

Determination of mercury and selenium in hair samples of Brazilian Indian populations living in the Amazonic region by NAA

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Biomonitoring of mercury contamination of Brazilian Indian population groups living in the Xingu Park, a reservation situated in the Amazonic region, has revealed very high levels of mercury in hair samples as compared to controls. Total mercury was determined by INAA in most of the tribes living in the Park and methylmercury was determined by CVAAS in samples with total mercury above 10 mg/kg. Due to the fact that selenium seems to protect animals against the toxic effects of methylmercury, it was considered also of interest to determine its concentrations in the hair samples with very high mercury levels. Selenium was determined by INAA via the short-lived radionuclide ^{77m}Se ($T_{1/2} = 17.45$ s). The correlations between selenium and mercury concentrations in Brazilian controls and in the Indian population groups are discussed.

Introduction

Intensive gold exploration activities started in Brazil in the 1980's in the Amazonic region. Ever since, awareness of the general public and of authorities has been growing to the dangers of environmental contamination by disposal of metallic mercury used for extraction of gold by amalgamation. This process of gold extraction is considered as being of low efficiency and the amount of mercury released to the environment to produce 1 kg of gold in the Amazonic gold mines is estimated to be in a range from 1.1 to 5 kg, according to PFEIFFER and LACERDA.¹ Most of this mercury is evaporated and reaches the atmosphere, although 30 to 45% is released to river sediments, soils and drainage systems.

It is estimated by MALM et al.² that around 2000 tonnes of mercury have been released in the Amazon in the last 20 years as a consequence of these activities. Many studies have been conducted by Brazilians and researchers from other countries like Japan, Sweden, Canada, on the environmental impact of mercury pollution in the Amazon basin and the health effects on the riverine populations.³ Several of these studies have shown that high amounts of mercury can be found in hair samples of these populations, as well as in different fish species consumed by them.^{4,5} Also speciation of mercury has been carried out in samples of human hair, blood and urine,^{6,7} mainly in the Tapajos river basin.

On the basis of a comprehensive evaluation of the great number of scientific papers published on the presence of mercury on several compartments of the Amazonic region, it can be concluded that, instead of acute signs of contamination, the main impact of the

gold mining activities is of increasing the general background of mercury in soils, sediments and biota of the region.

LEBEL et al.⁸ applied neurobehavioural tests for manual dexterity, muscular strength and fatigue to a group of residents of Brasilia Legal, in the area of the Tapajos river, whose sole source of mercury is through consumption of contaminated food. Multiple regression analysis showed in this study that manual dexterity decreased significantly with the concentration of total mercury in the hair of these populations. Also it was observed that persons presenting disorganized movement of hands had significantly higher mercury in hair. The authors conclude that these findings suggest, that if mercury continues to accumulate in this population, there is a potential risk of occurrence of neurological disorders and that some neurotoxic alterations of motor functions can be detected even below the threshold value of 50 mg/kg set by WHO.

The Radiochemistry Division of IPEN/CNEN-SP has been engaged for several years in an IAEA Coordinated Research Programme concerning studies on the risk of mercury environmental contamination of populations living in developing countries, using nuclear techniques such as instrumental neutron activation analysis. Beside a control group of individuals not exposed to mercury occupationally or environmentally two main population groups were studied in the Brazilian project: one consisted of individuals living near the Billings Dam which receives effluents from one of the most industrialized regions of the country, and the second consisted of groups of Indians living in the Xingu Park, an Indian reservation situated in the Amazonic region. The first results obtained were published elsewhere.⁹

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In this work, the Indian population groups living in the Xingu Park were studied for the first time as regards mercury contamination, because the Park is not situated in the gold exploration activities region and is still considered as an environmentally clean area. The analysis of hair samples of the first group of Indians⁹ already revealed very high concentrations of mercury, with arithmetic mean, median and geometric means about 20 times higher than the controls. It should be noted that sampling of other biomonitors, e.g., body tissues or body fluids (blood, urine) of these populations was not possible for practical reasons. Quality assurance of the hair analysis for mercury was carried out by analysis of several reference materials.¹⁰

Further developments of the Project resulted in the analysis of hair samples of 13 of the 17 Indian tribes living in the Park and all of them presented values of total mercury much higher than the controls; although the Indians apparently did not show signs of mercury intoxication. Determination of methylmercury was also carried out in hair samples of some of the Indian tribes, at the Jozef Stefan Institute (Ljubljana, Slovenia) using cold vapor atomic absorption (CVAAS) after separation of the mercury species in an anionic exchange resin.¹¹ It was verified that generally from 70 to 100% of the mercury found in the Indian hair was in the form of methylmercury.

These findings led to another step in the work, with respect to the analysis of selenium in hair samples of these populations. Selenium, while being an essential element and a normal component of several enzymes, seems also to have a protecting effect against the toxic effects of methylmercury. Selenium was determined in the present work, in hair samples of the control group, in the population group living by the Billings Dam and in some of the Indian tribes, using instrumental neutron activation analysis via the short-lived nuclide ^{77m}Se ($T_{1/2} = 17.45$ s).

Experimental

Collection and washing of hair samples

The hair samples were collected and washed according to the protocol recommended by the IAEA. The samples were cut using stainless steel scissors, from the occipital area of the head and as close as possible to the scalp in an amount corresponding to about 2 g. The hair was then cut with the scissors into segments as short as possible and transferred to a glass vial to be submitted to the recommended procedure of sequential washing with acetone and water, followed by drying at room temperature.

Determination of total mercury in hair and reference materials by instrumental neutron activation analysis (INAA)

About 100–200 mg of the prepared hair samples and of the reference materials (RMs) were weighed in clean polyethylene envelopes and submitted to a thermal neutron flux of about $10^{16} \text{ m}^{-2} \cdot \text{s}^{-1}$, in the IEA-R1 nuclear research reactor, together with mercury standards. The more detailed procedure has already been described elsewhere.^{9,10}

Determination of total mercury and methylmercury in hair by cold vapor atomic absorption spectroscopy

Part of the hair samples collected from the Indians of the Xingu Park was sent to the Nuclear Chemistry Department of the Jozef Stefan Institute (Ljubljana, Slovenia), for analysis of total mercury and methylmercury. The method used for hair analysis is basically the technique described by MAY et al.¹² using an anion exchange separation of extracted inorganic from organic mercury species, followed by destruction of organic species by UV irradiation, with the usual CV-AAS finish.

Determination of selenium in hair by instrumental neutron activation analysis (INAA)

Selenium was determined in the prepared hair samples using the short-lived radioisotope ^{77m}Se ($T_{1/2} = 17.45$ s). The Indian hair samples were analyzed using the fast-rabbit system assembled at Interfaculty Reactor Institute (Delft, The Netherlands). Samples were irradiated in a thermal neutron flux of $1.5 \cdot 10^{17} \text{ m}^{-2} \cdot \text{s}^{-1}$ for 17 seconds and measured during 30 seconds, after a waiting time of about 20 seconds. Measurements were carried out using a coaxial 20% Ge detector (FWHM 1.63 keV at 1332 keV of ⁶⁰Co) equipped with a loss free counting module.

Results and discussion

In Table 1 are presented the results obtained for analysis of total mercury in several Indian population groups and also in the control group and in the Billings Dam group. Most of the analysis have been made by instrumental neutron activation analysis and some by cold vapor atomic absorption spectrometry.

Table 2 shows the results for methylmercury in six of the Indian groups, as determined by atomic absorption after ion exchange separation. Some of the results have been presented elsewhere.¹¹

Table 1. Summary of the results obtained for total mercury contents in the hair of the Brazilian population groups studied (in mg/kg)

Population group	Mean	Standard deviation	Median	Geometric mean	Range
Controls	1.1	0.6	1.0	0.9	0.3–2.9
Billings	0.88	0.68	0.74	0.71	0.30–3.04
Indian group 1*	18.5	5.9	18.0	17.1	6.9–34.3
Indian group 1**	17.5	4.6	17.7	16.7	5.4–26.5
Indian group 2*	12.0	4.0	10.7	11.4	6.5–21.6
Indian group 2**	13.2	4.0	12.1	12.8	10.1–21.7
Indian group 3	8.7	3.0	8.2	8.2	4.5–18.5
Indian group 4	13.2	3.8	13.0	12.7	4.8–25.3
Indian group 5	10.6	3.9	11.5	9.4	1.7–15.1
Indian group 6	20.6	10.0	18.8	19.0	8.1–57.3
Indian group 7	16.5	5.5	15.8	15.5	2.5–30.2
Indian group 8	17.2	6.0	16.2	16.3	2.1–31.7
Indian group 9*	21.8	6.1	20.8	21.0	12.4–34.2
Indian group 9**	17.7	4.1	16.6	17.3	10.9–25.0
Indian group 10*	8.1	9.0	2.8	4.7	1.5–33.1
Indian group 10**	4.7	5.8	2.4	3.2	1.5–26.1
Indian group 11	18.2	7.8	16.2	16.7	5.5–41.8
Indian group 12	12.2	3.1	12.5	11.8	6.6–18.8
Indian group 13	3.6	2.4	2.6	3.1	1.2–11.1

* Results obtained by INAA.

** Results obtained by CVAAS.

Table 2. Summary of the results obtained for methylmercury contents in the hair of the Brazilian population groups studied (in mg/kg)

Population group	Mean	Standard deviation	Median	Geometric mean	Range
Indian group 1	15.6	4.5	15.0	14.9	4.8–25.7
Indian group 2	10.2	1.8	10.5	10.1	7.6–12.9
Indian group 9	15.9	3.9	15.1	15.5	10.0–23.7
Indian group 10	12.4	8.3	10.0	10.6	5.5–24.2
Indian group 11	16.9	7.0	14.2	15.5	4.4–32.8
Indian group 12	10.6	2.8	11.2	10.1	4.3–15.3

The results obtained for total mercury in the hair samples of the Indian groups show that the mercury contents of these populations are significantly higher than the controls, in all the groups analyzed, as tested using ANOVA at 95% confidence level.

As for methylmercury, it can be observed in Table 2 that the amounts are also very high and comprise almost the totality of the mercury found in hair. Nevertheless, no cases were detected of methylmercury concentrations higher than 50 mg/kg, which is the minimum threshold set by WHO for mercury toxicity.

Table 3 presents the results obtained for analysis of selenium in hair samples from the control group, the group of the Billings Dam and some of the Indian groups of the Xingu Park.

In Table 4 are presented the results of mercury and selenium concentrations in nmol/g and the calculated Se/Hg and Hg/Se ratios.

Figure 1 presents the results of linear regression of the molar ratios Hg/Se found in the hair samples against mercury concentrations. It can be observed that the molar ratios are very close to one for low mercury concentrations and increase linearly with the mercury concentrations. These results agree with those of DRASCH et al.¹³ who determined mercury and selenium concentrations in kidney cortex samples of 195 autopsies. The authors point out that, since in vitro mercury and selenium form relatively stable adducts, these results suggest the formation of a 1:1 Hg-Se compound that may explain the mercury detoxification by selenium.

Table 3. Summary of the results obtained for selenium contents in the hair of the Brazilian population groups studied (in mg/kg)

Population group	Mean	Standard deviation	Median	Geometric mean	Range
Controls	0.43	0.04	0.43	0.43	0.34–84.3
Billings	0.38	0.12	0.36	0.36	0.17–26.8
Indian group 1	0.47	0.01	0.43	0.45	<0.28–0.86
Indian group 2	0.352	0.033	0.352	0.351	0.328–0.375
Indian group 3	0.28	0.04	0.28	0.3	0.25–0.32
Indian group 4	0.563	0.401	0.429	0.498	0.402–1.63
Indian group 5	0.314	–	–	–	0.314
Indian group 6	0.372	0.028	0.372	0.371	0.34–0.41
Indian group 7	0.329	–	–	–	0.329
Indian group 8	0.343	0.032	0.351	0.691	0.28–0.39

Table 4. Results of the selenium and mercury ratios found in the hair of the Brazilian population groups studied

Population group	Mean Hg, $\mu\text{mol/kg}$	Mean Se, $\mu\text{mol/kg}$	Se/Hg	Hg/Se
Control	5.49	5.45	0.993	1.007
Billings	4.39	4.82	1.098	0.911
Indian group 1	92.3	5.96	0.0646	15.49
Indian group 2	59.85	4.46	0.0745	13.42
Indian group 3	43.40	3.55	0.0818	12.23
Indian group 4	65.84	7.14	0.108	9.221
Indian group 5	52.87	3.98	0.0753	13.28
Indian group 6	102.74	4.71	0.0458	21.81
Indian group 7	82.29	4.17	0.0507	19.73
Indian group 8	85.79	4.35	0.0507	19.72

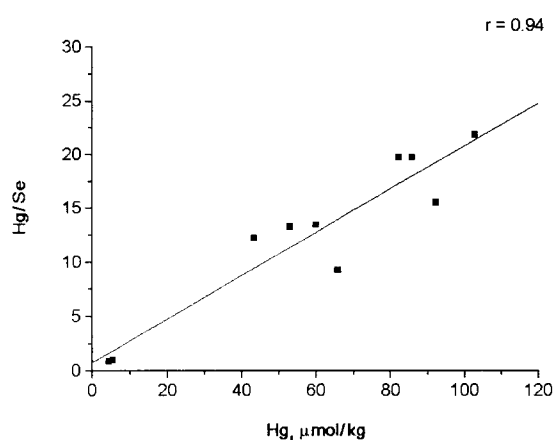


Fig. 1. Linear regression of the Hg/Se molar ratios of the Brazilian population groups studied against Hg concentrations

In an animal study on the other hand, female monkeys were exposed to methylmercury¹⁴ for up to 18 months and the concentrations of selenium and mercury were measured in brain. The results obtained indicated an association between concentrations of mercury and

selenium in both occipital pole and thalamus in the methylmercury exposed animals and the conclusion was that an important role for selenium in the retention of mercury in brain could be indicated.

Conclusions

Very high amounts of mercury were found in hair samples of Indian populations living in the Xingu Park, in a reservation far from gold exploration activities. The mercury found in hair is almost totally as methylmercury, which suggests incorporation by fish consumption. The Hg/Se ratios are very close to one for the control and Billings Dam populations and increase with the mercury concentrations, which agrees with results found by other authors in organs of autopsies.

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