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Evaluation of As, Se and Zn in octopus samples in different points of sales of the distribution chain in Brazil

Marildes Josefina Lemos Neto · Elizabeth de Souza Nascimento · Vera Akiko Maihara · Paulo Sergio C. Silva · Mariza Landgraf

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Abstract Shellfish such as squid and octopus, class Cephalopoda, has high commercial value in restaurants and for export. As, Se and Zn concentrations were determined in 117 octopus acquired in different points of the distribution chain in 4 coastal cities of São Paulo state (Guarujá, Santos, São Vicente and Praia Grande)—Brazil. The methodology for elemental determination was Instrumental Neutron Activation Analysis (INAA). The element concentration in the octopus samples (wet weight) range from: 0.184 to 35.4 mg kg^{-1} for As, 0.203 to 2.26 mg kg^{-1} for Se and 4.73 to 37.4 mg kg^{-1} for Zn. Arsenic and Se levels were above the limit for fish established by Brazilian legislation, while Zn concentrations were in accordance with literature values.

M. J. Lemos Neto
Instituto de Pesca- Avenida Bartolomeu de Gusmão, Santos, SP 192 CEP:11030-906, Brazil
e-mail: marildes@pesca.sp.gov.br

E. de Souza Nascimento · M. Landgraf
Faculdade de Ciências Farmacêuticas – USP, Núcleo de Apoio à Pesquisa em Alimentos e Nutrição – USP, Av. Prof. Lineu Prestes, 580 Bl.14, São Paulo, SP 05508-000, Brazil
e-mail: esnaci@usp.br

M. Landgraf
e-mail: landgraf@usp.br

V. A. Maihara (✉) · P. S. C. Silva
Instituto de Pesquisas Energéticas e Nucleares, (IPEN/CNEN), Av. Prof. Lineu Prestes 2242, São Paulo/SP CEP 05508-000, Brazil
e-mail: vmaihara@ipen.br

P. S. C. Silva
e-mail: pscsilva@ipen.br

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Introduction

Fisheries are big business on a national and global scale. Indeed, this industry, as well as related industries such as restaurants and grocery store providers, is a key determinant of the amount, type, and form of fish that people consume by affecting the cost, availability, and desirability of different fish [1].

Data from the Food and Agriculture Organization [2] show that fisheries and aquaculture production worldwide have reached approximately 148.5 million tons and a fish consumption of 18.4 kg per capita in the world. About 86 % of total fishery production (128.3 million tons in 2010) was used for direct human consumption.

From 2005 to 2007 the average consumption of fish, in Brazil, was 6.5 kg per capita [3]. According to the Fishery and Aquaculture Ministry, the fish consumption has been increasing annually and in 2009 the estimated consumption was 9 kg/year [4]. This consumption is still considered low, probably due to the high price of the product and a deficient distribution system in wholesale and retail networks [5]. There are also large differences in fish consumption according to region: for instance, while the fish consumption per capita in the São Paulo metropolitan region is 15.1 kg/year [6], in Manaus, in the Amazonas region, it is 33.7 kg/year [7] and 18.5 kg/year in Rio de Janeiro [8].

Recent studies have reported an increase in world fish consumption per capita, due to the diversification and provision of convenience products by the fishing industries that are responsible for this trend [9]. Another factor contributing to this increase is the search for products with high nutritional value and proven good health effects, such as fish and shellfish [10].

Shellfish such as squid and octopuses, Class Cephalopoda, has a high commercial value in restaurants and for export. Fish, including octopus, is an important source of high-quality animal protein, vitamins and long-chain polyunsaturated fatty acids. On the other hand, octopuses may also be a potential source of toxic elements due to its higher trophic position on the food chain [11–13].

The major route of intoxication by organic and inorganic pollutants associated with aquatic systems is the consumption of contaminated fish and seafood [14]. The health risks associated with eating contaminated seafood are upto 20–40 times higher than that of drinking contaminated water [15]. This is due to aquatic organisms' ability to concentrate elements upto ten times more than that observed in the environment [16]. Thus, seafood can be considered as bio-indicators of metal pollution and a potential risk if contaminated seafood is consumed [17, 18].

There are several studies reporting the presence of contaminants in the top predator species in the food chain which can be a result of biomagnification or of bioaccumulation [19]. While the former is a successive and progressive accumulation of pollutants at different steps of the food chain, the latter is the increase in the concentration of pollutants in tissues throughout the life of first animal in the food chain. In this case for the same species, the larger the individual the higher the level of pollutant.

Octopuses feed on other cephalopods, crustaceans, mollusks and fish, which give them great potential as a bioindicator organism, providing qualitative information on environmental contamination [20]. There are many reports of toxic element contamination in various regions of the Brazilian coast, due to high emission rates by industrial activities that is concentrated in urban drainage basins and coastal areas since the 1980s [21–26].

With the purpose of verifying the levels of As, Se and Zn in octopus samples acquired in different points of the production chain, Instrumental Neutron Activation Analysis (INAA) was applied. This analysis (INAA) has been used for determination of these elements in several food matrices, such as fish samples due to its high specificity and its few sources of bias in common with non-nuclear techniques. INAA is also highly important and extensively applied for the certification of arsenic and other elements in biological reference materials [27–29].

Materials and method

Sampling

In the period from 2005 to 2007, during the fishing seasons of "summer" (September–February) and "winter" (March–August), 117 samples of raw octopus were obtained in the following points of the production chain: street markets (12 samples), markets/fishmongers (27), supermarkets (25), industry (23) and fishing terminals or warehouses (30) in 4 municipalities (Guarujá, Santos, São Vicente and Praia Grande) of São Paulo State, in Brazil, which have the most representative octopus trade in Sao Paulo state, Brazil.

In street markets, markets and fishmongers, octopus samples were chosen, when possible, weighing over 1.5 kg. The animals were eviscerated and packed in plastic bags. In some supermarkets, the same procedure was used, but in others, the samples were purchased as commercialized, i.e., already packed. In industries, the samples were collected in their original packages as approved by the Federal Inspection Service. In the fishing terminals, the samples were taken directly from the boat at the time of its arrival and placed in plastic bags. All samples were transported in a cold box to the laboratory located in the city of Sao Paulo. After that, octopus samples were placed in plastic bags and kept at −20 °C before analysis.

Instrumental neutron activation analysis (inaa)

Preparation of octopus samples

Octopus samples were cut in small pieces and freeze dried for 10–15 h at −51 °C in a ModulyD Model freeze-dryer (Thermo Electron Corporation, Milford, MA, USA). After the freeze-drying process, the samples were ground and homogenized in a domestic blender with Ti blades. These samples were stored in pre-cleaned polyethylene vials until analysis. The range of moisture varied from 70.0 to 93.4 %.

Preparation of As, Se and Zn standards

Standards of As, Se and Zn were prepared from appropriate dilutions of high purity standard solutions (SPEX Certiprep Inc., Metuchen, NJ, USA) using Milli-Q water 18.2 MΩ cm^{-1} (Millipore Corporation, Milford, MA, USA), in concentrations of 1000 mg L^{-1} of As, Se and Zn. Aliquots (50–100 μL) of these solutions were pipetted onto Whatman 40 filter papers and dried under infrared lamp. After drying, filter papers were transferred to polyethylene bags, previously cleaned with 10 % nitric acid, analytical grade (Merck, Darmstadt, Germany) and Milli-Q water.

Sample and standard irradiations

About 150–200 mg of octopus samples and 200 mg of reference materials (Oyster Tissue NIST SRM 1566b and Dogfish NRCC DORM-2) were weighted in polyethylene

Table 1 As, Se and Zn concentrations in the Certified Reference Materials

Element	Reference materials (mean ± standard deviation)[a]			
	Oyster tissue (SRM 1566b)[b]		NRC-DORM-2[c]	
	This study	Certified value	This study	Certified value
As (mg kg^{-1})	7.39 ± 0.37	7.65 ± 0.65	16.8 ± 0.8	18.0 ± 1.1
Se (mg kg^{-1})	2.04 ± 0.22	2.06 ± 0.15	1.31 ± 0.09	1.40 ± 0.09
Zn (mg kg^{-1})	1428 ± 97	1424 ± 46	22.4 ± 2.0	25.6 ± 2.3

[a] Mean and standard deviation of five determinations ($n = 5$)

[b] *SRM* Standard Reference Material, National Institute of Standards and Technology(NIST), Gaithersburg, USA

[c] *NRC* Dogfish Muscle DORM-2, National Research Council Canada

bags. For irradiation, samples, reference materials and element standards were simultaneously irradiated for 8 h under a thermal neutron flux of 4.5 × 10^{12} n cm^{-2} s^{-1} at the nuclear research reactor IEA-R1 of the Nuclear and Energy Research Institute, IPEN/CNEN-SP, Sao Paulo, Brazil.

Gamma spectrometry

After appropriate decay periods, γ-ray spectra of octopus samples, reference material and element standards were measured using a high-purity Ge detector POP TOP model (EG&G, ORTEC, Oak Ridge), TN, USA) with 20 % efficiency and 1.9 keV resolution for 1,332.49 keV peak of ^{60}Co. The detector is coupled to an electronic system composed of multi-channel analyzer, source of high tension, amplifier and a compatible microcomputer. The gamma-ray spectra were analyzed using the VISPECT 2 software, in TURBOBASIC language.

Results and discussion

Analytical quality control

The reference materials Oyster Tissue NIST 1566b and Dogfish Muscle DORM-2 were used for quality control purposes and to evaluate the effectiveness of the applied methodology. The mean value concentration and standard deviation obtained for As, Se and Zn in these reference materials were compared to certified values, as shown in Table 1. The analytical values were obtained after a correction for residual moisture, obtained according to drying instructions from the certificates; the values obtained were in accordance with the certified values, showing adequate precision and accuracy.

The detection and quantification limits (DL and QL) for As, Se and Zn were determined by the Currie criterion [30] in the experimental conditions and are present in Table 2.

Table 2 Detection and quantification limits for As, Se and Zn in the octopus samples as determined by INAA

Element	DL	QL
As (mg kg^{-1})	0.22	0.75
Se (mg kg^{-1})	0.15	0.47
Zn (mg kg^{-1})	0.29	0.87

Table 3 As, Se and Zn concentrations in 117 octopus samples (wet weight mg kg^{-1})

	As	Se	Zn
Mean ± SD	7.89 ± 6.92	1.43 ± 0.34	14.4 ± 4.6
Median	5.67	1.40	14.2
Range	0.184–35.38	0.203–2.26	4.7–37

As, Se and Zn concentrations in octopus samples

In the present study, the following element concentration ranges in fresh octopus were: for As from 0.184 to 35.38 mg kg^{-1}; Se from 0.203 to 2.26 mg kg^{-1} and for Zn from 4.7 to 37 mg kg^{-1}. Table 3 shows the mean, standard deviation, median, maximum and minimum values for the As, Se and Zn concentrations in the 117 octopus analyzed samples.

As a result of naturally occurring metabolic processes in the biosphere, arsenic occurs as a large number of organic or inorganic chemical forms in food. The adverse health effects of arsenic have long been recognized. The acute toxicity of arsenic ranges from very toxic to completely non-toxic, inorganic arsenic being more toxic than organic arsenic compounds. Methylated forms of arsenic have a low acute toxicity; arsenobetaine which is the principal arsenic form in fish and crustaceans is considered nontoxic. Especially in the marine environment, arsenic is often found in high concentrations as organic forms, upto 50 mg kg^{-1} of As on a wet weight basis in some seafood, including seaweed, fish, shellfish and crustaceans [31].

Selenium is an essential trace element for humans, displaying a narrow boundary between essentiality and

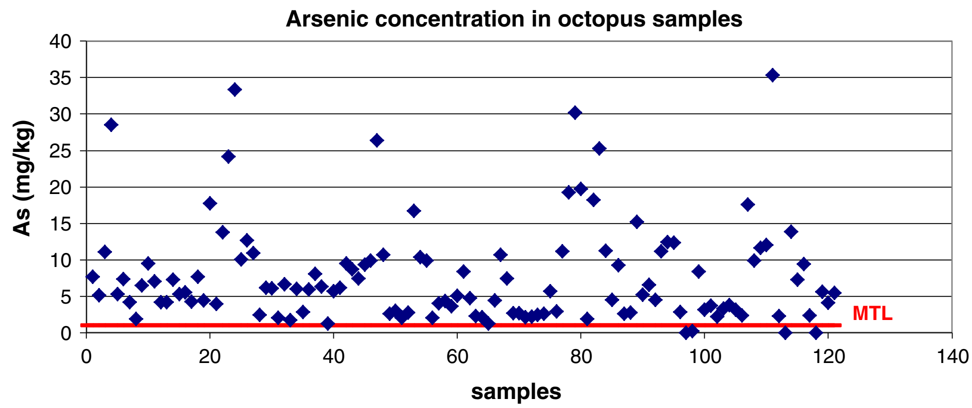

Fig. 1 As concentration (mg kg^{-1}) in fresh octopus samples and maximum tolerance level (MTL)

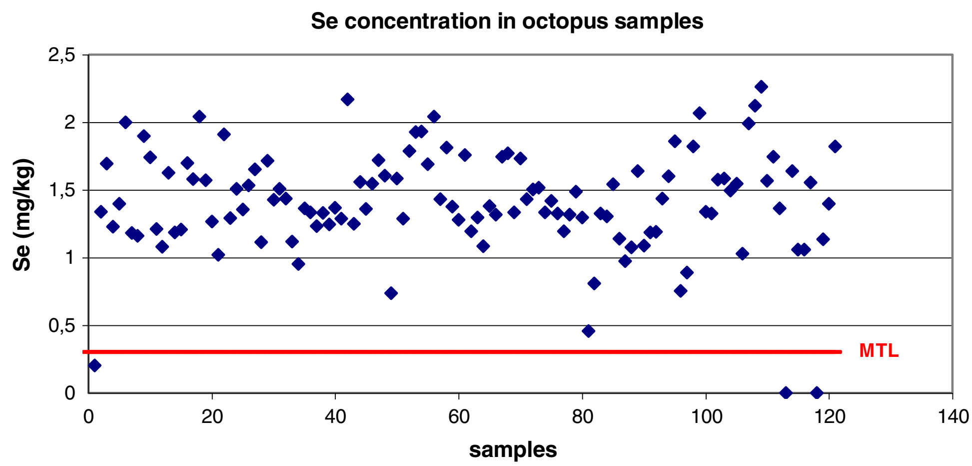

Fig. 2 Se concentration (mg kg^{-1}) in fresh octopus samples and maximum tolerance level (MTL)

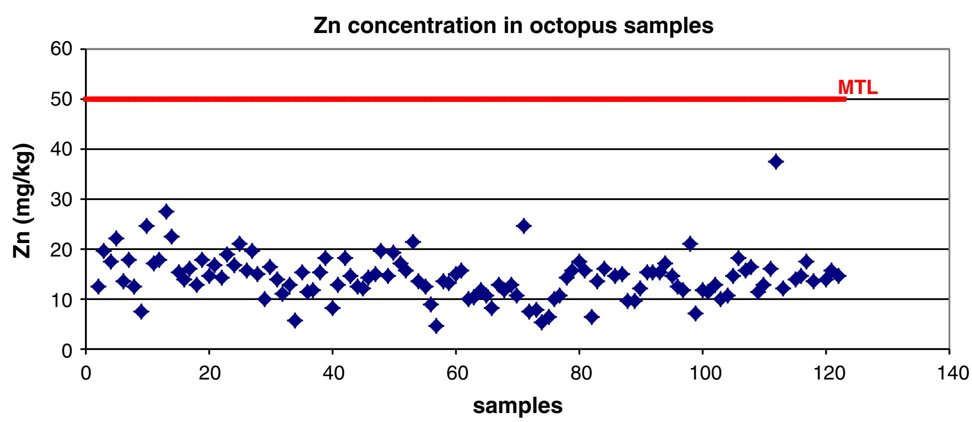

Fig. 3 Zn concentration (mg kg^{-1}) in fresh octopus samples and maximum tolerance level (MTL)

toxicity. Se is part of the selenoproteins such as glutathione peroxidase. Zinc is also an essential element, but toxic concentrations can be reached due to environmental contamination.

Brazilian legislation [32] determines the maximum tolerance level (MTL) of 1.0 mg kg^{-1} for arsenic in fish and fishery products and for Se and Zn 0.30 mg kg^{-1} and 50.0 mg kg^{-1}, for solid foods, respectively.

Figures 1, 2 and 3 show the dispersion of As, Se and Zn in all analyzed octopus samples, respectively. It can be observed that virtually all samples presented concentration of As and Se above the MTL established by Brazilian legislation while for the same samples Zn concentration were below the MTLs.

The results obtained in this study are in accordance with those observed by Denobile [33] who found As levels

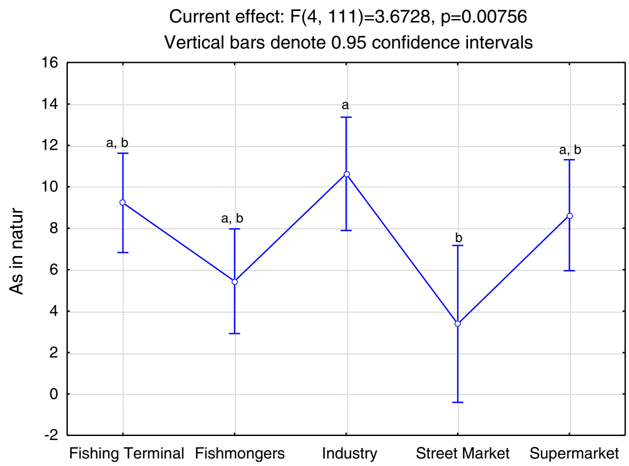

Fig. 4 As concentrations (mg kg^{-1}) in fresh octopus samples from Guaruja, Santos, São Vicente and Praia Grande cities, SP, Brazil, in different points of the distribution chain

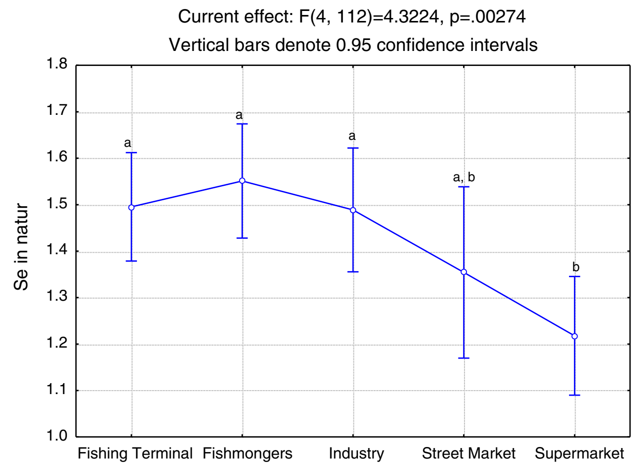

Fig. 5 Se concentrations (mg kg^{-1}) in fresh octopus samples from Guaruja, Santos, Sao Vicente and Praia Grande cities, SP, Brazil, in different points of the distribution chain

ranging from 2.10 to 33.53 mg kg^{-1} in 51 fish samples collected in São Paulo city.

Pedreira Filho et al. [21] concluded that the levels of Zn and other elements, such as Al, Cd, Cr, Cu, Ni, Pb, Fe, Hg and As, presented by samples of octopus commercialized in Sao Paulo city, posed no risk to the population since they were in the range of 10.1–16.3 mg kg^{-1} dry weight. Concentrations of As were also studied but although the description of the methodology used, no results or comments were described.

Catharino et al. [22] assessing mussel contamination in five different regions of the Sao Paulo coast, reported the following concentrations: As 0.90–3.23 mg kg^{-1}, Se 0.32–0.77 mg kg^{-1} and Zn 15.1–24.7 mg kg^{-1}, which are similar to those presented in this study.

Curcho et al. [23] analyzed different species of fish marketed in the municipalities of Cananeia and Cubatão, Sao Paulo State, and found the following contents for fish wet weight: As ranging from below the low detection limit (LD) to 6674 μg.kg^{-1}, Se ranging from 100 to 1889 μg.kg^{-1} and Zn from 1.6 to 17.9 mg.kg^{-1}, also showing samples with levels above the tolerance limits established by Brazilian legislation.

Seixas et al. [20] studied octopus samples from the Portuguese coast and observed that the average concentrations in octopus tentacles ranged from 1 to 2 mg kg^{-1} for Se, 1.5 to 2.5 mg kg^{-1} for Mn, 40 to 50 mg kg^{-1} for Fe, 4 to 203 mg kg^{-1} for Cu, and 50 to 300 mg kg^{-1} for Zn, the average concentrations of As, Hg and Pb in those tentacles being 40–130 mg kg^{-1}, 0.15–0.45 mg kg^{-1} and 3–4 mg kg^{-1}, respectively. In this study, almost all As was in the arsenobetaine form, which is the less toxic form.

Analysis of variance was applied to the results considering points of sales. The results are shown in Figs. 4, 5 and 6. To determine the significant differences between group means the Tukey test was also applied. In the figures equal letters mean no significant differences and different letters indicate significant differences. It can be observed that for As results significant differences were noted between the octopus samples collected in industry and street markets. However, no significant differences were observed among these two points and the other collection points.

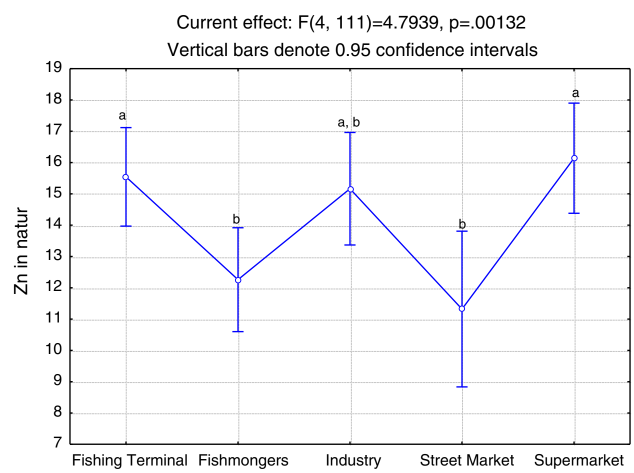

Fig. 6 Zn concentrations (mg kg^{-1}) in fresh octopus samples from Guaruja, Santos, Sao Vicente and Praia Grande cities, SP, Brazil, in different points of the distribution chain

For Se, only the supermarket octopus samples showed significant statistical differences. For Zn, fishmongers and street market octopus sample results indicate no significant statistical differences either, while results from fishing terminal, industry and supermarket were statistically the same.

These differences observed could be related to many factors such as place of fishing, treatment after fishing, local storage, water and ice used for storage. Therefore, this study should be continued in order to explain some of the reported results. In the future research, aspects such as place of capture, the quality of the ice used to store the samples during marketing, the type of packaging, equipment and utensils, among others, should also be considered.

Octopuses were considered inadequate for consumption in view of the high levels of As and Se showed by the samples, with concentrations above those allowed by the current Brazilian legislation. However, it is not possible to assess the risk associated with octopus ingestion due to the lack of data on the consumption of this food in Brazil.

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