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*TECHNICAL NOTE*

# Fractionation and Spatial Distribution Analysis of Trace Elements in Wild and Farmed Shrimp from Northeast Brazil

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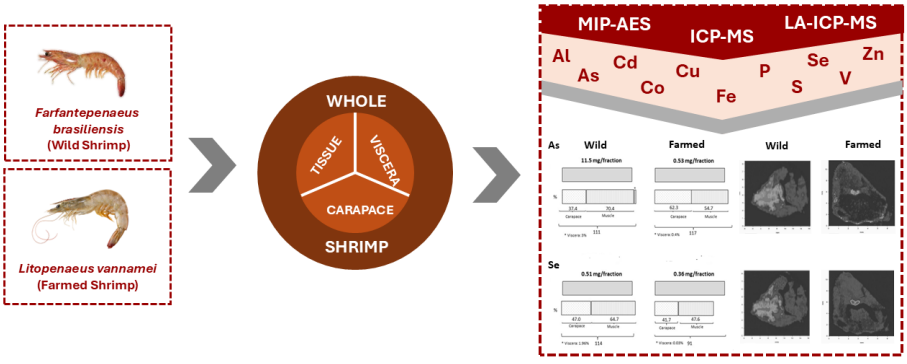


Shrimp is an important commodity, and its production has been increased through aquaculture systems. This animal can bioaccumulate trace elements in their tissues, including toxic ones. The entire body of the animal can be used in culinary processes in food preparations. Thus, the distribution of trace elements in shrimps is a concern. The whole and fractionated (muscle tissue, carapace and viscera) samples of wild and farmed shrimps (*Farfantepenaeus brasiliensis* and *Litopenaeus vannamei*, respectively) from Northeast of Brazil were analyzed. As, Cd, Co, Cu, Mn, Mo, Se and V were quantified by ICP-MS, while MIP-OES was used for Al, Fe and Zn. Spatial distribution of the muscle tissue fraction cross-section of the shrimps was performed by LA-ICP-MS to assess the distribution of As, Cu, Fe, P, S and Se in both shrimp species. Wild and farmed shrimps show different content for almost all analytes, except Co and Mn. The levels of Cu, Mo, and Zn were higher in farmed shrimp. The vanadium content in wild shrimp was around one order of magnitude higher than that found in farmed shrimp. Arsenic (11.5 mg $kg^{-1}$) and Cd (1.94 mg $kg^{-1}$) in the wild sample exceed around 10 times and 4 times, respectively, the limit established by Brazilian legislation. The trace elements distribution in fractions of shrimps are similar for both species, the majority of these elements are mainly found in

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carapace. Cd and Mn are almost completely present in the carapace. The most part of As, Se and Zn is in muscle tissue for both shrimp species, except As in farmed shrimp that is mostly in carapace. Similar spatial distribution was found for As, Se, P and S, probably due to the chemical similarity among them. Relevant information is obtained only through LA-ICP-MS analysis, which showed the correlation between the elements through their spatial distribution.

**Keywords:** laser ablation, aquaculture, minerals, toxic elements, ICP

## INTRODUCTION

Shrimp is considered a high-quality food since it is a source of protein, carbohydrate, vitamins, and other required nutrients for human health. Besides that, it is rich in omega-3 which reduces the risk of heart diseases and provides healthy brain growth in children.[1,2] This crustacean is consumed all over the world and it is considered as a primary food source in some regions of many countries, especially on the coast.[3] Thus, shrimp is an important food commodity, and in the last years, it has been also produced in aquaculture systems to avoid the depletion of wild shrimp stocks caused by its growing consumer demand and international trade.[4] The major species of shrimp in the aquaculture system is the white-leg pacific shrimp (*Litopenaeus vannamei*).[5]

Marine invertebrates, such as shrimps, can bioaccumulate trace elements in their tissues.[1,6] Trace elements play important roles in the biochemical functions in living beings, such as a cofactor in enzymes, responses of oxidative stress, the building of protein and lipidic synthesis.[7] However, some elements can be toxic or potentially toxic, depending on their concentration and/or chemical form. Thus, the possible bioaccumulation of toxic or potentially toxic elements in different organs of shrimp through the water, sediments, and food chain has become a big health concern.

The bioaccumulation of trace elements in an organism is controlled by the balance between uptake and elimination.[8,9] It depends essentially on the concentration of these elements in water, levels in prey or commercial feed, and chemical uptake and elimination kinetics.[10] Other factors such as growth cycle, environmental parameters (salinity, temperature), age, size, body weight, sex, population density, and trophic position of the animal also can determine the extent of trace element accumulation in organism.[11]

The level of inorganic contaminants in food is in general limited by regulatory bodies in different countries. In Brazil, this is done by the National Health Surveillance Agency of Brazil (ANVISA) through “Resolução da Diretoria Colegiada - RDC nº 722, IN-160 de 1º de julho de 2022”.[12]

In general, shrimps are commercially available in three different forms: whole shrimp, de-headed shrimp, and peeled shrimp.[13] The most edible part of this crustacean is the muscle tissue. However, the whole animal body can be used in culinary processes and food preparations. The shells, for example, are used for shrimp stock preparation and the production of shrimp flavor bouillon cubes.[13,14] Hence, the assessment of trace elements distribution in shrimps is required.

In this study, Al, As, Cd, Co, Fe, Mn, Mo, Se, V and Zn were quantified in whole and fractionated samples (muscle tissue, carapace and viscera) of wild and farmed shrimps (*Farfantepenaeus brasiliensis* and *Litopenaeus vannamei*, respectively) from Northeast of Brazil. These shrimp species are the most important resources from fisheries and aquaculture in Brazilian Northeast. The literature extensively describes fractionation studies in white-leg shrimp, but this type of study in pink shrimp was not found.[15–18] Comparison of trace elements concentrations between wild and farmed shrimp is an issue of great concern regarding regulations and monitoring farmed shrimp food contamination.

The elements As, Cd, Co, Cu, Mn, Mo, Se and V were determined by ICP-MS while for Al, Fe and Zn determination, MIP-OES was employed. Spatial distribution of the muscle tissue fraction cross-section of the shrimps was performed by LA-ICP-MS to assess the distribution of As, Cu, Fe, P, S and Se in the sample. The data obtained with LA-ICP-MS analysis were compared with ICP-MS and MIP-OES elemental analysis.

## MATERIALS AND METHODS

### *Sample and reagents*

About 50 wild (*Farfantepenaeus brasiliensis*) and 50 farmed (*Litopenaeus vannamei*) shrimps were acquired from a local supermarket in Fortaleza, Ceará, Brazil in April 2019. This resulted in about 2 kg of each species of shrimp. Half was kept as bought while the other half was dissected into subsamples of the edible muscle tissue, carapace (exoskeleton + head) and viscera using previously decontaminated plastic utensils. The shrimps had an average size of 10.4 cm and 8.8 cm for wild and farmed species, respectively. All samples were freeze-dried (Liobras L 108, São Carlos, SP, Brazil). Three whole shrimps (wild and farmed) were separated for LA-ICP-MS analysis, while the remaining samples were ground using a coffee grinder equipped with stainless steel blades and stored in bags at -20 °C to be digested and subsequently analyzed by ICP-MS and MIP-OES. The ground samples were homogenized so that pooled samples were analyzed in triplicates. The moisture percentage in the fresh shrimps was about 70% w $w^{-1}$, which was monitored throughout the freeze-drying process.

Certified reference material samples DORM-4 (Fish protein, National Research Council of Canada, Canada) and TORT-2 (Lobster hepatopancreas, National Research Council of Canada, Canada) were used for trueness test.

Solutions were prepared using ultrapure water (18.2 MΩ cm resistivity) obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). All plasticware was immersed in $HNO_3$ 10% v $v^{-1}$ (Vetec, Rio de Janeiro, Brazil) for 24 h and thoroughly rinsed with ultrapure water.

All sample digestions were performed using 70% w $w^{-1}$ $HNO_3$, (p.a., Fisher Scientific, UK) and 30% w $w^{-1}$ hydrogen peroxide ($H_2O_2$) (p.a., Fisher Scientific, UK).

### *Instrumentation*

A CEM Mars 5 non-pressurized system (Mars-5, CEM instrument, UK) was used for sample digestion. ICP-MS (Agilent 8800 ICP Triple Quad, ICP-QQQ) was used for quantification. After digestion, As, Cd, Co, Cu, Mn, Mo, Se and V were analyzed in whole shrimp sample, muscle tissue, carapace and viscera fractions using ICP-MS. The ICP-MS parameters are shown in Table I.

**Table I**. Instrumental settings used for As, Cd, Co, Cu, Mn, Mo, Se and V analyses by ICP-MS

| ICP-MS settings | |
|---|---|
| RF power (W) | 1600 |
| Nebulizer gas flow (L $min^{-1}$) | 1.18 |
| Plasma gas flow (L $min^{-1}$) | 15 |
| Nebulizer | Cross Flow |
| Auxiliary gas flow (L $min^{-1}$) | 1 |
| Spray chamber | Double-pass |
| Interface cones | Nickel |
| Lens voltage (V) | 3.5–4.0 |
| Mass resolution (u) | 0.8 |
| Integration time (ms) | 1000 |
| *m/z*+ | $^{75}As$, $^{111}Cd$, $^{59}Co$, $^{63}Cu$, $^{55}Mn$, $^{95}Mo$, $^{78}Se$, $^{51}V$ |

Aluminium, Fe, and Zn determination was carried out using a microwave-induced plasma optical emission spectrometer model (MIP-OES) (4200 Agilent Technologies, Santa Clara, CA, USA). Sample introduction comprised an Agilent SPS 3 autosampler, a double-pass cyclonic spray chamber, and inert OneNeb nebulizer. A liquid $N_2$ Dewar model 4107 (Agilent Technologies) was used as the plasma gas source. Background correction was automatically performed using the MP Expert software (Agilent Technologies). The peristaltic pump speed was set at 15 rpm. Stabilization time was 15 s, uptake time was 60 s and rinse time was 30 s. The nebulizer gas flow rate and the viewing position were automatically optimized by the MP Expert software using a multi-element solution containing the analytes and internal standards. The wavelengths 396.152 nm, 371.993 nm, and 213.857 nm were used for Al, Fe, and Zn, respectively.

Arsenic, Cu, Fe, P, S and Se were also determined on a thin tissue of the shrimp cross section using laser ablation inductively coupled plasma mass spectrometry, LA-ICP-MS (New Wave UPS123 and 7900 ICP-MS Agilent technologies). The instrument parameters are shown in Table II. The construction of elements images was performed by Sigma Plot program.

**Table II.** Instrument setting for LA-ICP-MS for As, Cu, Fe, P, S, and Se mapping in cross-section shrimp

| **LA parameter** | |
|---|---|
| Laser | Nd:YAG |
| Laser Fluence (J $cm^{-2}$) | 25 |
| Wavelength (nm) | 213 |
| Repetition rate (Hz) | 20 |
| Scan speed (µm $s^{-1}$) | 50 |
| Spot size (µm) | 100 |
| Output energy (%) | 40 |
| Scanning type | Line by line |
| **ICP-MS Parameter** | |
| RF power (W) | 1550 |
| Nebulizer gas flow rate (L $min^{-1}$) | 1.26 |
| Spray chamber temperature ( ºC) | 2 |
| Skimmer cone | Nickel |
| Sampling cone | Nickel |
| Reaction cell $H_2$ flow rate (mL $min^{-1}$) | 3.2 |
| *m/z*+ | $^{75}As$, $^{63}Cu$, $^{57}Fe$, $^{31}P$, $^{32}S$, $^{78}Se$ |

### *Wet digestion of shrimp samples*

Shrimp samples were digested using microwave oven cavity in an open vessel system. Approximately, 0.100 g of sample was transferred to a Falcon® tube and 2 mL of 65% w $w^{-1}$ $HNO_3$ was added. This mixture was left overnight, then 2 mL of 30% w $w^{-1}$ $H_2O_2$ was added to the samples which were submitted to the heating program presented in Table III. After this, the vessels were allowed to cold to room temperature and the samples were diluted to 25 mL with ultrapure water and analyzed by ICP-MS and MIP-OES.

**Table III.** Heating programming used for wet digestion of shrimp samples in a microwave cavity oven

| Power (W) | Ramp (min) | Temperature (ºC) | Hold (min) |
|---|---|---|---|
| 800 | 2 | 50 | 5 |
| 800 | 2 | 75 | 5 |
| 800 | 5 | 95 | 30 |

Certified reference materials DORM-4 and TORT-2 were also digested under the same procedure applied to shrimp samples for trueness test.

### *Sample preparation for spatial distribution analysis*

Dried shrimps were frozen and sliced to thin sections using Microtome cryostat (Bright 5030, Bright Instruments, UK) at -20 ºC and left overnight on a glass slide at room temperature before analysis. The cross-section of the shrimp sample and thin tissue is shown in Figure 1.

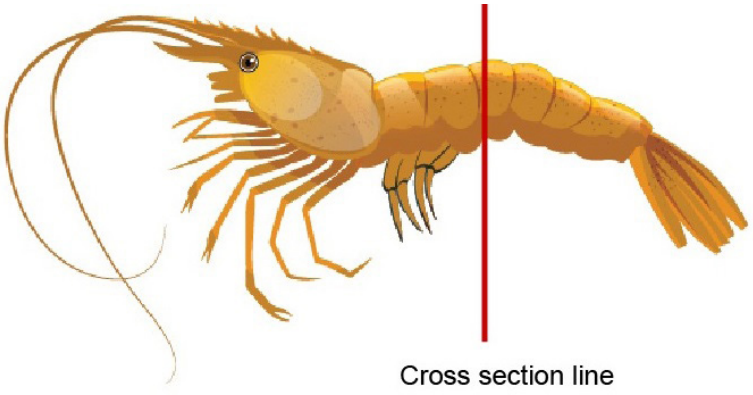


**Figure 1.** Cross-section line of shrimp for LA-ICP-MS analysis.

## RESULTS AND DISCUSSION

### *Trueness test*

In order to verify the trueness of the analytical method used for elemental determination in shrimps, samples of the certified reference materials (CRM) DORM-4 and TORT-2 were analyzed. The CRMs samples were digested using the same procedure as the shrimp samples. The results of CRMs analyses are shown in Table IV.

**Table IV.** Quantification of Al, As, Co, Cu, Mn, Mo, Se, V and Zn (mg $kg^{-1}$) in DORM-4 and TORT-2 CRMs samples after digestion using microwave cavity oven. Al, Fe, and Zn were quantified by MIP-OES while the other elements were quantified by ICP-MS. (mean ± sd, n=3)

| | Samples | | | | | |
|---|---|---|---|---|---|---|
| | DORM-4 | | | TORT-2 | | |
| Elements | Proposed Method (mg $kg^{-1}$) | Reference Value* (mg $kg^{-1}$) | Recovery (%) | Proposed Method (mg $kg^{-1}$) | Reference Value* (mg $kg^{-1}$) | Recovery (%) |
| Al | 1279 ± 53 | 1280 ± 340 | 100 | 20.2 ± 2.3 | - | - |
| As | 7.00 ± 0.45 | 6.87 ± 0.44 | 102 | 21.9 ± 2.5 | 21.6 ± 1.8 | 101 |
| Co | 0.23 ± 0.01 | (0.25) | 92 | 0.53 ± 0.07 | 0.51 ± 0.09 | 104 |
| Cu | 14.9 ± 0.7 | 15.7 ± 0.5 | 95 | 111 ± 5 | 106 ± 10 | 105 |
| Fe | 358 ± 13 | 343 ± 20 | 104 | 91.6 ± 0.8 | 105 ± 13 | 87 |
| Mn | 3.14 ± 0.17 | 3.17 ± 0.26 | 100 | 13.5 ± 0.3 | 13.6 ± 1.2 | 100 |
| Mo | 0.29 ± 0.01 | (0.29) | 100 | 1.09 ± 0.15 | 0.96 ± 0.10 | 112 |
| Se | 3.64 ± 0.05 | 3.45 ± 0.40 | 105 | 6.57 ± 0.73 | 5.63 ± 0.67 | 115 |
| V | 1.31 ± 0.06 | 1.57 ± 0.14 | 83 | 1.84 ± 0.21 | 1.64 ± 0.19 | 112 |
| Zn | 50.3 ± 3.7 | 51.6 ± 2.8 | 97 | 170 ± 8 | 180 ± 6 | 94 |

*mean ± U, k=2

The recovery of the analytes in the certified reference materials was satisfactory (83-115%) and the certified values are statistically similar to the determined values according to Student's *t*-test (95% confidence). The precision of the data is also satisfactory, obtaining a relative standard deviation (RSD) below 20% for all elements.

It is important to highlight that despite a non-pressurized microwave system was used for sample digestion, a heating program with mild temperatures (maximum 95 ºC) was applied. Therefore, if any loss of some potentially volatile elements (As, Se, V, Zn) occurred, it was not significant. This could be verified by statistical analysis (*t*-test) described in the paragraph above.[19]

The trace elements content of Al, As, Cd, Co, Fe, Mn, Mo, Se, V and Zn was quantified in the whole animal and different fractions of wild and farmed shrimp samples (Table V and Figure 2).

**Table V.** Fractionation of trace elements in wild and farmed shrimp quantified by ICP-MS (As, Cd, Co, Cu, Mn, Mo, Se and V) and MIP-OES (Al, Fe and Zn) after wet digestion (Mean ± SD, n=3)

| | | WILD SHRIMP | | | | FARMED SHRIMP | | | |
|---|---|---|---|---|---|---|---|---|---|
| **ELEMENT** | | **TOTAL** | **TISSUE** | **CARAPACE** | **VISCERA** | **TOTAL** | **TISSUE** | **CARAPACE** | **VISCERA** |
| Al | Mean (mg kg$^{-1}$) | 6.14 ± 0.61 | 3.17 ± 0.35 | 26.8 ± 0.6 | 595 ± 39 | 1.75 ± 0.22 | 1.73 ± 0.26 | 6.62 ± 0.62 | 331 ± 27 |
| | mg/fraction | 6.14 | 0.38 | 3.06 | 2.47 | 1.75 | 0.22 | 0.81 | 0.22 |
| | Mass balance (%) | | | 96 | | | | 71 | |
| As | Mean (mg kg$^{-1}$) | 11.5 ± 0.5 | 16.3 ± 0.3 | 8.9 ± 0.2 | 19.5 ± 0.01 | 0.53 ± 0.02 | 0.57 ± 0.03 | 0.67 ± 0.01 | 0.07 ± 0.01 |
| | mg/fraction | 11.5 | 8.1 | 4.3 | 0.34 | 0.53 | 0.29 | 0.33 | 0.002 |
| | Mass balance (%) | | | 111 | | | | 117 | |
| Cd | Mean (mg kg$^{-1}$) | 1.94 ± 0.38 | 2.53 ± 0.09 | 18.6 ± 0.6 | 10.4 ± 0.06 | <LQ | <LQ | <LQ | <LQ |
| | mg/fraction | 1.94 | 0.31 | 2.12 | 0.04 | | | - | |
| | Mass balance (%) | | | 127 | | | | | |
| Co | Mean (µg kg$^{-1}$) | 22.6 ± 1.3 | 43.5 ± 3.1 | 211 ± 11 | 836 ± 41 | 24.0 ± 2.3 | 22.3 ± 3.4 | 166 ± 16 | 713 ± 25 |
| | mg/fraction | 22.6 | 5.29 | 20 | 3.46 | 24.0 | 2.82 | 20.4 | 0.50 |
| | Mass balance (%) | | | 127 | | | | 99 | |
| Cu | Mean (mg kg$^{-1}$) | 10.5 ± 0.7 | 32.5 ± 1.7 | 87.5 ± 2.2 | 72.4 ± 0.8 | 19.6 ± 2.8 | 39.3 ± 5.9 | 122 ± 4 | 123 ± 43 |
| | mg/fraction | 10.5 | 2.94 | 8.98 | 0.30 | 19.6 | 5.0 | 15.1 | 0.08 |
| | Mass balance (%) | | | 116 | | | | 103 | |
| Fe | Mean (mg kg$^{-1}$) | 24.0 ± 1.7 | 11.4 ± 1.3 | 118 ± 5 | 2298 ± 21 | 6.44 ± 0.54 | 2.84 ± 0.10 | 47.6 ± 4.1 | 957 ± 15 |
| | mg/fraction | 24.0 | 1.39 | 13.4 | 9.53 | 6.44 | 0.36 | 5.85 | 0.62 |
| | Mass balance (%) | | | 101 | | | | 106 | |

(continues on next page)

**Table V.** Fractionation of trace elements in wild and farmed shrimp quantified by ICP-MS (As, Cd, Co, Cu, Mn, Mo, Se and V) and MIP-OES (Al, Fe and Zn) after wet digestion (Mean ± SD, n=3) (continuation)

| | | WILD SHRIMP | | | | FARMED SHRIMP | | | |
|---|---|---|---|---|---|---|---|---|---|
| **ELEMENT** | | **TOTAL** | **TISSUE** | **CARAPACE** | **VISCERA** | **TOTAL** | **TISSUE** | **CARAPACE** | **VISCERA** |
| Mn | Mean (mg $kg^{-1}$) | 2.00 ± 0.11 | 1.56 ± 0.07 | 18.2 ± 0.5 | 43.7 ± 2.6 | 2.93 ± 0.54 | 1.55 ± 0.24 | 24.6 ± 1.6 | 57.8 ± 21.6 |
| | mg/fraction | 2.00 | 0.19 | 1.98 | 0.18 | 2.93 | 0.20 | 2.83 | 0.04 |
| | Mass balance (%) | | | 117 | | | | 111 | |
| Mo | Mean (µg $kg^{-1}$) | 22.7 ± 1.6 | 44.3 ± 5 | 164 ± 3 | 188 ± 11 | 124 ± 13 | 43.7 ± 5.7 | 173 ± 7 | 371 ± 31 |
| | mg/fraction | 22.7 | 5.39 | 17.8 | 0.77 | 33.6 | 5.58 | 21.3 | 0.24 |
| | Mass balance (%) | | | 105 | | | | 81 | |
| Se | Mean (mg $kg^{-1}$) | 0.51 ± 0.01 | 2.73 ± 0.30 | 2.10 ± 0.11 | 3.21 ± 0.17 | 0.36 ± 0.04 | 1.33 ± 0.06 | 1.25 ± 0.27 | 2.20 |
| | mg/fraction | 0.51 | 0.33 | 0.24 | 0.01 | 0.36 | 0.17 | 0.15 | 0.001 |
| | Mass balance (%) | | | 114 | | | | 91 | |
| V | Mean (µg $kg^{-1}$) | 49.3 ± 0.1 | 60.0 ± 1.0 | 350 ± 40 | 2140 ± 80 | 8.90 ± 1.00 | 13.4 ± 2.8 | 55.4 ± 11 | 1174 ± 228 |
| | µg/fraction | 49.3 | 7.0 | 39.4 | 8.9 | 8.90 | 1.8 | 6.8 | 0.76 |
| | Mass balance (%) | | | 112 | | | | 105 | |
| Zn | Mean (mg $kg^{-1}$) | 3.76 ± 0.14 | 21.2 ± 0.99 | 10.3 ± 0.63 | 125 ± 1 | 5.57 ± 0.70 | 26.7 ± 2.0 | 22.8 ± 2.9 | 90.1 ± 3.3 |
| | mg/fraction | 3.76 | 2.55 | 1.18 | 0.52 | 5.57 | 3.41 | 2.80 | 0.06 |
| | Mass balance (%) | | | 113 | | | | 112 | |

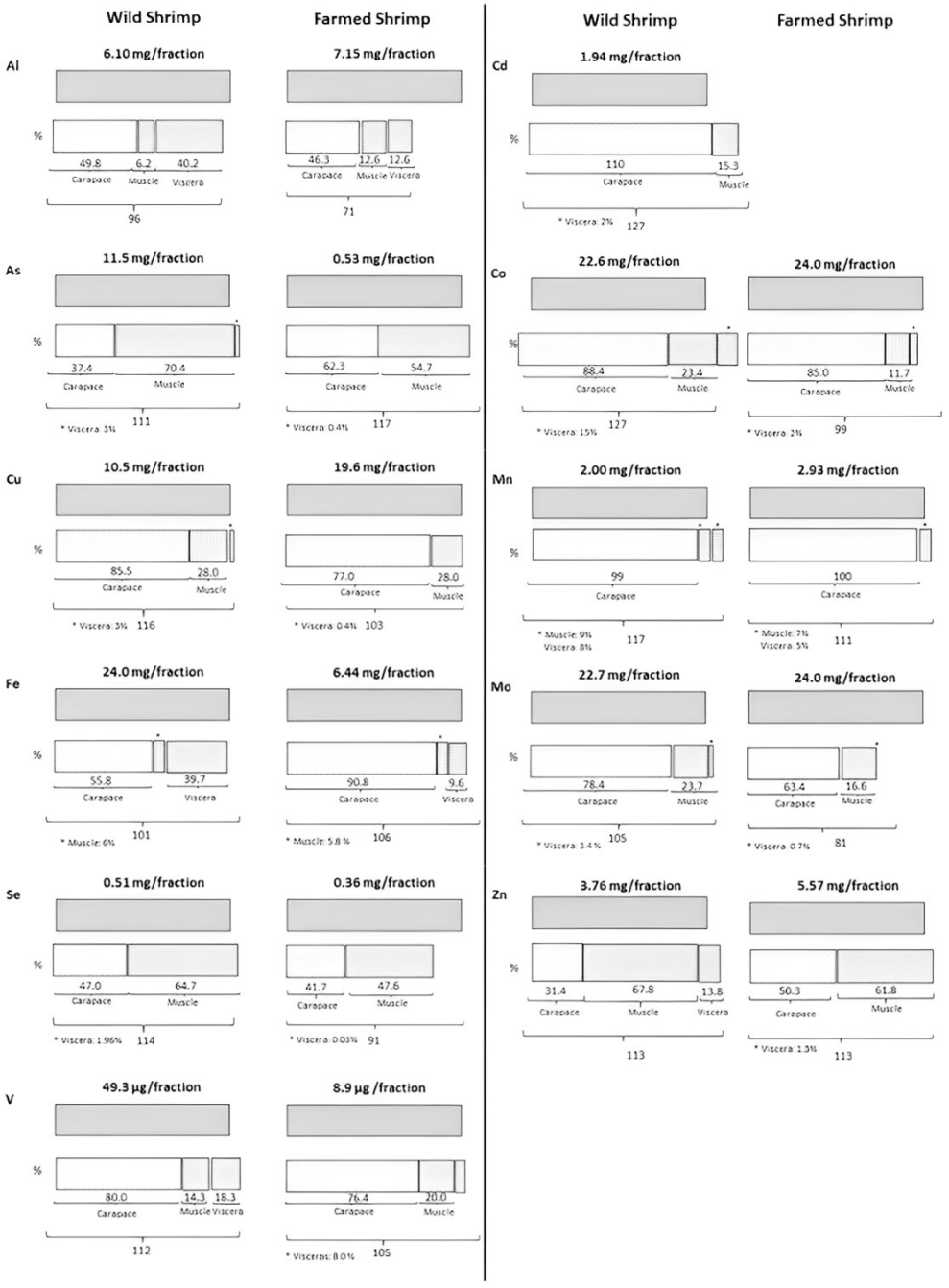


**Figure 2.** Distribution of Al, As, Cd, Co, Cu, Fe, Mn, Mo, Se, V and Zn in fractioned shrimp.

The recovery percentages shown in Table V are related to a mass balance between the total concentration of the elements found in the whole shrimp samples and the content of the elements found in the fractions of shrimp samples (muscle tissue, carapace and viscera). For this, 2 kg of each species of shrimp was divided into two groups of around 1 kg each. In one group it was kept the whole body of the animals, while in the other the animals were fractionated and each fraction was weighed. The mass values of the fractions used in the mass balance calculations are described in Table VI.

**Table VI.** Values of mass of the wild and farmed samples

| Sample | Mass of the dried fraction (g) |
|---|---|
| whole (wild shrimp) | 238.0245 |
| whole (farmed shrimp) | 269.9673 |
| muscle tissue (wild shrimp) | 121.2777 |
| muscle tissue (farmed shrimp) | 127.5393 |
| carapace (wild shrimp) | 114.1142 |
| carapace (farmed shrimp) | 122.9314 |
| viscera (wild shrimp) | 4.1453 |
| viscera (farmed shrimp) | 0.8574 |

Mass balance was calculated using Equation 1.

$$Mass\ balance\ = \frac{\{(C_{tissue}\ \times\ m_{tissue}) + \ (C_{carapace}\ \times\ m_{carapace}) + (C_{viscera}\ \times\ m_{viscera})\}}{C_{total}} \qquad \text{Equation 1}$$

where *C* is the concentration of the element in the fraction, *m* is the mass of each fraction

The patterns of the trace elements that occur in the whole shrimps can be written in descending order as follows:

- wild shrimp: Fe > Mo > Co > As > Cu > Al > Zn > Mn > Cd > Se > V
- farmed shrimp: Mo > Co > Cu > Al > Fe > Zn > Mn > As > Se > V > Cd

Wild and farmed shrimps show different content for almost all analytes, except Co and Mn (ANOVA with Turkey Test, 95% of confidence). The levels of Cu, Mo, and Zn were higher in farmed shrimp. High concentrations of Cu and Zn compounds are used in shrimp farms for different purposes like antibiotics, fungicides, and food supplementation.[20] Some researches related that Cu and Zn are the main elements found in shrimp farms waste.[21]

The vanadium content in wild shrimp was around one order of magnitude higher than that found in farmed shrimp. Vanadium is a common trace element found in the sea water and marine plants, therefore marine animals, in general, have higher concentration of V than terrestrial ones.[22] However, the source of this element can also be related to anthropogenic activities like pollution from steel or oil industries.[23]

Cadmium was detected only in wild shrimp and its concentration is higher than that recommended for crustaceans by Brazilian legislation (0.5 mg $kg^{-1}$ Cd). As well as As that is more than 10 times above of the limited value (1.0 mg $kg^{-1}$ As). However, for this last element, our research group revealed that only 1.2% of the As in wild shrimp is as inorganic As, the most toxic As species; and that arsenobetaine, the non-toxic arsenic form, predominates in this matrix.[1]

The patterns of the trace elements occurrence in the fractions of the shrimp samples are presented in descending order as follows:

- Muscle tissue (Wild Shrimp): As > Mo > Co > Cu > Zn > Fe > Al > Se > Cd > Mn > V
- Muscle tissue (Farmed Shrimp): Cu > Mo > Zn > Co > Al > Fe > As > Mn > Se > V > Cd
- Carapace (Wild Shrimp): Co > Mo > Fe > Cu > As > Al > Mn > Cd > Zn > Se > V
- Carapace (Farmed Shrimp): Co > Mo > Cu > Fe > Al > Mn > Zn > As > Se > V > Cd

- Viscera (Wild Shrimp): Co > Al > Fe > Mo > Zn > As > Cu > Mn > Cd > Se > V
- Viscera (Farmed Shrimp): Al > Fe > Co > Mo > Mn > Cu > Zn > As > V > Se > Cd

The trace elements distribution in fractions of shrimps are similar for both species, the majority of these elements are mainly found in carapace. This distribution of elements in the fractions is better shown in Figure 2. Some elements can be adsorbed on exoskeleton due to their interaction with the polysaccharides chitosan and/or chitin that make up the exoskeleton of crustaceans.[24,25] Although the exoskeleton can contain a significant proportion of the total body burden of inorganic elements, this can be reduced after to moulting.[15] Cadmium and Mn are almost completely present in the carapace. The literature reports that Cd in decapods is preferentially accumulated in hepatopancreas and its presence in substantial concentration in exoskeleton can be attributed to the involvement of this tissue in the excretion of this element.[26] The toxicity of Cd in seafood requires more studies, especially regarding information about chemical species. Cadmium may be found binding with metallothionein. In general, the complexation of Cd with thionein decreases the toxicity of this element. However, more studies must be done.[27] It is noteworthy that Cd could not be detected in farmed shrimps. Manganese can substitute Ca in $CaCO_3$,[28] this may explain why this element is predominantly found in the calcified fraction of the animal; furthermore, this element could complement or substitute $Mg^{2+}$,[15] and is a cofactor of the superoxide dismutase enzyme.[29] There is also evidence that Mn is found when high concentrations of Cu are achieved in seafood.[30] Aluminum, Co, Cu, Fe, Mo, and V are also mainly found in carapace.

In contrast, the most part of As, Se and Zn is in muscle tissue for both shrimp species, except As in farmed shrimp that is mostly in carapace. Though inorganic As is toxic, arsenobetaine (non-toxic As form) is predominant in shrimps.[31] Selenium is an essential element that is tightly bound to amino acids in the form of selenomethionine and selenocysteine.[32] And Zn is an important cofactor in some enzymes such as SOD, which controls the oxidative stress process.

The distribution of Al, Fe, and V shows that after carapace fraction, the major content of these elements is found in viscera. This represents the capability of crustaceans digestion system to excrete excess and/or toxic elements. This phenomenon is well discussed in the literature.[10,15,16,33,34]

***LA-ICP-MS***

Laser ablation coupled with ICP-MS (LA-ICP-MS) was used to obtain a mapping of some elements ($^{75}As$, $^{63}Cu$, $^{57}Fe$, $^{31}P$, $^{32}S$, $^{78}Se$) using cross-sections of the whole body of wild and farmed shrimp. It was possible to observe the distribution of these elements in a cross-section of the animal without fractioning the sample and with minimum sample treatment.

The elemental maps for wild and farmed shrimp (Figure 3) show that As (3A and 3B) is predominantly found in the tissue sample followed by the exoskeleton for wild shrimp and is practically equally distributed between muscle tissue and exoskeleton for farmed shrimp, as discussed in fractionation analyses with ICP-MS after sample digestion.

Selenium presented similar profile of As in shrimps (Figures 3K and 3L). The relationship between Se and As is well discussed in the literature. This is based on the mutual interaction of these elements in the attempt to decrease the toxicity of As inorganic forms.[32,35,36] There are pieces of evidence that the formation of an As-Se complex may serve to reduce the uptake of the inorganic forms of As and Se in the tissues.[36]

Although there are few papers about the association between organic species of As and Se, it is fact that the major fraction of As found in seafood is arsenobetaine (AsB).[31,37] Some papers report several organic forms of Se in this type of matrix, such as selenoproteins, selenomethionine, and trimethylselenonioum cation.[38] Among these Se species, selenomethionine (SeMet) is an important pathway that may explain the Se and As interactions observed in this study. Several biological pathways that use Se as cofactor are known in environmental and biological systems. In water and sediments the presence of dimethylselenopropionate $(CH_3)_2Se^+CH_2COO$ (DMSeP) is common, this substance is analogous to AsB ($(CH_3)_3As^+CH_2COO$) and it is a metabolite of the SeMet pathway.[39] It is important to mention that DMSeP is found in several plant

and algae samples[40] that could be a food source for shrimp. Besides that, the trimethylselenium $(CH_3)_3Se^+$ cation found in oysters and mussels is similar to trimethylarsine $(CH_3)_3As$ and tetramethylarsonium ion $(CH_3)_4As^+$ (TMA). These As species are used in arsenosugar synthesis in algae and microorganisms.[39,41] More studies in this special relationship are needed to understand the mechanisms of interaction between As and Se organic forms.

Looking at Figures 3G and 3H, it can be seen that phosphorus occurs in a similar spatial distribution to As in shrimp tissues, with a high concentration in the carapace, especially in the farmed sample. This relationship is less clear for wild shrimp, but still follows this analogous pattern. Phosphorus and As are from the same family in the periodic table and, therefore, they have similar chemistry. In marine organisms, the As uptake pathway is the same as that for phosphate,[8] therefore it is expected that they will have an equivalent spatial distribution. In fact, there is some evidence of the possibility of As substituted P in complex molecules such as DNA and RNA.[42–44] Phosphorus found in the exoskeleton is probably as calcium phosphate once it was already found in this fraction.[45]

Sulfur has the same tendency found for As, P and Se (Figures 3I and 3J). It occurred mainly in tissue of shrimps. Selenium could substitute S in several organic structures, such as in amino acid cysteine forming the selenocysteine.[38,46] Sulfur has a high affinity with As species[47] and some sulfured arsenic compounds were found in the literature.[48] This affinity and relations between As and Se may explain their similar spatial distribution.

Copper in the farmed sample is mainly found in the carapace (Figures 3C and 3D), as indicated by ICP-MS results. It is important to observe that according ICP-MS data, Cu concentration in farmed shrimp is almost 2 times higher than wild shrimp. The biological function of this element is well known in the literature,[49] and probably the Cu found in tissue is related to the capability of this element to be a cofactor in several enzyme classes.[50] The different concentrations among shrimp species could be related to the use of some products (antibiotics and insecticides) based on Cu in the shrimp farms, besides that Cu is a common element found in effluents of shrimp farms.[21]

Iron is a trace element found in hemoglobin complexes, however, in crustaceans, this mechanism can be replaced by Cu.[49] In imaging data (Figures 3E and 3F), it is possible to observe the major fraction of Fe is in carapace as Cu. Iron is found in the carapace probably due to the molting stage when the concentrations of this element increase.[51]

The use of LA-ICP-MS expands the discussion about trace element spatial location in shrimp sample, showing some relations not observable in ICP-MS analysis. More studies should be done to explain some trends observed in the present manuscript and the spatial relationship with species of elements.

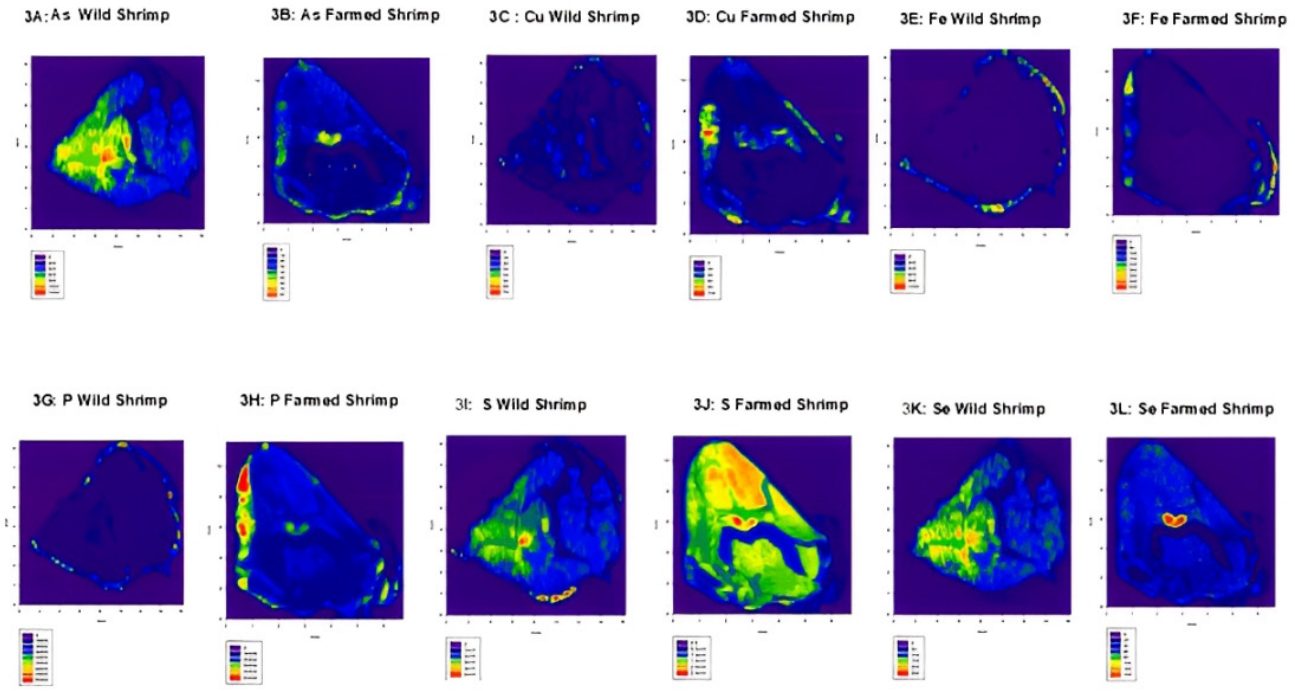


**Figure 3**. Concentration mapping (μg $kg^{-1}$) of As (**A, B**), Cu (**C, D**), Fe (**E, F**), P (**G,H**), S (**I, J**) and Se (**K, L**) in wild and farmed shrimp using LA-ICP-MS.

## CONCLUSIONS

The trace elements content of Al, As, Cd, Co, Fe, Mn, Mo, Se, V and Zn was quantified in different fractions of wild and farmed shrimps from Northeast Brazil. Wild and farmed shrimps show different content for almost all analytes, except Co and Mn. The levels of Cu, Mo, and Zn were higher in farmed shrimp. The vanadium content in wild shrimp was around one order of magnitude higher than that found in farmed shrimp. For Brazilian and international legislation, As and Cd in wild sample exceed around 10 times and 4 times, respectively, the allowance limit.

The distribution of trace elements in fractions of shrimps are similar for both species, the major of these elements are mainly found in carapace. Cadmium and Mn are almost completely present in the carapace. The majority of As, Se and Zn is found in muscle tissue for both shrimp species, with the exception of As in farmed shrimp, which is mainly found in the carapace.

Similar spatial distribution was found for As, Se, P and S, probably due to the chemical similarity among them. Arsenic and Se have a known interaction between their inorganic forms, but not with organic species. Speciation analysis studies are required to understand this interaction. The LA-ICP-MS results given equivalent information obtained by ICP-MS fractionation but required minimal sample preparation.

### Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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