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ARSENIC ABSORPTION BY MEMBERS OF THE BRASSICACEA FAMILY, ANALISED BY NEUTRON ACTIVATION, k_0-METHOD – PRELIMINARY RESULTS

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ABSTRACT

Natural arsenic contamination is a cause for concern in many countries of the world including Argentina, Bangladesh, Chile, China, India, Mexico, Thailand and the United States of America and also in Brazil, specially in the Iron Quadrangle area, where mining activities has been contributing to aggravate natural contamination.
Brassicacea is a plant family with edible species (arugula, cabbage, cauliflower, cress, kale, mustard, radish), ornamental ones (alysssum, field pennycress, ornamental cabbages and kales) and some species are known as metal and metalloid accumulators (Indian mustard, field pennycress), like chromium, nickel, and arsenic.
The present work aimed at studying other taxa of the Brassicaceae family to verify their capability in absorbing arsenic, under controlled conditions, for possible utilisation in remediation activities.
The analytical method chosen was neutron activation analysis, k_0 method, a routine technique at CDTN, and also very appropriate for arsenic studies.
To avoid possible interference from solid substrates, like sand or vermiculite, attempts were carried out to keep the specimens in ¼ Murashige & Skoog basal salt solution (M&S). Growth was stumped, plants withered and perished, showing that modifications in M&S had to be done.
The addition of nickel and silicon allowed normal growth of the plant specimens, for periods longer than usually achieved (more than two months); yielding samples large enough for further studies with other techniques, like ICP-MS, and other targets, like speciation studies.
The results of arsenic absorption are presented here and the need of nickel and silicon in the composition of M&S is discussed.

1. INTRODUCTION

Natural arsenic contamination is a cause for concern in many countries of the world including Argentina, Bangladesh, Chile, China, India, Mexico, Thailand, United States of America [1] and also in Brazil, specially in the Iron Quadrangle area, where mining activities has been contributing to aggravate natural contamination [2].

The discovery that a fern, *Pteris vittata*, hyperaccumulates arsenic [3] led to the search of other pteridophyte species that might have this capacity [4-6], and so could be used for phytoremediation of arsenic contaminated areas.

Phytoremediation is a technology that can be considered as a relatively recent one, although it is based on knowledge that has been gathered and accumulated for a long time. According to

the United States of America Environmental Protection Agency (EPA), Phytoremediation, is the direct use of living green plants for *in situ* risk reduction for contaminated soil, sludges, sediments, and ground water, through contaminant removal, degradation, or containment [7].

One advantage of phytoremediation is its relatively low cost, but, as it must rely on plant growth, its results might be quite slow to be achieved, specially in countries with temperate climate, which is not the case of vast areas of Brazilian territory.

One of the aims of phytoremediation is the search of plants that are hyperaccumulators, i.e., that are capable of accumulating pollutants and/or contaminants in high concentrations, of which the above mentioned fern *Pteris vittata* is a fine example, considering arsenic accumulation [3].

The possibilities of exploring Brazilian native flora for this purpose demand no further comments.

The reclamation of degraded areas demands might not necessarily rely only on species and/or varieties that are accumulators, but also on taxa that are resistant to a given pollutant, a mixture of pollutants, a specific soil condition, and/or all combinations that are possible of the situations described. In this case, a precise analysis of the environment is fundamental, specially to ascertain, for sure, that some toxic element is not present.

The process of selection of plant species suitable for the activities above mentioned demands the utilisation of analytical tools. Elemental determination of a certain plant sample can be achieved through different analytical techniques [8-12], such as:
· Atomic Absorption Spectrometry (AAS);
· High Performance Liquid Chromatography (HPLC);
· Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP/AES);
· Inductively Coupled Plasma Mass Spectrometry (ICP/MS) and
· Neutron Activation Analysis (NAA).

Among these techniques, NAA distinguishes itself by being a non-destructive technical procedure, if necessary, it allows the determination of several elements in a single sample. Neutron Activation Analysis is a sensitive analytical technique useful for performing both qualitative and quantitative multi-element analysis of major, minor, and trace elements in samples from several types of matrices. For many elements and applications, NAA offers sensitivities that are superior to those attainable by other methods, in order of parts per billion or better.

To determine the chemical composition of a sample applying this technique, the sample is bombarded with reactor neutrons. After the irradiation, the radionuclides formed by the (n, γ) reaction are analysed through gamma spectrometry; the elemental concentrations are then calculated.

The application of purely instrumental procedures is commonly called Instrumental Neutron Activation Analysis (INAA) and it does not require any chemical process neither during preparation nor during the determination of the elemental concentration.

The most powerful instrumental method currently used is the k_0- standardisation method [12-14]. It is a technique in which instead of standards of the analysed elements, monitors are used to calculate the elemental concentrations. Besides, the nuclear data, which are unknown, are replaced by nuclear constants called k_0 factors.

In Belo Horizonte, the Laboratory for Neutron Activation, located at Centro de Desenvolvimento da Tecnologia Nuclear/Comissão Nacional de Energia Nuclear, CDTN/CNEN, has been applied the k_0- method as a routine procedure. At CDTN/CNEN, roughly, 70% of the known elements can be determined by this analysis [13,14], as shown in the Periodic Table of Elements, in Figure 1.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															
			Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu	
			Th	Pa	U	Np	Pu	Am	Pu	Bk	Cf	Es	Fm	Md	No	Lr	

Figure 1. Elements determined by Neutron Activation Analysis at CDTN/CNEN are in red

The need to start researches aiming at phytoremediation and reclamation of degraded areas, appeared during the development of our research activities, as stated in [15], after samples of soil, sediment, plants and crops cultivated close to a mining area were analysed to verify the concentration of metals and metalloids, in order to verify possible risks to human health [16-18].

Such researches led to a preliminary screening of some ferns from the Iron Quadrangle, to verify which species might accumulate arsenic [19] and to the establishment of a methodology to expose, under controlled conditions, different plant species to a Murashige & Skoog basal salt solution (M&S) [20] with arsenic in order to verify which might absorb the metalloid and have a possible utilisation in remediation activities [21].

This methodology also allows the selection of species that are can grow in the presence of arsenic, without absorbing the metalloid, and that can be used in restoration programs. The methodology was established primarily using a species known to accumulate arsenic, Indian mustard (*Brassica juncea*) [9, 22] and another species introduced in the Iron Quadrangle, *Mirabilis jalapa*.

Brassica juncea specimens, right after germination, were exposed to M&S with 100 $\mu g L^{-1}$ of sodium arsenate for 16 days, and presented 61 ± 2 mg kg^{-1} of arsenic, in dry weight. 14 day old specimens of *Mirabilis jalapa* were treated for 30 days, and presented 483 ± 17 61 ± 2 mg kg^{-1} of arsenic, in dry weight.

Control specimens of both species presented < 1 mg kg^{-1} of arsenic, in dry weight; all specimens were kept in washed sand, and the samples were formed by as many specimens as necessary to achieve 0.3 g of dry weight.

In order to verify the capacity of absorbing arsenic of other taxa of the Brassicaceae family, further experiments were carried out; their results are presented and discussed here.

2. MATERIAL AND METHODS

The taxa studied were:

- Ornamental: carpet-of-snow (white) and violet queen (purple) alyssum (*Lobularia maritima*) and
- Edible: arugula (*Eruca sativa*); cabbage early Jersey Wakefield (*Brassica oleracea* var. *capitata*); cauliflower snowball early (*Brassica oleracea* var. *botrytis*); kale or collards Georgia Southern (*Brassica oleracea* var. *acephala*); long scarlet short top radish (*Raphanus sativus*) and radish Minowase (*Raphanus sativus* var. *acanthioformis*).

Specimens were obtained by germination of Isla Pak seeds, bought in the local market. Seeds were sown in vermiculite, watered with tap water, transferred to flasks with vermiculite; healthy specimens were then divided in two groups (control and for arsenic exposure).

Control groups were kept with ¼ M&S; treated ones were provided with 100 $\mu g\ L^{-1}$ of arsenic acid ¼ M&S. All groups were kept in open air; with weekly changes of the nutrient solution; the length of time for treatment depended on the growth of the specimens. In order to avoid possible interference of the substrate, attempts to grow specimens in M&S, without substrate, were also carried out.

Specimens were harvested when their size might be enough for analysis; unhealthy specimens were discarded. After harvest, whole specimens were washed with deionized distilled water, freeze-dried, hand ground, transferred to irradiation vials and weighed.

The k_0-standardization method was applied to determine the arsenic concentration in the samples. The irradiation was carried out in the carrousel IC-7 of the TRIGA MARK I IPR-R1 reactor at CDTN/CNEN, under a thermal neutron flux of 6.35×10^{11} cm^{-2} s^{-1}, 100 kW power. The parameters *f* and a in the IC-7 are (22.32 ± 0.2) and (-0.0022 ± 0.0002), respectively. The samples were irradiated simultaneously with neutron flux monitor Al-Au (0.1%) IRMM-530RA foil.

The usual neutron activation analysis including the gamma spectroscopy comprised the scheme of 8 hours to determine arsenic. The gamma spectroscopy was performed on an HPGe detector with 15% efficiency and for the spectra analysis - peak area evaluation - the Hyperlab program [23] was used and for the calculation of elemental concentrations a software package KAYZERO/SOLCOI [24] was applied.

3. RESULTS AND DISCUSSION

Among the taxa studied, only the ones presented in Table 1 provided samples large enough to be analysed. Other taxa did not yield enough material to be analysed; growth, when achieved, was stumped, and showing nutrient deficiencies. Attempts to keep the material in M&S, without any substrate were not successful; *Lobularia* specimens did not survive in order to be treated. Algae proliferation was reduced, or practically null, with the dilution of M&S to ¼, and no fungal and/or bacterial growth was detected.

Table 1. Elemental arsenic concentration in plants

Species	Age (days)	Exposure (days)	Group	# specimens	Sample size (g)	As Dry weight ($mg.kg^{-1}$)
Brassica oleracea var. *capitata*	54	12	control	5	0.0858	< 1
			treated	5	0.1010	22 ± 1
Brassica oleracea var. *acephala*	65	49	control	23	0.2552	< 0.06
			treated	15	0.1235	15.0 ± 0.5

The results presented in Table 1 show that *B. oleracea* var. *capitata* and *B. oleracea* var. *acephala* are capable of surviving in an environment with arsenic, absorbing and accumulating arsenic. Considering that these taxa are edible, further experiments should be designed, aiming at the production of fully grown individuals, which was not possible up to now.

Maintenance of whole specimens in M&S for long periods is not an easy task to be achieved, even under axenic conditions [25]; other authors did not treat *B. juncea* for longer than 7 days [22], and, so far, it was not found treatments with M&S for periods as long as the ones that were achieved by our group. Growth and survival problems showed that the composition of M&S had to be altered, in order to have samples large enough to be properly analysed (see Table 1).

The utilisation of a substrate was necessary, but, most probably, when certain nutrients, present as contaminants in vermiculite, were spent, growth was stopped, leading to loss of material. In this sense, *Lobularia* was most sensitive, followed by *Raphanus sativus* (both kinds), *Eruca sativa*, and *B. oleracea* var. *botrytis*; this might indicate that studies with these species demand different approaches, which might lead even to different composition of fertilisers.

The use of vermiculite was necessary due to hygroscopic properties of this material, which are more appropriate than washed sand; plus, the presence of some elements like Ca, Fe, Si that are part of the its composition. The ideal experimental condition would be the one where there is absolute control on what is provided to the plants, since the presence of Fe, as an example, might interfere with arsenic absorption [26] and explain why samples of kale cultivated in the Iron Quadrangle, in a site with soil contaminated with arsenic, did not present arsenic in concentrations above the detection limits [17].

Also, it would be ideal to have samples large enough to analyse roots and aerial parts separately, since it is part of the resistance mechanisms of some species to adsorb the metalloid to the roots, without absorption to other parts of the plant [27].

A typical Ni deficiency was observed: leaves with brown tips; the inclusion of this element in the composition of M&S has been suggested quite recently [28], and its change of status, from beneficial element to micronutrient (essential element in trace amounts) is also recent (for a review, see [29]), and controversial. The fact that Ni is essential to Brassicaceae is not that surprising, considering that some other species of Brassicaceae are Ni hyper-accumulators [29]; but, so far, that was not found in the literature for the taxa studied.

Although the addition of Ni was beneficial, in a set of growth experiments, following the recommendations in [28], ideal growth was not achieved, denoting that some other deficiency had to be solved. The addition of silicon, according to [31-32], allowed normal growth of our specimens, including *Lobularia*.

Silicon is not considered, yet, as an essential element to plants, although its importance has been acknowledged for a certain time. It has been put in the background, in the initial investigations in Plant Nutrition, at the end of 19th century, due to difficulties in experimental design, since it is a common contaminant in water, even distilled water, in many laboratories; for a review in this matter, see [28; 30; 31].

4. CONCLUSIONS

At the present moment, after the addition of Ni and Si, all the taxa that did not provide samples enough, were planted again, in vermiculite, were successfully treated and are waiting to be analysed in the near future, not only by NAA, but also by other methods, allowing us to start arsenic speciation studies with the taxa proposed.

Preliminary experiments are being carried out, aiming at the growth in M&S, without any substrate, but with the addition of Ni and Si; so far, it seems to be feasible to grow plants in M&S alone, until their whole life cycle is completed.

Last, but not least, it should be noted that when analysing samples by neutron activation, they are not heated, nor dissolved by any chemical treatment, procedures that might lead to loss of arsenic that is present. Therefore, comparison with data from other authors, like the results from some Brassicaceae in [33], which were derived from 50º C oven-dried samples, becomes unreliable.

Furthermore, plants should be better identified, specially crops, considering that, like in cabbage and others, there are many kinds, suited for early or late production, for winter or summer, etc; this is a commonly overlooked information, as can be seen in [33]. One never knows which genes might be altered, selected, or eliminated, by chance, in the process of producing new crops, along with desired characteristics; some might be part of arsenic metabolism.

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