INAA applied to *Tradescantia pallida* plant study for environmetal pollution monitoring*)

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In this study, INAA was applied to analyse *T. pallida* plant in order to establish an adequate protocol for its sampling and treatment and so that it can be used for further environmental biomonitoring. Concentrations of most elements obtained in leaves, stems and flowers plus peduncles separately were found to have the same magnitude. Results obtained in leaf samples collected in several sites with distinct levels of pollution indicated that the cleaning of the sample must be done to eliminate dry and wet deposition on leaf surfaces. Morevover, concentrations of elements accumulated by plant depend on leaf age. Results obtained in this study have indicated the viability of using *T. pallida* in environmental pollution biomonitoring.

1 Introduction

Pollution increase in environment has led to experimental methods optimisation to evaluate contaminant levels and to identify its source. In this context, several plant species have been extensively investigated with the prospect to be used as toxic element biomonitors.

In the element analysis of plant samples, sampling and their preparation including cleaning, drying and homogenisation are very important steps in the analytical procedure to provide meaningful interpretation of the environmental elements. The errors caused during sampling and sample preparation are pointed out in several papers [1-4].

Among several plants present in our ecosystem, *Tradescantia pallida* (Rose) D.R. Hunt. cv. purpurea Boom from Commelinaceae family was chosen since it is easily sampled, cultivated and propagated. It is a decorative plant found in gardens and even in places with high pollution levels like streets and avenues.

Besides, in recent years *Tradescantia* plants have become widely acknowledged for the detection of clastogenic effects of heavy metal ions by micronucleus assay and it is suggested to biomonitor metal-contaminated soils [5]. The nuclei of plant DNA molecules, which are submitted to high pollution levels, are split in micronuclei so that *T. pallida* might be used as an environmental pollution indicator. The number of micronuclei increases with the air pollution, that is, the more DNA molecules split, the more polluted is the air.

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This work presents results obtained in the study of sampling and sample preparation of *Tradescatia pallida* plant chosen for toxic element biomonitoring in the atmospheric environment. The following parameters were studied to establish a protocol to use this plant species: selection of plant parts to be used for biomonitoring, treatment of the plant material including washing and grinding the sample and the effect of plant age on the elemental concentrations.

2 Experimental

The elemental concentrations obtained in stems, flowers plus peduncles, and leaves, separately, proved that the same elements can be detected in these three parts of *T. pallida* plant. Therefore leaves were selected since they can be more easily collected and prepared for the analysis than the other parts. The leaves were collected in the third knot from the edge of the stems and placed in a paper envelope instead of plastic bags to avoid the formation of mould on the sample surface.

Leaf samples were cleaned by wiping the leaf surfaces with a stuffing soaked with milliQ water, freeze dried and then ground manually in an agate mortar to obtain in a powder for analyses. In the drying process there was a mean weight loss of about 94.7%. Precautions were taken to avoid the contamination of the samples.

For INAA, 100-180 mg of each sample were weighed in polyethylene involucres and irradiated with synthetic elemental standards. A pneumatic transfer system of the IEA-R1 nuclear reactor with a thermal neutron flux of 4×10^{11} n cm^{-2} s^{-1} was used for short 5-minute irradiations in order to determine Ba, Cl, K Mg, Mn, Na and Sr. Longer irradiations of 16 h under thermal neutron flux of about 10^{12} n cm^{-2} s^{-1} were carried out for As, Ba, Br, Ca, Ce, Co, Cr, Fe, K, La, Rb, Sb, Sc, Sr, Th and Zn determinations.

After adequate decay times, gamma ray measurements were performed using a Canberra GX2020 hyperpure Ge detector which was coupled to Model 1510 Integrated Signal Processor and System 100MCA Card, both from Canberra. The detector used had a resolution (FWHM) of 0.9 keV for 122 keV gamma rays of ^{57}Co and 1.78 keV for 1332 keV γ rays of ^{60}Co. Samples and standards were measured at least twice and the sample-to-detector distances of 3.0 and 0.5 cm were used for first and second measurements, respectively. The γ-ray spectra were processed using VISPECT software[6] that evaluates peak areas (counting rates) and γ-ray energies. The standard comparative method was used for calculating the elemental concentrations.

Certified reference materials IAEA 336 Lichen and NIST 1547 Peach Leaves were irradiated with the samples and analysed to control the quality of the results. The relative standard deviations and relative errors were lower than 10 % for most of elements analysed.

3 Results and discussion

Results obtained in the analyses of leaves, flowers + peduncles and stems of *T. pallida* species indicated that the same elements can be detected in these three parts (Table 1) and most of elements were found at the same magnitude in these three parts of *T. pallida*. The exception was Mn that was found at the highest concentrations in stems. From the point

Table 1. Elemental concentrations found in the analysis of leaves, stems and flowers plus peduncles of *T. pallida* plant

Element	Leaves	Flowers + peduncles	Stems
Br, $\mu g\ g^{-1}$	38.8 ± 0.3*	20.3 ± 0.2	35.4 ± 0.2
Ca, %	6.5 ± 0.2	2.4 ± 0.1	6.9 ± 0.2
Cl, %	1.87 ± 0.04	1.64 ± 0.05	3.2 ± 0.1
Fe, $\mu g\ g^{-1}$	100 ± 20	273 ± 18	200 ± 19
K, %	1.5 ± 0.1	3.5 ± 0.1	1.24 ± 0.01
La, $\mu g\ k\ g^{-1}$	407 ± 3	221 ± 2	97 ± 2
Mg, %	1.03 ± 0.05	0.84 ± 0.03	1.40 ± 0.05
Mn, $\mu g\ g^{-1}$	60.1 ± 1.9	84.6 ± 4.1	652 ± 12
Na, $\mu g\ g^{-1}$	20.06 ± 0.06	49.03 ± 0.13	23.53 ± 0.09
Rb, $\mu g\ g^{-1}$	20.7 ± 1.1	40.4 ± 1.1	20.9 ± 0.9
Sb, $\mu g\ k\ g^{-1}$	42 ± 4	65 ± 3	20 ± 3
Zn, $\mu g\ g^{-1}$	122 ± 2	65 ± 1	112 ± 2

*- Uncertainties evaluated using statistical counting errors of standard and sample

of view of sample collection and treatment, our experiment work showed that leaves are the easiest ones to be collected, cleaned and obtained in a powder form and so leaves were chosen for our study.

The results obtained in replicate analyses for three leaf samples collected in different sites, presented in Table 2, shows that the reproducibility was better than 10% for most of elements analysed, which demonstrates the homogeneity of the samples prepared.

Table 3 shows the mean values obtained in the analyses of ten samples of young and old leaves of *T. pallida* plant cultivated in vases using the soil with the same composition and kept in the same place. These results showed that concentrations of Ce, Fe, La, Sb, Sc and Zn in old leaves were slightly higher than those presented in the young ones and for the elements Ca, Cl, Co, Cr, K, Mn, Rb and Sr there was no difference related to the leaf age. In order to avoid the effect of the leaf age in concentrations of elements, it was decided to collect leaf samples in the third knot from the edge of the stems

The cleaning effect of *T. pallida* leaves on the elemental concentrations were studied by analysing washed and unwashed samples collected in two places with different levels of pollution. The washing procedure consisted of wiping the leaf surfaces with a stuffing cotton soaked with milliQ water and then these leaves were kept for about 1 min immersed in milliQ water. Results obtained in the analyses of washed and unwashed samples are presented in Table 4. The unwashed samples presented concentrations of most elements of the same magnitude or slightly higher than the washed ones. The washing effect is important mainly for samples collected in polluted places. These results indicate that the washing procedure should be performed to eliminate dry and wet depositions on the leaf surfaces.

Table 2. Mean values of elemental concentrations obtained in replicate analysis of *T. pallida* leaves

Element	Sample 1		Sample 2		Sample 3	
	x ± s*	sr, % **	X ± s	sr, %	x ± s	sr, %
Br, μg g^{-1}	40 ± 3	7.5	57 ± 2	3,5	37 ± 1	2.7
Ca, %	3.3 ± 0.3	9.1	3.7 ± 0.3	8.1	3.8 ± 0.1	2.6
Ce, μg kg^{-1}	364 ± 16	4.4	754 ± 12	1.6	583 ± 14	2.4
Co, μ g kg^{-1}	48 ± 3	6.2	90 ± 2	2.2	83 ± 1	1.2
Cr, μ g kg^{-1}	219 ± 20	9.1	488 ± 5	1.0	202 ± 7	3.4
Fe, μ g g^{-1}	100 ± 10	10.0	323 ± 16	4.9	117 ± 2	1.7
Hf, μ g kg^{-1}	23 ± 3	13.0	104 ± 4	1.2	66 ± 4	6.0
K, %	4.3 ± 0.3	7.7	3.5 ± 0.2	5.7	2.2 ± 0.1	4.5
La, μ g kg^{-1}	142 ± 13	9.1	379 ± 9	3.8	253 ± 6	2.4
Na, μ g g^{-1}	2088 ± 236	11.3	157 ± 6	3.8	31 ± 4	12.9
Rb, μ g g^{-1}	40 ± 2	5.0	46 ± 6	13.0	22.1 ± 0.7	3.1
Sb, μ g kg^{-1}	29 ± 3	10.3	90 ± 3	3,2	34 ± 2	5.9
Sc, μ g kg^{-1}	13.6 ± 0.4	2.9	49 ± 2	4.0	8.1 ± 0.5	6.1
Sm, μ g kg^{-1}	5.7 ± 0.6	10.5	29.0 ± 0.3	1.0	16 ± 1	6.2
Th, μ g g^{-1}	20 ± 2	10.0	90 ± 1	1.1	23 ± 1	4.3
Zn, μ g g^{-1}	56 ± 4	6.1	193 ± 6	3.1	63 ± 4	6.3

x ± s = Arithmetic mean and standard deviation of 3 or 4 determinations; s_r = relative standard deviation

Table 3. Elemental concentrations in young and old leaves of *T. pallida*

Element	Young leaves		Old leaves	
	x ± s	Range	x ± s	Range
Br, g g^{-1}	42 ± 14	77 – 21	26 ± 7	36.5 – 14.8
Ca, %	2.5 ± 0.4	3.05 – 1.91	2.3 ± 0.3	2.7 – 1.9
Ce, μg kg^{-1}	471 ± 281	1174 – 281	974 ± 323	1396 – 251
Cl, %	0.88 ± 0.17	1.15 – 0.69	0.74 ± 0.23	1.12 – 0.41
Co, μ g kg^{-1}	180 ± 91	376 – 73	245 ± 97	445 – 130
Cr, μ g kg^{-1}	194 ± 125	502 – 94	254 ± 112	353 – 127
Fe, μ g g^{-1}	84.5 ± 30.4	172 –70	124 ± 31	167 – 64
K, %	4.36 ± 2.43	10.9 – 2.2	3.12 ± 1.04	5.27 – 1.84
La, μ g kg^{-1}	286 ± 205	806 – 136	568 ± 232	927 – 114
Mn, μ g g^{-1}	140 ± 57	245 – 89	133 ± 70	248 – 51
Na, μ g g^{-1}	50.7 ± 15.7	75.2 – 25.4	85.9 ± 42.1	163 – 35
Rb, μ g g^{-1}	37.8 ± 14.9	66.0 – 13.5	23.0 ± 10.4	38.4 – 10.9
Sb, μ g kg^{-1}	55 ± 45	173 – 17	136 ± 62	282 – 29
Sc, μ g kg^{-1}	8.2 ± 5.4	23.0 – 4.3	15.3 ± 5.1	25.8 – 6.6
Sr, μ g g^{-1}	286 ± 98	519 – 166	274 ± 65	392 – 211
Zn, μ g g^{-1}	109 ± 61	278 – 63	149 ± 35	192 – 77

* Arithmetic mean and standard deviation of results obtained in 10 samples cultivated in 10 different vases.

Table 4. Concentrations of elements in washed and unwashed *T. pallida* samples

Element	Sample from clean place (greenhouse)		Sample from polluted area	
	washed	unwashed	washed	Unwashed
As, $\mu g\ kg^{-1}$	n.d.	n.d.	95 ± 6	175 ± 6
Br, $\mu\ g\ g^{-1}$	40 ± 3	19 ± 2	57 ± 2	51 ± 1
Ca, %	3.3 ± 0.3	3.8 ± 0.6	3.7 ± 0.3	5.3 ± 0.2
Ce, $\mu\ g\ kg^{-1}$	364 ± 16	404 ± 43	754 ± 12	1323 ± 19
Co, $\mu\ g\ kg^{-1}$	48 ± 3	55 ± 8	90 ± 2	157 ± 2
Cr, $\mu\ g\ kg^{-1}$	219 ± 20	220 ± 20	488 ± 5	1404 ± 4
Fe, $\mu\ g\ g^{-1}$	100 ± 10	141 ± 11	323 ± 16	557 ± 29
Hf, $\mu\ g\ kg^{-1}$	23 ± 3	42 ± 2	104 ± 4	121.5 ± 0.7
K, %	4.3 ± 0.3	4.5 ± 0.3	3.5 ± 0.2	3.4 ± 0.2
La, $\mu\ g\ kg^{-1}$	142 ± 13	211 ± 9	379 ± 9	666 ± 26
Rb, $\mu\ g\ g^{-1}$	40 ± 2	30 ± 1	46 ± 6	44 ± 5
Sb, $\mu\ g\ kg^{-1}$	29 ± 3	29 ± 4	90 ± 3	153 ± 7
Sc, $\mu\ g\ kg^{-1}$	13.6 ± 0.4	26 ± 2	49 ± 2	111 ± 3
Th, $\mu\ g\ g^{-1}$	20 ± 2	44 ± 2	90 ± 1	202 ± 6
Zn, $\mu\ g\ g^{-1}$	56 ± 4	53 ± 4	193 ± 6	252 ± 9

n.d. = not detected

4 Conclusions

T. pallida is a plant of easy cultivation and propagation, so that, to avoid soil composition influence on elements uptaken by this plant, it can be cultivated in vases using the same lot of soil and after six months, distributed in different sites for environmental pollution monitoring.

Results obtained in this work indicate the feasibility of using *T. pallida* as biomonitor of environmental pollution and conditions established in this work for sampling and sample treatment can be adopted in its analysis.

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