DETERMINATION OF HEAVY METALS IN SEAFOOD

Andreea DRAGOI BRINZA[1], Raluca-Ioana STEFAN-VAN STADEN[2], Gabriela STANCIU[3], Catalina CIOATES NEGUT[4,*]

The presence of pollutants in food is a real danger for the human and animal body, therefore it is important to determine their concentration by accurate, modern methods using high performance equipment. This work aims to investigate and assay the concentration of heavy metals in domestic and imported seafood employing AAS, in order to assess the importance of their introduction into diets. Following the determinations carried out, it was observed that the seafood screened does not contain heavy metals in concentrations above the normal regulated limits, therefore they can be consumed without presenting a health hazard.

Keywords: Heavy metals, seafood, contamination, atomic absorption spectrometry

1. Introduction

Seafood accumulates a wide range of metals in the tissue, and therefore, determination of the amounts of accumulated heavy metals like lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) in seafood is essential because of their use and the potential adverse effects when consumed by people [1]. The contamination of the seas with high levels of heavy metals has a significant impact on seafood, creating a grave concern [2]. Due to their non-degradable nature, heavy metals are deposited, assimilated, or integrated into water, sediments, and aquatic organisms. Consequently, they can be transmitted to people through the food chain, especially by consuming fish and crustaceans. The harmful effect becomes apparent when a specific limit is surpassed, below which some metals like as cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) can even serve as necessary elements of

[1] PhD student, Faculty of Chemical Engineering and Biotechnologies, National University of Science and Technology POLITEHNICA Bucharest, Romania, e-mail: deealevantica@yahoo.com.
[2] CS I, Prof. habil., Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, and Faculty of Chemical Engineering and Biotechnology, National University of Science and Technology POLITEHNICA Bucharest, Romania, e-mail: ralucavanstaden@gmail.com.
[3] Prof., Department of Chemistry and Chemical Engineering, „Ovidius" University of Constanta, Romania, e-mail: gstanciu66@yahoo.com.
[3] Prof., Department of Chemistry and Chemical Engineering, „Ovidius" University of Constanta, Romania, e-mail: gstanciu66@yahoo.com
[4] * CS I, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania, e-mail: negutcatalina79@gmail.com

proteins participating in different metabolic pathways. Therefore, if foods were entirely devoid of any trace of metals, it would Pb to the development of nutritional deficiencies [3].

Various variables contribute to varying levels of heavy metal concentrations in soil, water, air, plants, and animal-derived food products. Air can serve as a means of pollution by facilitating the transportation and deposition of metals onto soil and plants. For example, emissions of Pb from autos can contribute to this process. The air becomes contaminated with heavy metals due to many human activities, such as burning coal and oil, producing non-ferrous metals, steel and Fe, cement, operating waste gas treatment plants, accumulating trash, and incineration [4]. A study has shown the effects of extracting heavy metals from seafood, tuna, and poultry during the process of cooking and preserving them. Researchers employed the inductively coupled plasma-optical emission spectrometry method to identify small amounts of heavy metals in the muscle tissue of uncooked, cooked, frozen, and preserved seafood, fish flesh, tuna species, chicken, and turkey. The concentration of heavy metals such as aluminum (Al), tin (Sn), Fe, Co, titanium (Ti), and manganese (Mn) increased by almost 100% when cooked using conventional methods. Conversely, preservation of mussels facilitated the extraction and accumulation of metals like Al, Fe, Sn, Zn, Cu, Hg, and As, resulting in a concentration rise of over 180% [5].

Guanabara Bay is a severely polluted estuary system and a significant fishing region in southeastern Brazil. The current study on the correlation between particular seafood pollutants, non-living elements, and evaluations of human health risks is insufficient. The results suggested that crabs are the primary organisms that accumulate high levels of toxins over time. Overall, the levels of contaminants were generally within the limits established by several international organizations, except As, which exceeded the Brazilian threshold of 1 mg kg^{-1}. Scientists have developed a hazard index to evaluate the potential health dangers that consumers may encounter when consuming fish. The study by Almeida Rodrigues et al. highlighted the significance of conducting a unified evaluation of many harmful substances to obtain a more precise evaluation of health hazards [6].

The enduring peril of metal pollution resulting from shipwreck activities has sparked much apprehension regarding its impact on both the maritime ecosystem and human well-being. An investigation was conducted to examine the distribution, features, dangers, sources, and potential impacts of metals in sediments and seafood from the Bay of Bengal and open wreck sites in Bangladesh. A total of 78 sediment samples and 208 seafood samples were gathered from both exposed and control sites between 2018 and 2020, encompassing both dry and wet seasons [7].

Some studies have examined the seasonal variations in fish diversity and the levels of heavy metals (like Hg, Pb, chromium (Cr), Cd, Cu, and Zn) in coastal regions. These studies also explored the potential health concerns for humans

connected with these heavy metals [1]. The soil can get contaminated with metals through the use of fertilizers and pesticides that include metals, such as fungicides that contain Hg, Cu, As, Zn, and so on. Depending on the soil type and geographical location, it can have elevated levels of heavy metals (such as in Baia Mare and Copșa Mică in Romania), or it may lack them. Water can serve as a significant conduit for pollution as a result of accidents, activities at sewage treatment and pre-treatment plants, sewage discharge, and domestic garbage. The water's hardness and the presence of organic substances can contribute to the accumulation of Pb in pipelines. Furthermore, a significant cause of heavy metal pollution in food can be attributed to the interaction with processing machinery, installations, or equipment, as well as the storage of canned food in metal packaging.

Water contamination by heavy metals poses a significant risk to both public water supplies and those who consume fish products [8]. These pollutants are considered a fundamental group of substances found in water due to their ability to accumulate in living organisms and their resistance to natural decomposition processes in the environment [9]. Consuming fish and other aquatic foods that are contaminated from this environment can expose humans to both organic and inorganic contaminants [10].

Research on the bioaccumulation of contaminants in fish is crucial for assessing the levels of various trace metals [11]. Metal buildup serves as a means to assess the influence of metal on the aquatic ecosystem, resulting in adverse effects on organisms [12,13]. Some studies [14,15] have discovered elevated amounts of heavy metals, including Pb, Zn, Cu, and Cd, in marine sediments. These findings suggest that the average total concentration of fish in the Kelantan River follows the order of Pb>Ni>Cd. Farago discovered that *Orechromis sp*. exhibits a greater ability to bioaccumulate Pb and Cd metals through biological processes [16]. Lee found that the levels of Cd in tilapia fish were greater compared to other metals [17]. The average composition of Cd in the fish was similar to the maximum level provided by the Commission Regulation [18].

Based on the Priority List of Hazardous Substances developed by the Agency for Toxic Substances and Disease Registry, the metals that pose the greatest damage to human health, in descending order, are Pb, Cd, Ni, Zn, Cu, and Mn [19,20]. Atomic absorption spectrometry (AAS) is the most commonly employed method for quantifying metals in low concentrations in environmental samples. This is because AAS offers several advantages, including the availability of measuring equipment, a straightforward approach, high sensitivity, accuracy, and a quick analysis time [21,22].

The present study aims to assess the levels of heavy metals in both domestic and imported seafood by employing various AAS techniques. The goal is to ascertain the significance of their inclusion in diets. The employed methodologies

included cold vapor atomic absorption (CVAAS), flame atomic absorption (FAAS), and graphite furnace atomic absorption (GFAAS).

2. Experimental

2.1. Materials and reagents

All reagents used were of spectral purity. Certified solutions of 1000 mg/L for Hg, Pb, Cd, supplied by Certipur and Sigma Aldrich, were used for calibration of the equipment. Appropriate reference material was used to validate the results:

- Hg: ERM-CE 278K muscle tissue, FAPAS T07221QC liver, FAPAS T07261QC canned fish, FAPAS TET045RM canned crab.
- Pb and Cd: muscle tissue ERM-CE 278, bovine liver 185R, freeze-dried liver T07221QC, freeze-dried bovine liver ERM-BB185

Ultrapure water was used for dilution. This was procured from suppliers Rotipuran and Roth.

Microwave digestion of the samples was carried out in sealed vessels in a Berghof MWS-2 microwave oven in a mixture of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2), and calcining in a Nabertherm 30-3000°C.

The VARIAN AA 240 FS hydride generator AAS was used for the determination of Hg, and the GBC Avanta FAAS and the VARIAN Spectra GFAAS spectrometer were used for the analysis of Cd and Pb.

2.2. Collection and preparation of samples for analysis

Between March and May 2022, 13 samples of domestic and imported seafood were taken from fishermen and supermarkets. These were mainly mussels (7 samples), shrimp (4 samples), octopus (1 sample), and squid rings (1 sample). In the case of mussels, the shells were removed and the shrimp were shelled. Samples were chopped and homogenized, then placed in polyethylene bags, numbered and stored in the freezer. In order to determine the concentration of the metals, the samples were brought into solution using microwave digestion (for the determination of Hg) or calcination followed by ash solubilization (for the determination of Cd and Pb).

Microwave digestion was used for the sampling. The principle of the method is based on the digestion of samples under pressure in a microwave oven at high temperature and pressure with 69% concentrated HNO_3 and H_2O_2. Known amounts of sample (approximately 500 mg) were weighed and introduced into the digestion vessel. Then 3.5 mL concentrated 65-69% HNO_3 and 1.5 mL H_2O_2 were added to prevent the sample from cementing to the walls of the digestion vessel and to achieve complete mixing of the sample with the acid. The reaction was allowed to react for about 20 minutes then the reaction vessel was tightly closed and placed in the microwave oven, where the digestion time was programmed (15-40 minutes). When the digestion program was finished, the oven door was opened, and one let

the digestion vessels cool. The walls of the flasks were rinsed with ultrapure water, and the content was transferred to a 25 mL volumetric flask and made up to the mark with ultrapure water.

Mineralization of the sample by drying, calcination, and ash solubilization was done in a porcelain capsule or crucible, and 10 mg-25 g of sample were weighed into it. For the assay of Pb and Cd, the crucible with the sample was placed in the electric furnace at an initial temperature of not more than 100°C and the temperature was raised by 50°C per hour until it reached 300-350°C, at which point it was maintained until complete carbonization. In the final phase of burning, charcoal may ignite. The temperature was raised to 450°C. The processed sample stood overnight (about 16 hours). The crucible was placed back in the oven at a temperature below 200°C, gradually increasing to 450°C, and baking at this temperature for 1 hour to 2 hours or more. Repeats of the operations were done until the sample was completely calcined, i.e., until the ash is white/grey or slightly colored. The number of repetitions varies according to the type of sample. Ash with charcoal residue was treated with 1-5 mL H_2O_2, depending on the amount of charcoal, covered with a watch bottle, and heated on the stove until no more gas bubbles were released and it evaporated to dryness. Repeat the operation until the ash turns white. Afterwards, treat with about 1 ml magnesium nitrate ($Mg(NO_3)_2$) at 10% and dry again, then place in the electric oven and burn for 1 hour at 450°C until the residue is free of charcoal. After cooling, the ash was treated with 5 mL hydrochloric acid (HCl) (0.1 mol/L), therefore, all the ash came into contact with the acid. The capsule was covered with watch glass and kept for 15 minutes on the electric hob to dissolve, evaporate the acid on the water bath or on the hob. The residue was melted in 0.1 mol/L HNO_3 and passed through filter paper into a 25 mL volumetric flask. The vessel was rinsed and filtered with a few mL of ultrapure water for quantitative passage of the mineralization. After cooling, the flask was filled with deionized water.

2.3. Determination of heavy metals

Calibration equations were determined for Cd, Hg, and Pb using their certified standard solutions. Table 1 shows the equations of calibration and the related parameters.

Table 1

Quality parameters of calibration curves

Heavy metal	Method	Concentration range (μg/ml)	R^2	LOD (mg/kg)	LOQ (mg/kg)
Cd	FAAS	0.01-0.5	0.9998	0.012	0.04
Hg	CVAAS	1.5- 40.0	0.9999	0.027	0.09
Pb	FAAS	0.1- 2.0	0.9999	0.030	0.10
	GFAAS	0.05-0.25	0.9998	0.005	0.02

After calibrating, the amounts of the three heavy metals in the solutions of the samples were determined using the technique outlined in Table 1. When the found concentration values are below the detection limit, the sample preparation procedure can be resumed by weighing a larger amount of sample. If the content of the element in the sample is higher than the highest value of the standard with which the calibration curve was drawn, then the initial sample solution was diluted, taking into account the dilution in the final calculation of the concentration of the element to be investigated.

3. Results and discussion

The study's objective was to quantify the levels of three hazardous metals in several seafood samples (mussels, shrimp, octopus, and squid) that are accessible to consumers in Romania, utilizing different AAS techniques. Hg, being volatile, was determined using the CVAAS hydride generation technique, Cd by FAAS flame atomic absorption and Pb by both FAAS flame atomic absorption and atomic absorption after atomization in the more sensitive GFAAS graphite furnace.

Table 2 displays the concentration limits permitted by European regulations, which are also applicable in Romania.

Table 2

Concentration limits of toxic metals studied according to Regulation EC1881/2006 as amended by Regulation EC629/2008[23]

Sample	Metal concentration, mg/kg wet weight		
	Cd	Hg	Pb
Crustaceans	0.5	0.5	0.5
Bivalve molluscs	1		1.5
Cephalopod	1		1

Tables 3 and 4 present the amounts of the heavy metals detected using AAS in samples of seafood.

Table 3

Cd, Hg, Pb concentration in mussels in Romania (mg/kg wet weight)

Sample no.	Month	Cd	Hg	Pb
1.	March	0.09 ± 0.01	-	<LOQ (0.10)
2.	June	-	-	<LOD (0.03)
3.	July	0.18 ± 0.02	<LOD (0.027)	0.13 ± 0.01
4.	September	0.10 ± 0.01	<LOD 0.027)	-
5.	October	0.16 ± 0.02	-	0.13 ± 0.02
6.	November	-	-	0.12 ± 0.02
7.	December	0.1 ± 0.01	-	<LOD (0.03)

Table 4

Cd, Hg, Pb concentration in imported seafood (mg/kg wet weight)

No.	Seafood, provenance	Month	Cd	Hg	Pb
1.	Caracatite, India	April	0.53±0.05	<LOD (0.03)	<LOD (0.01)*
2.	Squid, China	February	0.2±0.02	<LOD (0.03)	<LOQ (0.02)*
3.	Prawns, India	February	<LOQ (0.04)	<LOD (0.03)	<LOD (0.03)
4.	Prawns Vietnam	April	<LOQ (0.04)	<LOD (0.03)	<LOQ (0.01)
5.	Prawns Vietnam	July	-	-	<LOD(0.03)
6.	Shrimps Netherlands	Sept	-	-	<LOD (0.03)

* Pb determination were done using the GFAAS method;
LOQ - limit of quantification; LOD - limit of detection

Comparing the values shown in Tables 2 and 3, it can be seen that the measured concentration values do not exceed the regulated values and are often below the limit of quantification or detection of the determination methods. The results are similar to those reported by other researchers [20]. As a result the seafood is not contaminated with the three heavy metals, and can be consumed.

4. Conclusions

The concentration of Cd, Hg, and Pb was determined with AAS. The concentrations found are within the permissible limits (0.5 -1.5 mg/kg) and are similar to those reported before. Following the determinations carried out, it was observed that the seafood studied does not contain concentrations of toxic metals above the normal regulated limits, which means that they can be consumed without presenting a danger to the health of human and animal organisms, but as a result of the gradual build-up of heavy metals in the body it is advisable to consume seafood with moderation: they should not be consumed in excess and should not become the main food of a diet.

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