL (every volume added was recorded and counted at the end of the experiment) each of lead solutions everyday or every other day during humid conditions, to ensure that there were no losses during watering.

To determine further if the plants did absorb lead from where they were planted, a separate set of fifteen pots containing sand only was set up having exactly the same treatment as the pots described above.

Sampling

After three months from the time of planting, all the leaves from each pot were harvested, washed, and oven-dried at 50°C to constant weight. The leaves were then manually shredded, weighed, and divided into six; three replicates were used for the acid-digestion method and three replicates for the dry-ashing method (Fig. 1). A total of fifty-four determinations resulted from the three batches. Similarly, sand samples were taken from each pot using the coning and quartering method of sampling, air-dried, passed through a 20-mesh sieve and analyzed for soluble lead.

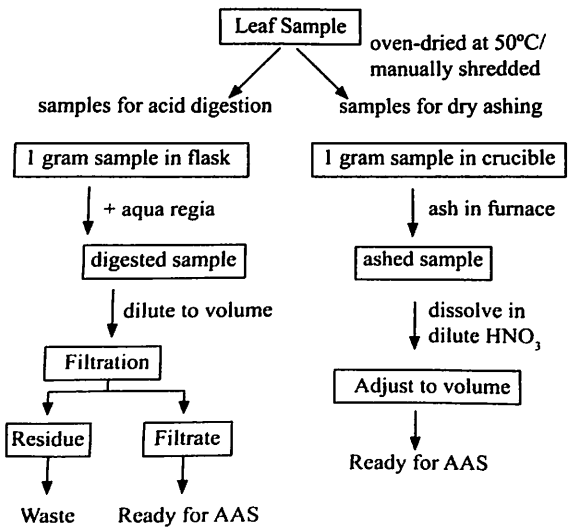

Figure 1. Leaf sampling and sample preparation by the acid-digestion and dry-ashing methods.

Wet Acid Digestion Method of Plant Samples

Three replicates containing one gram of the shredded sample (third replicate spiked with known concentration of $Pb(NO_3)_2$ solution) were digested in aqua regia in an erlenmeyer flask and warmed over a hot plate. The temperature was kept below boiling to prevent splattering, and the flask was covered with an inverted funnel to prevent impurities from entering the flask. The solution volume was kept to a minimum without allowing the solution to dry out. The digestion took up to 5 hours to completely digest and solubilize the material. When done, the digest was diluted to 25 mL with distilled-deionized water and was ready for AAS (Dayrit 1989).

Dry Ashing Method of Plant Samples

Similarly, three replicates of one gram of the shredded leaf samples (third replicate spiked with known amount of $Pb(NO_3)_2$ crystals) were weighed in a crucible with cover. The crucibles were placed in a muffle furnace and the temperature was set to 300°C for one hour. After the temperature had stabilized, it was raised gradually to 500°C and this temperature was maintained for four to five hours. After cooling, the ashed samples were dissolved in dilute nitric acid and diluted to 25 mL for AAS analysis (Bureau of Soils Methods).

Analysis of Sand

Sand was sampled by coning and quartering. Five grams of sand were extracted with 50 mL of 0.43 M nitric acid (1:10 sand/solution ratio), filtered and measured in the AAS. This procedure gives information on the quantity of lead in the soil (Houba 1985).

Spiking

For the acid-digestion method, spiking was done by adding a known concentration of lead solution into the flask containing the sample. digesting the material in a low flame hotplate. diluting to the appropriate volume and filtering the digested samples.

For the dry-ashing method, a microbalance was used to measure the desired amount of lead nitrate crystal which was added to the sample, ashed in the furnace, dissolved, and diluted to appropriate volume (Garfield 1991).

Lead in Fertilizer, Tap Water and Concentrated Nitric Acid

A post-analysis of the fertilizer used was done after the analysis of leaves from the control group revealed that it contained lead even if it was not treated with the lead nitrate solutions. Five grams of complete fertilizer were digested with dilute nitric acid and made up to 50 mL volume, filtered and analyzed by atomic absorption spectrophotometry using calibration standards with the same matrix as the sample (AAS Analytical Methods 1996).

Analysis of the tap water used in treating the control group and of the nitric acid used in acid-digestion and dissolution of ashed samples was done to determine the other possible sources of lead contamination in the control group. A water sample from the faucet was aspirated directly into the AAS. A sample of the nitric acid used in the sample preparation was also analyzed for lead by the AAS method.

RESULTS AND DISCUSSION

Lead Analysis of Water, Nitric Acid, Sand and Fertilizer

The tap water used in the treatment of the control group was not found to contain any traces of lead ions. The nitric acid used in the digestion and dissolution of samples was found to contain 0.78 mg/L. This was corrected for in the calculations and the background correction.

The sand used for planting was found to contain 3.3 mg/kg lead, which is a substantial amount considering the weight of sand used (approximately 25.0 kg). This means that initially, there were already 82.5 mg of lead in each pot prior to treatments with fertilizers and lead nitrate solutions. After analyzing the fertilizer, a bigger concentration of lead was found (13.9 mg/kg) but the fertilizer was still considered a minor source since the amount used was minimal, about 20 grams or 0.02 kilogram.

Analysis of Leaf Samples

Results of analysis show uptake of lead in the leaves for both 5 ppm and 10 ppm treatments. For the control, a total of 82.78 milligrams of lead was initially present, the 5 ppm treatment accounted for about 107.78 milligrams while the 10 ppm treatment contained a total of about 132.78 milligrams of lead per pot. This is calculated by multiplying the concentration of lead in sand by the total weight of sand used (25.0 kg) plus the amount of lead from the fertilizer added and the known quantities of lead from the total volume of 5 ppm and 10 ppm treatments with lead nitrate solutions. The total lead in leaves was obtained by multiplying the concentration of lead in leaves by the total weight of the dried leaves; For the control, an average weight of 7.8 grams of dried leaves per pot was obtained; 7.0 grams of dried leaves for the 5 ppm treatment and an average of 6.9 grams of dried leaves per pot was obtained. The percentage uptake was calculated from the total lead in leaves and the total lead in sand. The percentage uptake in leaves ranged from 0.03 to 0.07%.

Table 1. Average concentration of lead present in each treatment and the Approximate Percentage Assimilation in leaves (based on the acid-digestion method).

Treatment	mg Pb/kg sand	mg Pb/kg leaves	% Uptake
Control	3.3112 (±0.10)	5.14 (±0.95)	0.048
5 ppm	4.3112 (±0.10)	8.42 (±1.33)	0.054
10 ppm	5.3112 (±0.10)	10.78 (±0.85)	0.056

Table 1 summarizes the average concentration of lead in each treatment, the concentration of lead in leaves and the approximate percentage assimilation in leaves. The lead concentration in sand was based on the baseline value of 3.3 (±0.10) mg Pb/kg sand. The amounts of lead in sand and in fertilizer are approximations as these were calculated indirectly by analyzing the sand density, the volume of pot used and the remaining fertilizer. (Same sizes of pots were used, lagundi seedlings were planted at the same depths, and watering was done similarly for all the pots.

Comparison of the Two Methods

The two methods used in preparing the leaf samples for AAS analysis were found to be both efficient and are comparable. Both methods have high percentage recovery in terms of the known amount of lead added to the sample (spike) which shows that either method can be used in future studies or in lead analysis of plant tissue.

Table 2 summarizes the results of lead analysis in lagundi leaves using the two methods of sample preparation. From the table, absorption of lead ions by the plant is evident from the concentration of lead found in the leaf samples from the control, 5 ppm, and 10 ppm treatments, which shows an increasing average concentration of 5.14, 8.42, and 10.78 µg Pb/g, respectively (for acid-digestion method).

Table 2. Arithmetic mean of the concentration of lead present in lagundi leaves analyzed using the acid-digestion and dry-ashing methods of sample preparation.

Leaf Sample from the Treated Plants	Arithmetic Mean (mg Pb/g)	Arithmetic Mean (mg Pb/g)
	Acid-digest-ion Method	Dry-ashing Method
Control	5.14 (±0.95)	5.27 (±0.44)
5 ppm	8.42 (±1.33)	7.15 (±0.81)
10 ppm	10.78 (±0.85)	8.86 (±0.76)
Average % recovery	102.5	99.1

Statistical Analysis

One-way ANOVA for the two methods show that the plants took up lead and translocated this to the leaves. Since the computed F values (64.09 and 61.16 for the acid-digestion and dry-ashing methods, respectively) are greater than the tabulated F (3.40 and 3.44 for the acid-digestion and dry-ashing methods respectively), the null hypothesis that the three concentration means are equal is rejected. Using the Duncan's Multiple Range Test (DMRT), it was found that there is a significant difference between the mean of the control versus the mean of the 5 ppm treatment; there is also significant difference between the mean of the control versus the mean of the 10 ppm treatment and a significant difference is also observed between the mean of the 5 ppm versus the 10 ppm treatment .

Using the t-test, the two sample preparation methods were found to be both efficient in dissolving the mineral phase of the leaf samples for AAS analysis. The two methods of sample preparation do not vary significantly at 95% confidence level.

Summary

The results of the study show uptake of lead in the leaves of *V. negundo*. This is seen from the increasing concentration of lead in the control group, 5 ppm and 10 ppm treatments, which gave values of 5.14, 8.42, and 10.78 µg Pb/g respectively for the acid-digestion method and 5.27, 7.15, and 8.86 µg Pb/g for the dry-ashing method. The lead in the control was attributed to its initial presence in sand and in the fertilizer used.

Based on the total amount added to the sand and the initial lead present in sand and fertilizer, the lead found in leaves accounts for about 0.03 to 0.07% only. The sand was identified as the major source of lead and the fertilizer was a minor source for the control group, 5 ppm and 10 ppm treatments.

The two methods of sample preparation do not vary significantly. Both methods are found to be efficient in dissolving the mineral phase of the leaves for AAS analysis. However, the dry-ashing method is more convenient than the acid-digestion method since the latter requires the use of aqua regia which has an irritating odor even with the use of a fume hood.

Conclusion

This study proves that lagundi takes up lead from soil and translocates this to the leaves. The approximate percentage uptake ranges from 0.03 to 0.07% based on the initial lead present and the lead found in leaves.

The acid-digestion method and dry-ashing method of dissolving the leaf samples for AAS were both efficient and are comparable. Both methods gave high percentage recovery of spike which means that either method can be used in sample preparation.

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