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NATURAL RADIOACTIVITY DETERMINATION IN SAMPLES OF *Peperomia pellucida* COMMONLY USED AS MEDICINAL HERB

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ABSTRACT

The radionuclide concentration in different environmental matrixes is reported in literature; however, studies of the distribution of ^{238}U and ^{232}Th decay products in plant species are sparse. In this study concentration of naturally occurring radionuclides ^{238}U, ^{234}U, ^{232}Th, ^{230}Th, ^{226}Ra, ^{228}Ra and ^{210}Pb was determined in sample of *Peperomia pellucida* and in the surrounding soil. *Peperomia pellucida* has a rich history of medicinal uses. The content of U and Th isotopes in the leaves, aerial parts, roots and soil was determined by alpha spectrometry after with radiochemical separation by ionic exchange resins and measurement with a silicon surface-barrier detector. The radionuclides measurement of ^{226}Ra, ^{228}Ra and ^{210}Pb were carried out by gross alpha and beta counting after radiochemical separation. The radionuclide activity concentrations mean in samples analyzed ranged from 4.3 to 38 Bq kg^{-1} for ^{238}U, from 42 to 129 Bq kg^{-1} for ^{234}U, from 2.1 to 38 Bq kg^{-1}for ^{230}Th, from 1.7 to 124 Bq kg^{-1} for ^{232}Th, from 8.5 to 37 Bq kg^{-1}for ^{226}Ra, from 3.2 to 46 Bq kg^{-1}for ^{228}Ra, from 39 to 93 Bq kg^{-1} for ^{210}Pb. It was also determined the percentage of radionuclides ^{226}Ra, ^{228}Ra and ^{210}Pb in the extraction and infusion of the drug analyzed. The arithmetical mean value recovery was from 23% to 60% in maceration and 24% to 75% in infusion.

1. INTRODUCTION

The therapeutic use of medicinal plants in human health is as ancient practice [1]. In recent times, the study of medicinal plants has become the focus of research ever more extensive in all over the world, due to the diversity and potential that plants have as source of medicinal products.

Generally, the studies related with therapeutic plants aim to characterize the active compound of the plant for scientific evidence of its therapeutic properties [2]. Therefore, studies of the distribution of ^{238}U and ^{232}Th natural radionuclides series in plant of the species are sparse.

An emerging problem in many development countries is industrial pollution that threatens the health of the local environment and can lead to contamination of vegetation with heavy

metals, pesticides, or radioactivity [3]. There are several sources contributing to plants contamination which can result from direct deposition of radioactive particles from the atmosphere onto the above-ground parts, indirect sorption of radionuclides from the soil by the root system, also resuspension and deposition of radionuclides in the soil.

The presence of radionuclides in plants constitutes the pathway for their migration to the human. A medicinal plant containing high concentrations of these radionuclides can cause health problems, since medicinal plants are commonly used for long periods of oral treatment and via uptake of remedies made with medicinal herbs.

In this study, concentration of naturally occurring radionuclides ^{238}U, ^{234}U, ^{232}Th, ^{230}Th, ^{226}Ra, ^{228}Ra and ^{210}Pb was determined in sample of *Peperomia pellucida* and in the surrounding soil. *Peperomia pellucida* (L.) KUNT, whose popular name in Brazil is “erva-de-jabuti”, is a plant known by its medicinal properties like healing property, analgesic activity, antibacterial activity and antifungal activity against food fungi [4,5,6].

The content of U and Th isotopes in the aerial parts, roots and soil was determined by alpha spectrometry after radiochemical separation by ionic exchange resins and measurement with a silicon surface-barrier detector. The radionuclides measurement of ^{226}Ra, ^{228}Ra and ^{210}Pb were carried out by gross alpha and beta counting after radiochemical separation. The percentage of ^{226}Ra, ^{228}Ra and ^{210}Pb extraction from medicinal plant into maceration and infusion is also evaluated in this study.

2. EXPERIMENTAL

2.1. Samples

Peperomia pellucida specimens and the surrounding soil were collected at the Botanical Garden in Rio de Janeiro, Brazil, in December 2010 and identified by Dr. Elsie F. Guimarães. A voucher specimen was deposited at the Ibirapuera Park Herbarium.

2.1.1. Preparation of the samples

Samples of *P. pellucida* were washed with ultrapure water to remove impurities and soil particles present in all plant structures, air-dried and separated into leaves, aerial parts (including stems and leaves) and roots. After this processes, the samples of *Peperomia pellucida* and soil were dried at 60°C and ground into powder.

2.1.2. Preparation of the maceration (ethanolic extract) for radiochemical

Dried *Peperomia pellucida* aerial parts were softening in 70% ethylic alcohol during seven days at room temperature. The extract was filtered through Wattman filter paper and the residue was discarded. The filtrate was evaporated near dryness and separated for analysis of radionuclides.

2.1.3. Preparation of the infusion (aqueous extract) for radiochemical

The powder material of *P. pellucida* aerial parts was stored in centrifuge tubes with subsequent addition of 50 ml of Milli-Q® water boiling on the tube. This mixture was homogenized and left to stand for 5 minutes. After this period, the solution was filtered and the residue was discarded. The final solution was transferred to a beaker, placed on plate heated at 150°C, evaporated to near dryness and separated for analysis.

2.2. Analytical methods

Two different analytical techniques were used to determine the natural radionuclides: alpha spectrometry for ^{238}U, ^{234}U, ^{232}Th, ^{230}Th and gross alpha and beta counting after radiochemical separation for ^{226}Ra, ^{228}Ra and ^{210}Pb.

2.2.1. Alpha spectrometry

To determine ^{238}U, ^{234}U, ^{232}Th, ^{230}Th by alpha spectrometry [7], it is necessary to separate these radionuclides from the solution obtained after the complete dissolution of *P. pellucida* (leaves, aerial parts and roots) and the soil samples. To achieve this aim, after addition of a known activity of ^{232}U and ^{229}Th as the yield internal standard, the samples were dissolved in hot plate with $HNO_{3conc.}$ and 30% H_2O_2 Then the solutions were evaporated three times to dryness. After, the precipitates were dissolved with HCl 9 mol L^{-1}, evaporated almost to dryness and re-dissolved in HCl 9 mol L^{-1}. The obtained solution was passed through a pre-conditioned anionic exchange resin column in HCl 9 mol L^{-1} media. The eluate was evaporated to dryness and re-dissolved with HNO_3 8 mol L^{-1}, and passed through a pre-conditioned anionic exchange resin column in HNO_3 mol L^{-1} media. Both, U and Th were eluted with HCl 0,1 mol L^{-1}. After the elution, the samples were evaporated and electroplated in a steel disk during one hour using NH_4Cl as electrolyte [8]. The detection of alpha particles was done with a silicon barrier detector and counted for 60.000 seconds. The mean counting efficiency was 33.6 ± 0,1 and the background was approximately 3 10^{-5} cps in the energy region of interest. The chemical yield resulted from 31% to 61% for U and 30% to 80% to Th. The minimum detectable activity mean was 1.6 10^{-3} Bq kg^{-1} for ^{238}U, 1.7 10^{-3} Bq kg^{-1} for ^{234}U, 1.8 10^{-3} Bq kg^{-1} for ^{232}Th and 1.9 10^{-3} Bq kg^{-1} for ^{230}Th.

2.2.2. Gross alpha and beta counting after radiochemical separation for ^{226}Ra, ^{228}Ra and ^{210}Pb

For the radiochemical separation, the samples of *Peperomia pellucida* (leaves, aerial parts and roots), the ethanolic extract, infusion and soil were dissolved in hot plate with $HNO_{3conc.}$ and 30% H_2O_2 till total elimination of organic matter. Carries of Ba^{2+} and Pb^{2+} were added before the dissolution. The solutions were treated with citric acid for iron and lead complexation. Sulfuric acid was added for sulfate precipitation of Ra^{2+}, co-precipitation as $Ba(Ra)SO_4$ and $PbSO_4$. The precipitate was dissolved with NTA and 6M NaOH was added to achieve a basic medium. The addition of $(NH_4)_2SO_4$ (25 mg mL^{-1}) and glacial acetic precipitates $Ba(Ra)SO_4$ leaving Pb^{2+} in solution. The precipitate is separated in two steps of centrifugation and washing, dissolved with EDTA and precipitated as $Ba(Ra)SO_4$ and filtered

in Millipore filter and stored for counting. Counting was performed after 21 days of precipitation. The solution containing Pb^{2+} was treated with 1M NaS_2 to precipitate PbS. The precipitate is centrifuged, dissolved in nitric acid and filtered for sulfur separation. The addiction of 30% Na_2CrO_4 precipitate $PbCrO_4$ that is filtered in Millipore filter and stored for counting. Counting was performed after 10 days of precipitation. Counts were made in a gas flow proportional detector of low background, Berthold, model Lb 770, during 200 minutes. The procedure was taken from Moreira [9] and Oliveira [10].

3. RESULTS AND DISCUSSION

3.1. Radionuclide concentration

Radionuclides concentrations were determined in reference materials IAEA-300 and IAEA-326. Results obtained were in good agreement with the certified values.

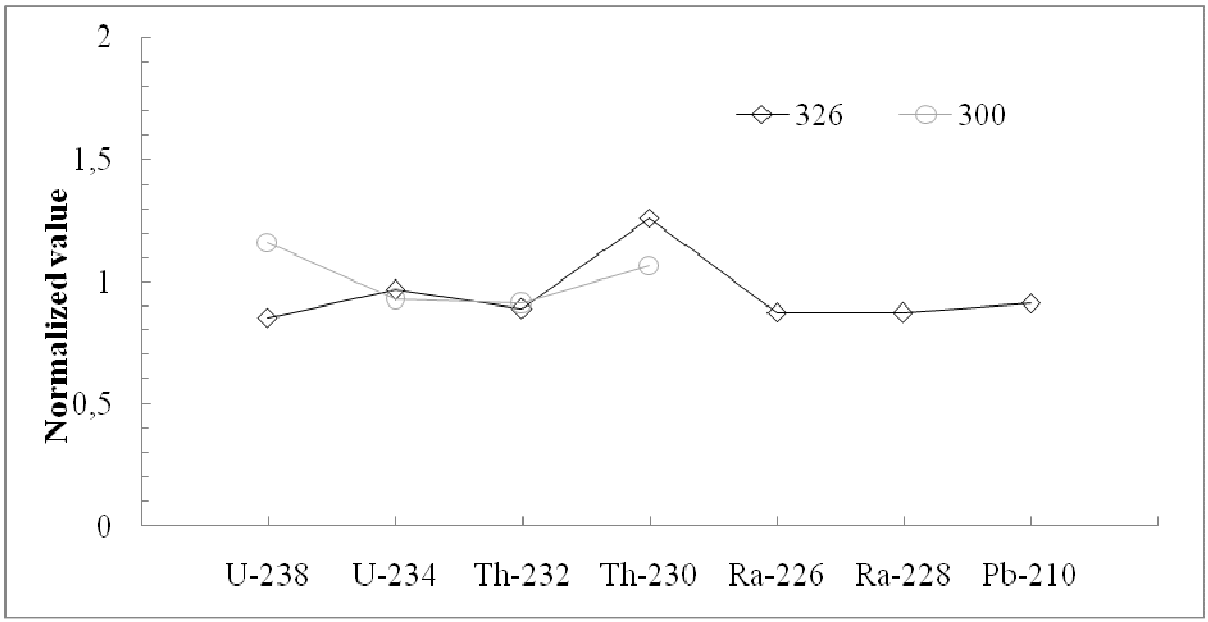

Figure 1. Concentration values measured for reference materials IAEA-300 and IAEA-326 normalized by the respective concentration certified values.

Table 1 shows the activity concentration (Bq kg^{-1}) of the natural radionuclides ^{238}U, ^{234}U, ^{232}Th and ^{230}Th, ^{226}Ra, ^{228}Ra and ^{210}Pb determined in the medicinal plant selected in this study and the surrounding soil (SPEP). The medicinal plant was subdivided into leaves (FPEP), aerial parts (PPEP) and roots (RPEP).

Table 1. Activity concentration (Bq kg^{-1}) of the radionuclides ^{238}U, ^{234}U, ^{232}Th, ^{230}Th determined in aerial parts (PPEP), roots (RPEP) and soil (SPEP).

Samples	^{238}U	^{234}U	^{230}Th	^{232}Th
PPEP	4.1 ±0.7	38 ±3	1.9 ±0.5	1.5 ±0.4
	4.6 ±0.6	45 ±3	1.8 ±0.4	1.9 ±0.5
	4.2 ±0.8	44 ±3	2.7 ±0.6	1.8 ±0.5
Mean ± RSD	4.3 ±0.3	42 ±4	2.1 ±0.5	1.7 ±0.2
RPEP	7.5 ±1.6	126 ±11	7.4 ±1.3	7.8 ±1.4
	7.7 ±1.2	133 ±13	6.9 ±1.4	6.5 ±1.3
	7.3 ±1.4	129 ±10	7.1 ±1.4	6.7 ±1.3
Mean ± RSD	7.5 ±0.2	129 ±4	7.1 ±0.3	7.0 ±0.7
SPEP	43 ±3	62 ±4	40 ±6	125 ±12
	34 ±4	65 ±6	40 ±4	125 ±8
	38 ±3	64 ±7	34 ±4	122 ±9
Mean ± RSD	38 ±5	64 ±2	38 ±3	124 ±2

The data shows that activity concentration mean ranged from 4.3 Bq kg^{-1} to 38 Bq kg^{-1} for ^{238}U. The highest activity concentration was found in the roots comparing the plant parts. ^{234}U activity concentration ranged from 42 Bq kg^{-1} to 129 Bq kg^{-1}. The generally trend of the radionuclides concentration is the following: soil > roots > aerial parts. ^{230}Th ranged from 2.1 Bq kg^{-1} to 38 Bq kg^{-1}. The concentration trend of these radionuclides is the following: > soil > roots > aerial parts. The activity concentration of ^{232}Th ranged from 1.7 Bq kg^{-1} to 124 Bq kg^{-1}. Average concentration for ^{232}Th in plants is about 0.2 Bq kg^{-1}, one order of magnitude lower than those found is the studied plant [11]. The world average of ^{238}U and ^{232}Th radionuclides content in soil is 35 Bq kg^{-1} and 30 Bq kg$^{-1,}$ respectively [12]. The level of ^{232}Th analyzed in this study present much higher concentration than that presented in soil content.

Table 2 shows the activity concentration (Bq kg^{-1}) of the natural radionuclides ^{226}Ra, ^{228}Ra and ^{210}Pb determined in leaves (FPEP), aerial parts (PPEP), roots (RPEP) and soil (SPEP).

Table 2. Activity concentration (Bq kg^{-1}) of the radionuclides ^{226}Ra, ^{228}Ra and ^{210}Pb determined in leaves (FPEP), aerial parts (PPEP), roots (RPEP) and soil (SPEP).

Samples	^{226}Ra	^{228}Ra	^{210}Pb
FPEP	nd	nd	39 ±5
PPEP	8.5 ±0.2	3.2 ±0.3	52 ±3
RPEP	23.2 ±0.2	10 ±1	58 ±6
SPEP	37 ±1	46 ±4	93 ±3

Activity concentrations of ^{226}Ra are higher than ^{228}Ra in the samples, except for soil (SPEP). As the former belong to ^{238}U and the last to ^{232}Th decay series, these results are in accordance with those obtained for ^{238}U and ^{232}Th in table 1. Radium in nature exists in soil, rock, surface water, plants and animals at low concentration generally lower than 37 Bq kg^{-1} [11].
The ^{210}Pb concentration ranged from 39 Bq kg^{-1} to 93 Bq kg^{-1}. The concentration trend of these radionuclides in the samples of *Peperomia pellucida* is the following: RPEP > PPEP > FPEP. Leads occur naturally in plants as a result of uptake, but the incorporation of ^{210}Pb by above-ground plant-part occurs mainly via atmospheric deposition, and that the transfer via the root system is rather small [13].
For comparison purpose, reference values for diary intake of some kind of foods are presented in Table 3 [12].

Table 3. Reference activity concentrations of natural radionuclides in food.

	Activity Concentration (mBq kg^{-1})					
	^{238}U - ^{234}U	^{230}Th	^{232}Th	^{226}Ra	^{228}Ra	^{210}Pb
Milk	1	0.5	0.3	5	5	15
Meat	2	2	1	15	10	80
Grain	20	10	3	80	60	50
Leafy vegetables	20	20	15	50	40	80
Root vegetables and fruits	3	0.5	0.5	30	20	30
Drinking water	1	0.1	0.05	0.5	0.5	10

The Figure 2 shows a comparison of the activity concentration of ^{238}U, ^{234}U, ^{232}Th, ^{230}Th, ^{226}Ra, ^{228}Ra and ^{210}Pb in medicinal plant parts.

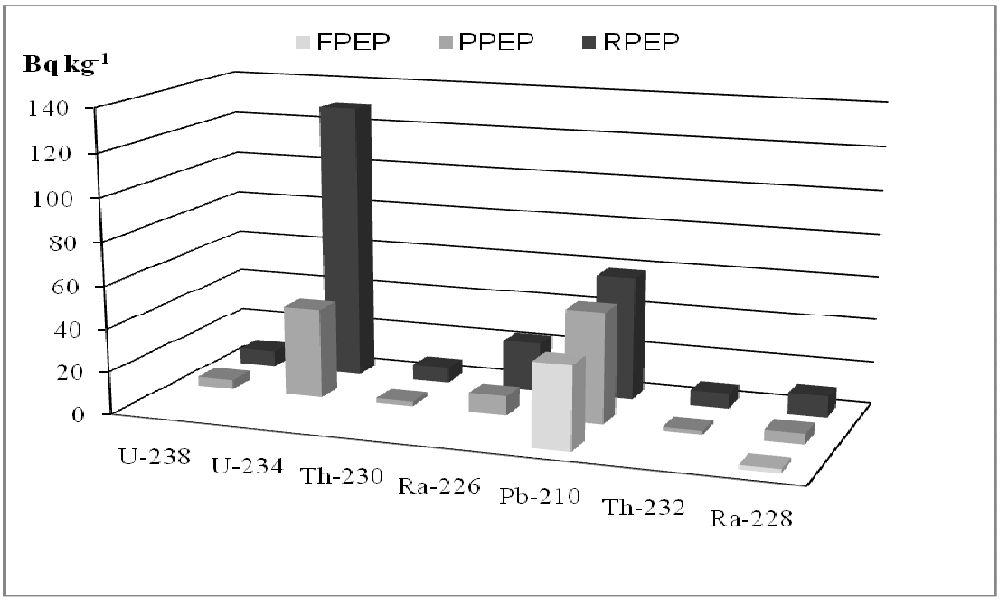

Figure 2. Activity concentration of ^{238}U, ^{234}U, ^{232}Th, ^{230}Th, ^{226}Ra, ^{228}Ra and ^{210}Pb in medicinal plant parts.

Although there are many uncertainties about the specificity of metals absorption mechanisms by the plants, usually the content and the accumulation of the elements in plant tissue and roots depending on their availability in the soil solution [14]. It is observed that there is a greater accumulation of radionuclides in the roots and with higher concentration for ^{234}U. This accumulation could be related to immobilization of these elements in organic complexes present in the radicular tissue [14]. *P. pellucida* has higher concentrations of ^{210}Pb in all the analyzed plant part. This can be explained because this radionuclide is a decay product of ^{222}Rn, a noble gas which emanate continuously from the soil to the atmosphere, and can be deposited on vegetation and incorporated by foliar or root absorption [9].
The results show that the ^{238}U and ^{232}Th radionuclides series are not in secular equilibrium.
Figure 3 shows the results of percentage of ^{226}Ra, ^{228}Ra and ^{210}Pb extraction in maceration (EPEP) and infusion (IPEP) carried out on sample of *Peperomia pellucida* and the comparison between maceration and infusion extraction. The percentage trend is following: IPEP > EPEP.
This difference is related to the interaction of metals with substances in plant structures, such as macromolecules and small molecules and the solubility of metals in different solvents [15].

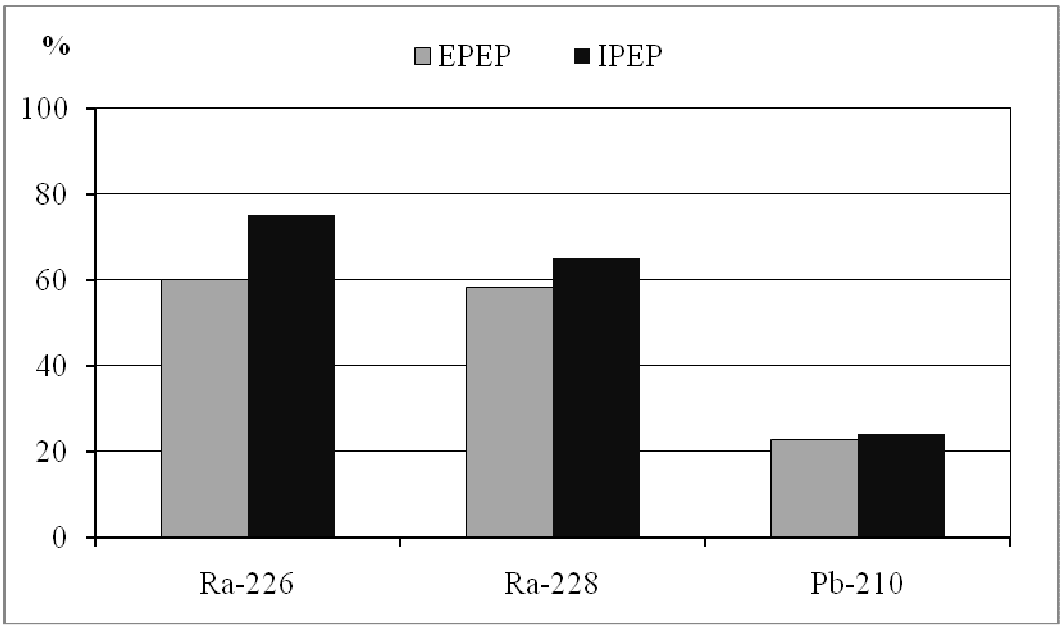

Figure 3. Percentage of ^{226}Ra, ^{228}Ra and ^{210}Pb extraction in maceration and infusion.

4. CONCLUSIONS

In this paper, a study of the natural radioactivity in sample of *Peperomia pellucida* is presented. ^{238}U, ^{234}U, ^{232}Th and ^{230}Th were determined by alpha spectrometry and ^{226}Ra, ^{228}Ra and ^{210}Pb were determined by gross alpha and beta counting after radiochemical separation. The percentage of ^{226}Ra, ^{228}Ra and ^{210}Pb extraction in maceration and infusion was also determined.

As no reference values for radionuclides in medicinal herbs are available, the reference values for the analyzed elements in some foods presents in a normal diet, were also presented. The level of radionuclides analyzed in this study present much higher concentrations than that presented in the day-to-day diet.
Considering the method used for extracting of active compound from medicinal plants for the production of phytotherapics and the way the herbs are consumed by the population, the study of radionuclides concentration in medicinal plants has great significance.

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REFERENCES

1. Alvim, N. A. T., Ferreira, M. A., Cabral, I. V., Filho, A. J. A., "O uso de plantas medicinais como recurso terapêutico das influências da formação profissional às implicações éticas e legais de sua aplicabilidade como extensão da prática de cuidar realizada pela enfermeira," *Rev Latino-am Enfermagem,***14,** pp.3 (2006).
2. Naidu, G.R.K., Denschlag,H.O., Mauerhofer, E., Porte, N., Balaji T., "Determination of macro, micro nutrient and trace element concentrations in Indian medicinal and vegetable leaves using instrumental neutron activation analysis," *Appl. Radiat. Isot*, **50**, pp.947-953 (1999).
3. Desideri, D. Meli, M. A. Roselli, C., "Natural and artificial radioactivity determination of some medicinal plants," *Journal of Environmental Radioactivity*, **101**, pp. 751-756 (2010).
4. Bayma, J. C., Arruda, M. S. P., Müller, A. H., Arruda, A. C., Canto, W. C., "A dimeric ArC_2 compound from *Peperomia pellucida*," *Phytochemistry*, **55**, pp.779-782 (2000).
5. Khan, A., Rahman, M., Islam, M. S., "Neuropharmacological effects of *Peperomia pellucida* leaves in mice," *Daru*, **16**, n.01 (2008).
6. Arrigoni-Blank, M. F. A., Dmitrieva, E. G., Franzotti, E. M., Antoniolli, A. R, Andrade, M. R, Marchioro, M., "Anti-inflamatory and analgesic activity of *Peperomia pellucida* (L.) HBK (Piperaceae)," *Journal of Ethnopharmacology*, **91**, pp.215-218 (2004).
7. Alencar, M. M., "Determinação de isótopos de urânio e tório em amostras de liquens *Carnoparmelia texana,"* Dissertação de Mestrado, Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brasil (2008).
8. Silva, P. S. C. "Determinação de radionuclideos pertencentes às séries naturais de ^{238}U e ^{232}Th nos fosfatos do Arquipélago de Abrolhos (BA)," Dissertação de Mestrado, Instituto Astronômico e Geofísico da Universidade de São Paulo, Brasil (1998).
9. Moreira, S. R. D., "Determinação de ^{210}Pb em águas minerais da cidade de Águas da Prata," Dissertação de Mestrado, Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brasil (1993).
10. Oliveira, J., "Determinação de ^{226}Ra e ^{228}Ra em águas minerais de região de Águas da Prata", Dissertação de Mestrado, Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brasil (1993).
11. ANL. Aragonne National Laboratory, Human Health Fact Sheet (2005).
12. UNSCEAR (2000) Exposures from natural radiation sources.

13. Bunzl, K., Trautmannsheimer, M., “Transfer of ^{238}U, ^{226}Ra and ^{210}Pb from slag-contaminated soils to vegetables under field conditions,” *The Science of the Total Environment*, **231**, pp.91-99 (1999).
14. Silva, M. L. S., Vitti, G. C., Trevisan, A. R., “Concentração de metais pesados em grãos de plantas cultivadas em solo com diferentes níveis de contaminação,” *Pesq. agropec. bras*, **42**, pp.527-535 (2007).
15. Veiga, M. A. M. S., Silva, A. A., Nascimento, A. N., Barreiros, A. L. B. S., Yoshida, M., “Avaliação da distribuição de metais em folhas de Copaifera multijuga por extração sequencial e detecção por ICP-MS,” 30ª Reunião Anual da Sociedade Brasileira de Química, Águas de Lindóia, São Paulo, 2007.