Neutron Activation Analysis of Biological Samples at the Radiochemistry Division of IPEN-CNEN/SP

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ABSTRACT

Neutron activation analysis is a very useful method for determination of a great number of elements in biological samples. At the Radiochemistry Division of the IPEN-CNEN/SP, this method is being extensively applied to study several materials, such as extracts from medicinal plants, human hair, snake venoms, human lungs, foodstuffs, and corn samples. Both instrumental neutron activation analysis (INAA) and radiochemical neutron activation analysis (RNAA) are used to analyze real samples, as well as biological standard reference materials to evaluate the accuracy and precision of the results.

Index Entries: Neutron activation analysis (NAA); medicinal plants; human hair; human lung; snake venoms; foodstuffs.

INTRODUCTION

With the increasing knowledge of the function of elements in living organisms, the determinations of trace elements in biological systems have become of great interest. In this respect, NAA plays an important role owing to its accuracy, precision, sensitivity, multielemental character, and for some elements, speed of analysis.

The purely instrumental mode of NAA based on measurement of γ activities of radionuclides produced by neutron irradiation is widely used for the determination of several elements in biological materials.

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However, for the determination of some elements of interest, such as As, Cr, Hg, Sb, and Se, present at very low concentrations, radiochemical separation is required to eliminate interfering activities and to improve in this way sensitivity and accuracy of analysis.

This article describes the most recent investigations using INAA and RNAA of biological materials performed at the Radiochemistry Division of IPEN-CNEN/SP in collaboration with universities, research institutions, and private medical clinics. Extracts from Brazilian medicinal plants were analyzed as a contribution to studies of their toxicity, medicinal action, or active components *(1)*. Investigations concerning medicinal plants have been of great importance, since their use is widespread in Brazil.

Human head hair was studied as an indicator of nutritional or health status of subjects by determining toxic and essential elements of interest. Total Hg in hair was also determined in order to detect population groups suspected of contamination by this element, mainly by ingestion of contaminated fish *(2)*. Determination of trace elements in snake venoms was carried out to study a role of metals in their toxic action and to characterize snake species *(3)*. Lungs from smokers, nonsmokers, and one stillborn were analyzed to determine normal levels of elements in hilum lymph nodes and pulmonary tissues, as well as to examine correlation between trace elements in these tissues and the smoking habit of the subjects *(4)*. Results of this study are also reported in detail elsewhere in this volume and will not be discussed here *(5)*.

Toxic and other elements were analyzed in Brazilian foodstuffs, such as bread, milk powder, and rice (essential components of the Brazilian diet *[6,7]*), as well as in gelatine samples used by the population for cooking *(8)* and in corns obtained as a result of studies aimed at increasing a nutritional value of the protein contained in this foodstuff *(9)*.

EXPERIMENTAL

Preparation of Synthetic Standards of the Elements

Standard solutions were prepared by dissolving high-purity metals, oxides, or salts of elements with appropriate reagents. Aliquots of multielemental standard solutions were pipeted onto a small sheet of Whatman filter paper and dried in a dessicator at room temperature. In the case of the Hg standard for short irradiation of 1 h, standard solution of this element was pipeted together with thioacetamide solution according to Noguchi et al. *(10)* to avoid losses of Hg during irradiation. The sheets of filter paper were put into polyethylene bags for irradiation. Long irradiations of standard solutions of volatile elements such as Hg, Cr, As, and Se were carried out in the same conditions used for samples, in quartz ampules.

Sample Collection and Preparation

Dried extracts were obtained from the Brazilian medicinal plants: *Cordia Verbenacea DC* (Boraginaceae) with antiinflammatory properties; *Folidago Microglossa DC* (Compositae), known by the name Brazilian Arnica and used as an antiinflammatory and cicatrizing drug; and *Petiveria Alliacea* (Phytolaccaceae), known by the name "Guiné" used in popular medicine "to calm nerves" and as an anesthetic, diuretic, and analgesic drug, too. Fresh materials of these plants were extracted using a 70% alcoholic solution at room temperature. The dark green solution was filtrated and concentrated under reduced pressure to obtain aqueous viscous material that was dried afterward.

Hair samples were collected from patients of a medical clinic and from control group formed by friends, relatives, and students. Hair strands were cut close to the scalp of the occipital region, and the length did not exceed 5 cm. In the laboratory, hair filaments were cut in lengths, smaller than 2 mm, and then they were washed with a solution of nonionic detergent (Triton X 100), acetone, and water *(11)*.

Also the procedure recommended by the IAEA *(12)* was used for washing of hair collected for total Hg determination in two population groups in Brazil that could be at risk with respect to Hg contamination. The two population groups selected for this work were: Indian tribes that consume fish from rivers located in Xingu Park (Amazonic region) and the population that frequently consume fish from the Billing Dam in São Paulo, which is situated near chlor-alkali and other industries.

Snake venoms were obtained at the Instituto Butantan-SP by squeezing the glands and collecting the liquids in Petri dishes. Then the samples were freeze-dried. Special care was taken to avoid their contamination before irradiation, as well to prevent breathing the venoms in or to prevent their contact with the skin. Snake venoms were analyzed from the following species: *Bothrops jararacussu, Crotalus durissus terrificus*, and *Bothrops jararaca*.

Foodstuffs (bread, milk, rice, and gelatine) were collected in the supermarkets of São Paulo city. Corn samples provided by Agronomy School, Piracicaba-SP and by Brazilian Company for Agropecuary Research (EMBRAPA) were sampled by quartering process, rinsed with water and dried at 50°C for 72h.

Irradiation and Counting

Irradiations have been carried out at the IEA-R1 swimming pool-type reactor of IPEN-CNEN/SP in a thermal neutron flux that ranged from 10^{11} to 10^{13} $n/cm^2/s$. Time of irradiation varied from 5 min to 16 h. Samples and standards were counted in close geometry using two counting systems: EG & G Ortec Model GEM 20190 Ge detector or ENERTEC hyperpure Ge detector coupled to an EG & G Ortec 4096 channel pulse height analyzer and EG & G Ortec Model GMX20190 Ge detector coupled to an ADCAM 918A multichannel buffer. Analyses of γ-ray spectra were carried out by using appropriate computer programs.

Instrumental and Radiochemical Neutron Activation Analyses

INAA was applied to analyze several elements in medicinal plant extracts, human head hair, snake venoms, human lung samples, and foodstuffs. To determine As, Cr, Hg, Sb, and Se at very low concentrations, several radiochemical procedures were used after wet ashing of the irradiated samples.

Hg and Se in Brazilian rice grains were determined using distillation in HBr medium and subsequent precipitation of Se by sodium metabisulfite and Hg by thiocetamide *(6,7)*. Inorganic exchanger (HAP) was also used in order to eliminate interferences from ^{24}Na and ^{42}K radioisotopes *(6)*. For determination in gelatine, a radiochemical separation procedure based on solvent extraction of Cr(VI) into tribenzylamine/chloroform solution *(8)* was used.

RNAA using inorganic exchangers (TDO and HMD), anionic resin (Dowex 2X8), and extraction of diethyldithiocarbamates were used for analysis of biological reference materials *(13)*.

RESULTS AND DISCUSSION

In medicinal plant extracts, concentration of the elements Ca, Cl, Mg, and Na was in the percent range, the elements Al, Br, Mn, Rb, and Zn were present at the ppm level, and the elements Co, Cs, and Sb at the ppb level *(1)*. Concentrations of Cl found in extracts from aerial parts were almost the same as those determined in extracts from roots. This fact was not expected, since Cl was found in a higher concentration in leaves than in roots. Extracts analyzed in this article exhibited high concentrations of K and Mg. It is important to note that potassium salts actively participate in constipation and the cardiac rhythm, whereas Mg and Ca can neutralize the acidity and avoid side effects of stomach lesions.

Results of hair analyses from patients of a medical clinic and control group are presented in Table 1. A comparison of geometric means indicates no significant difference between the two groups for concentration of the elements As, Br, Ca, Cr, Cu, Fe, Hg, K, Mg, Mn, and Zn. Nevertheless, the highest concentrations of As, Br, Cl, Cu, K, Mn, and Se as well as the highest variability of results for Cu, Cd, and K were found in the patient group. These hair analyses were used by physicians from the orthomolecular medicine field, together with other clinical exams, to determine how the element variations may contribute to health problems of the patients.

Table 2 presents results obtained for Hg determination of the group of Indians living in the Xingu Park, the group of people living near the

Table 1
Elemental Concentrations in Hair Samples from Patient and Control Groups Obtained by INAA (in ppm Unless Otherwise Indicated)

Element	Patients			Controls		
	Range	X_G x : S_G	n	Range	X_G x : S_G	n
As, ppb	5.5 — 125	27.9 x : 2.0	22	10 — 40	21.8 x : 1.5	14
Br	1.0 — 15.6	3.3 x : 2.1	24	0.46 — 6.04	2.4 x : 2.2	14
Ca	87 — 1820	445 x : 2.5	21	140 — 1788	412 x : 1.9	14
Cd, ppb	0.1 — 590	63 x : 25	6	74 — 800	263 x : 2.4	7
Cl	40 — 2080	463 x : 3.3	24	62.5 — 1339	263.4 x : 2.9	14
Cr, ppb	46 — 256	95 x : 2.3	24	69 — 692	143.9 x : 1.8	13
Cu	0.4 — 168	15.8 x : 3.5	21	4.0 — 50	19.8 x : 2.3	14
Fe	6.5 — 39	14.3 x : 1.7	23	7.9 — 30.0	13.9 x : 1.4	14
Hg	0.17 — 4.37	0.93 x : 2.7	22	0.46 — 4.8	1.3 x : 2.3	14
K	0.5 — 200	6.2 x : 4.3	23	0.53 — 15.4	2.5 x : 2.4	12
Mg	18 — 231	81.3 x : 2.3	9	21 — 251	60.1 x : 2.3	12
Mn	0.13 — 1.9	0.43 x : 2.1	24	0.125 — 0.670	0.4 x : 2.2	14
Na	1.0 — 95	6.7 x : 3.7	24	2.1 — 7.0	3.3 x : 1.5	14
Sb, ppb	6.3 — 120	24.0 x : 2.2	24	11.3 — 125	32.6 x : 2.5	14
Se	0.20 — 150	0.5 x : 3.9	24	0.034 — 9.03	0.47 x : 3.7	13
Zn	102 — 224	160x : 1.2	24	109 — 264	158 x : 1.2	14

X_G x:S_G = Geometric mean and standard deviation.
n = Number of individuals.

Table 2
Summary of the Results Obtained for Mercury Contents in the Hair of the Population Groups Studied *(2)* (in ppm)

Population Group	X + - s	Median	X x : sG	Range
Controls (n = 25)	1.06 + - 0.55	0.96	0.93 x : 1.71	0.20 - 2.5
Indians from Xingu Park (n = 27)	18.5 + - 5.9	18.0	17.6 x : 1.38	6.9 - 34
Population from the Billing Dam (n = 28)	0.88 + - 0.68	0.74	0.71 x : 1.85	0.30 - 3.0

Billing Dam, and for the control group. A comparison of these results shows highly elevated Hg contents in the Indian group, with the Hg mean concentration about 20 times higher than the mean of the control group. Table 3 presents the concentration ranges of the elements found in snake venom analyses, and shows significantly higher Zn concentra-

Table 3
Concentration Range of Elements Obtained in Brazilian Snake Venoms by INAA

Element	*Bothrops jararacussu* (n = 4)	*Crotalus durissus terrificus* (n = 4)	*Bothrops jararaca* (n = 4)
Br, ppm	4.24 — 19.45	2.3 — 8.6	1.3 — 13.6
Ca, ppm	625 — 1661	325 — 441	919 — 1052
Cl, ppm	1184 — 3906	505 — 1256	368 — 768
Cs, ppb	101 — 319	17 — 66	7.8 — 34.6
K, %	1.00 — 1.15	< 0.3	0.04 — 2.62
Mg, ppm	< 970	3340 — 3830	< 970
Na, %	0.763 — 0.947	2.04 — 2.17	1.28 — 2.31
Rb, ppm	56.2 — 82.1	5 — 12	4.4 — 26.3
Sb, ppb	91 — 472	5 — 29	24 — 2296
Se, ppm	1.66 — 3.55	3 — 6	1.83 — 3.57
Zn, ppm	537 — 678	96 — 142	587 — 803

n = Number of venom pool samples analyzed for each specie.

tions in *Bothrops* genera venoms than in venoms of *Crotalus durissus terrificus*.

Results obtained by INAA method in some foodstuffs, as an example, are reported in Table 4. Corn grains studies showed that the tryptophan content in the improved corn samples (EMBRAPA: BR451) was twice as high as in normal samples, whereas varying element concentration changes were found mainly for the elements Br, Ca, and I.

Results obtained for gelatine, bread, rice, and milk powder samples by employing various radiochemical procedures are presented in Table 5. The levels of toxic elements Hg, As, and Se found in rice grains were below the values established by the national legislation *(7)*.

In order to evaluate the accuracy and precision of INAA and RNAA results, several biological reference materials were analyzed. These results are given in Table 6. By comparing our results with literature data, it can be concluded that these analyses performed are accurate and precise within the limits expected for trace element analyses.

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Table 4
Elemental Concentrations in Brazilian Foodstuffs Determined by INAA
(µg/g dry wt Unless Otherwise Indicated)

Element	Industrialized Bread	Milk Powder	Rice		Corn	
			Polished Grain (1)	Parboiled Grain (2)	ESALQ: VD-2 (normal)	EMBRAPA: BR 451 (white)
As	-	-	0.093 +- 0.014	0.310 +- 0.021	-	-
Br	10.9 +- 0.3	14.7 +- 0.4	0.43 +- 0.04	0.33 +- 0.02	15.2 +- 2.9	0.95 +- 0.06
Ca	-	10200 +- 1100	-	-	51 +- 3	83 +- 6
Cl	10400 +- 600	7600 +- 500	-	-	761 +- 77	716 +- 119
Co, µg/kg	-	-	25.4 +- 5.7	27.9 +- 4.3	-	-
Cr	0.30 +- 0.01	0.21 +- 0.03		-	-	-
Cu				-	13 +- 4	17 +- 4
Fe	75 +- 3	-	7.58 +- 0.83	12.6 +- 1.2	22 +- 3	21 +- 4
I, µg/kg	-	-	-	-	462 +- 69	210 +- 53
K	-	11500 +- 200	654 +- 41	1079 +- 58	3.3 +- 0.2	3.9 +- 0.2
Mg, µg/kg	-	1040 +- 25	-	-	1.03 +- 0.04	1.30 +- 0.10
Mn	10.2 +- 0.8	0.52 +- 0.04	-	-	6.6 +- 0.7	5.6 +- 0.4
Na	7700 +- 200	3230 +- 40	12.3 +- 1.6	16.2 +- 0.7	3.8 +- 0.5	2.7 +- 0.5
Rb	3.4 +- 0.4	24 +- 2	3.81 +- 0.22	11.0 +- 0.7	6.5 +- 1.1	7.8 +- 0.2
S	-	-	-	-	2.0 +- 0.3	2.0 +- 0.4
Sb	0.90 +- 0.01	1.07 +- 0.06	-	-	-	-
Sc, µg/kg	2.35 +- 0.56	-	2.16 +- 0.08	1.11 +- 0.08	-	-
U, µg/kg	-	-	-	-	23 +- 4	16 +- 3
Zn	14.9 +- 0.6	24 +- 3	16.4 +- 0.4	7.61 +- 0.66	17 +- 1	29 +- 1

(1) Long Fine 2, Cerejeira Brand; (2) Long Fine, Mingote Brand.

Table 5
Determination of Cr, Hg, Sb, and Se in Foofstuffs by RNAA Method *(6–8)*
Results in µg/kg of Dried Material

Sample	Element	Rad. Proc. *	Mean +- s
Rice - Polished Grains	Hg Se	1 1	14.70; 18.89 (a) 34.70; 18.67 (a)
Rice - Parboiled Grains	Hg Se	1 1	5.20; 2.67 (a) < 24
Natural Gelatine	Cr	2	478 +- 30
Flavoured Gelatine	Cr	2	85 +- 10
Industrialized Bread	Sb	3	1200 (a)
Milk Powder	Sb	3	910 (a)

* Radiochemical procedure: 1. distillation + precipitation; 2. solvent extraction with tribenzylamine in chloroform; 3. retention of interferents in HAP.
(a). Individual results

Table 6
Elemental Concentrations in Reference Materials Obtained by INAA and RNAA (in μg/g dry wt Dried Material)

Element	Reference Materials	This Work	Literature Values (14 - 16)
Al	NIST Citrus Leaves	137 +- 16	92 +- 15
As	Bowen's Kale	0.128 +- 0.014 (*)	0.131 +- 0.045
	SHINR-HH Human Hair	0.56 +- 0.06	0.59 +- 0.06
	NIST Citrus Leaves	2.97 +- 0.16	3.1 +- 0.3
	NIST Citrus Leaves	3.17 +- 0.54 (*)	3.1 +- 0.3
Br	NIST Bovine Liver	9.03 +- 0.43	9
	Bowen's Kale	24.2 +- 2.2	24.9 +- 2.5
Ca	SHINR-HH Human Hair	856 +- 94	790 +- 72
	Bowen's kale	40890 +- 1890	41060 +- 2217
Cl	NIST Bovine Liver	2769 +- 141	2800 +- 98
	Bowen's Kale	3571 +- 173	3560 +- 427
Co	NIST Citrus Leaves	0.0211 +- 0.0029	0.020
	SHINR-HH Human Hair	0.144 +- 0.007	0.135 +- 0.008
Cr	Mixed Human Diet	0.110 +- 0.040 (*)	0.150 +- 0.040
	Mixed Human Diet	0.117 +- 0.007 (*)	0.150 +- 0.040
	NIST Citrus Leaves	0.74 +- 0.07	0.8 +- 0.2
	NIST Citrus Leaves	0.813 +- 0.020 (*)	0.8 +- 0.2
	NIST Citrus Leaves	0.820 +- 0.150 (*)	0.8 +- 0.2
	SHINR-HH Human Hair	4.36 +- 0.75	4.77 +- 0.38
Cs	Bowen's Kale	0.085 +- 0.004	0.0763 +- 0.0058
	NIST Citrus Leaves	0.097 +- 0.003	0.098
Cu	NIES 10A Rice Flour	4.6 +- 1.2	3.5 +- 0.3
	SHINR-HH Human Hair	22.9 +- 7.1	23.0 +- 1.4
Fe	NIST Citrus Leaves	84 +- 2	90 +- 10
	NIST Bovine Liver	193 +- 13	194 +- 19
	SHINR-HH Human Hair	69.1 +- 6.2	71.2 +- 6.6
I	NIST Citrus Leaves	2.2 +- 0.5	1.84 +- 0.03
Hg	NIST Bovine Liver	0.0054 +- 0.0014 (*)	0.004 +- 0.002
	Mixed Human Diet	0.0055 +- 0.0004 (*)	0.0048 +- 0.0014
	NIST Citrus Leaves	0.084 +- 0.004 (*)	0.080 +- 0.020
	Bowen's Kale	0.136 +- 0.022 (*)	0.171 +- 0.028
	Bowen's Kale	0.169 +- 0.019 (*)	0.171 +- 0.028
	SHINR-HH Human Hair	2.16 +- 0.19	2.16 +- 0.21
	NIES No.5 Human Hair	4.25 +- 0.24	4.4 +- 0.4
K	NIST Citrus Leaves	18000 +- 9000	18200 +- 600
	NIST Bovine Liver	10200 +- 700	9960 +- 70
	Bowen's Kale	24140 +- 1480	24370 +- 1462
La	Bowen's kale	87 +- 20	86.4 +- 13.0
Mg	NIST Citrus Leaves	6227 +- 177	5800 +- 302
	Bowen's Kale	1645 +- 49	1605 +- 176
Mn	SHINR-HH Human Hair	2.86 +- 0.14	2.94 +- 0.20
	NIST Bovine Liver	10.7 +- 0.8	9.9 +- 0.8
Na	NIES No.5 Human Hair	25 +- 2	26 +- 1
	Bowen's Kale	2127 +- 157	2360 +- 284
S	NIST Citrus Leaves	4.8 +- 1.3	4.07 +- 0.09
Sb	NIST Citrus Leaves	0.036 +- 0.006 (*)	0.040
	Bowens Kale	0.068 +- 0.006 (*)	0.0685 +- 0.0144
	Bowen's Kale	0.064 +- 0.008	0.0685 +- 0.0144
	SHINR-HH Human Hair	0.248 +- 0.028	0.210
Sc	NIST Citrus Leaves	0.0119 +- 0.0010	0.010
	Bowen's Kale	0.0152 +- 0.008	0.0948
Se	Bowen's Kale	0.123 +- 0.020 (*)	0.134 +- 0.020
	Bowen's Kale	0.147 +- 0.025	0.134 +- 0.020
	NIST Bovine Liver	0.638 +- 0.075 (*)	0.71 +- 0.07
	NIST Bovine Liver	0.72 +- 0.07	0.71 +- 0.07
Th	Bowen's Kale	0.0122 +- 0.0022	0.0104
Zn	NIST Citrus Leaves	30 +- 2	29 +- 2
	Bowen's Kale	32.9 +- 1.5	32.3 +- 2.7

Results in μg/g of dried material

* Results obtained by RNAA method.

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