REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No.2 (2024)
http://www.veterinaria.org
Received: 11/06/2024 Accepted: 16/10/2024 Published: 22/11/2024

Assessment and analysis of lead, cadmium, zinc, copper in *Aristeus antennatus* shrimp's from mediterranean coast of Algiers and evaluation of freshness by total volatile basic nitrogen test, before and after freezing

Chabha BENMOHAND[1,2*], Lynda AÏNOUZ[3,4], Fatma Amira HANI[1], Mohamed ZAOUANI[3,4], Sofiane BOUDJELLABA[2], Madjid AKKOU[5], Amel MILLA[2], Djamel KHELEF[1]

[1]Animal Health and Production Laboratory, Higher National Veterinary School, Algiers, Algeria (SPA-ENSV-Algiers)
[2] Research Laboratory Management of Local Animal Resources, Higher National Veterinary School, of Algiers, Algeria (GRAAL- ENSV-Algiers)
[3]Laboratory of Biology and Animal Physiology, ENS Kouba, Algiers, Algeria
[4]Research Laboratory of Food Hygiene and Quality Insurance System, Higher National Veterinary School, of Algiers, Algeria (HASAQ- ENSV-Algiers)
[5] Institute of veterinary Science,University of SaadDahlab Blida1 PB:09000, Blida, Algeria

*** Corresponding author:** Chabha BENMOHAND
c.benmohand@ensv.dz

Abstract
Seafood is the main source of exposure to environmental contaminants. However, risk-benefit analysis indicates that, in most of cases, the benefits associated to fish and seafood consumption outweigh the risks. The present study intended to determine the concentration of cadmium (Cd), lead (Pb), copper (Cu), zinc (Zn) and the Total Volatile Basic Nitrogen (TVB-N) level in *Aristeus antennatus* shrimp's, from the Mediterranean coast of Algiers, before and after freezing. After Freeze-drying and samples mineralization, Cd, Pb, Cu, Zn concentrations were determined by Atomic Absorption Spectrometry method. TVB-N level determination was performed following the EC N. 2074 (2005) regulation method, at different conservation temperatures. The results showed that the concentrations of Cd, Pb, Zn and Cu respectively, were below the Detection Limit (BDL), 0.072, 0.091 and 0.019 mg/kg in unpeeled shrimps and 0.048, 0.072 and 0.011mg/kg in the peeled ones. All trace metal elements concentrations were statistically different; however, they were below the maximum recommended limits for human consumption set by WHO and FAO(2017). Furthermore, the TVB-N levels were 5.64 mg/100 g in fresh shrimps, 17.95 mg/100g in shrimps frozen at -18°C, 23.998 mg/100g in shrimps frozen at -18°C and then chilled at 4°C for 5 days, and 42.64 mg/100 g in shrimps chilled at 4°C for 5 days. Statistical analysis revealed a significant difference. We conclude that metal trace elements were within the acceptable range, and freezing ensures good preservation of the quality of the shrimps.

Keywords: Shrimp, trace metal elements, total volatile basic nitrogen, Algerian coast.

Introduction

Seafood is classified as a nutritious and protein-rich food, providing heavy metals (also called trace metal elements: Zn, Fe, Cu…) as nutrients for human health(Nhu *et al.,* 2021).For this reason, the world consumption of seafood, such as marine fish and crustaceans (shrimp, crabs and lobster) and their products has increased. Regularly eating shellfish may increase immunity, and promote brain and heart health (Hosomi *et al.,* 2012). Seafood intake provides essential nutrients, especially for specific parts of the population, such as children, older adults, and women of reproductive age or pregnant. However, the safety of consuming this type of food is currently under debate as these may accumulate elements of toxicological importance in their tissues(Andayesh *et al.,* 2015).Many studies have pointed towards the adverse effects to human health that may occur from the consumption of the contaminated seafood with trace metal elements which are non-biodegradable, persistent and known to cause deleterious effects on human and animal health(Davydova, 2005). Trace metal elements are highly toxic to marine organisms and human, even at very low concentrations due to bioaccumulation. They can interfere with biological systems and induce inappropriate interactions with different intracellular structures(Mehouel *et al.,* 2019).Cadmium, lead, cupper and Zinc are traces of metal elements. Some of them (e.g., Cu, Se, and Zn) are nutritionally essential elements at low levels but toxic when high, whereas others (e.g., Pb, As, Cd and Hg) are exlusively toxic. Both acute and prolonged exposures to traces metal elements cause various diseases (Järup, 2003; Yousif *et al.,* 2021).
Trace metal elements are essential components of the aquatic environment, usually found in low concentrations. The largest part of the trace metal elements released into the earth finds its way into the fresh water and marine environment(Yousif *et al.,* 2021). Their long persistence, bioaccumulation, bio-magnification and toxicity in the food chain may cause a serious health hazard (Baki *et al.,* 2018). It is well established that the levels of trace metal elements are too high in the areas where domestic, mining, mechanical and cultivating activities are across natural ones(Bryan and Langston 1992; Baki *et al.,* 2018). Furthermore, some seafood have the ability to absorb and accumulate trace metal

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No.2 (2024)
http://www.veterinaria.org
Received: 11/06/2024 Accepted: 16/10/2024 Published: 22/11/2024

elements in their bodies, which then affects public health especially people consuming them(Tuzen, 2003). This situation greatly impacts the quality of seafood, and thus worrying for the public health(Baki *et al.*, 2018). However, the trace metal elements contamination of seafood is gradually becoming a global crisis given that sea water is vulnerable to the growing discharges of pollutants at the bay on almost every coast around the world(Ahmed *et al.*, 2015).
Shrimp, rich in proteins, minerals, vitamins, antioxidants, essential amino-acids, and unsaturated fatty acids, is considered as one of the most beneficial shellfish foodsfor human consumption(Biswas *et al.*, 2018). It is a perishable product that needs quick storage. Enzymatic and bacteriologic activity can rapidly decrease the protein content and then food quality becomes stale, producing some ammonia, trimethylamine, dimethylamine and others volatile basic nitrogenous compounds, which together are called TVB-N (Fallah *et al.*, 2015; Altissimi *et al.*, 2017).Environmental pollution associated with frequent breaks in the cold chain; make it mandatory to implement appropriate quality control tests for shrimps. In the present study, we aimed to assess seafood product quality by determining the Cd, Pb, Zn and Cu concentrations in unpeeled and peeled caught *Aristeus antennatus* shrimp's (Risso, 1816)from the Mediterranean coast of Algiers, and their freshness according to the different modes of conservation that reflect the domestic storage conditions.

Materials and Methods

Ethical approval

Ethical approval was not applicable to this study. Fresh shrimp samples used for the analysis were purchased and collected from the fishing ports of Algiers.

Reagents

The chemicals and reagents used were of analytical grade and high quality and mostly purchased from Sigma-Aldrich (St. Louis, MO) and Merck (Mannheim, Germany). Nitric acid Suprapur and standard solutions of lead, cadmium, zinc and copper were purchased from Merck (Darmstadt,Germany) and ultrapure water was used for all dilutions.

Samples collection

Sixty (60) fresh shrimps *Aristeusantennatus***,** were purchased in different days in Algiers' fishing ports. Immediately after the collection, samples were kept in an airtight insulating box and thereafter transported to the laboratory. Shrimp samples preparation for the analysis was carried out according to the requirements of the European standard EN 13804 (2005)[16]. Shrimps were quickly rinsed with tap water then with ultrapure water. Shrimps were randomly selected for testing and divided into several batches (n=10).

Samples preparation

For the trace metal elements assay, two batches of shrimps *Aristeus antennatus* with shells (unpeeled shrimps) and shrimps without shells (peeled shrimps) were congealed at -20°C. Freeze-drying of shrimp grinds has been conducted by freezing first at -20°C, then drying at - 40°C, under a pressure of 10^{-1} bar during 48 hours, in a LYOVAC GT 2.
For the determination of TVB-N, the edible parts of fresh shrimps were divided as follows: Two batches were frozen at -18°C, the first batch (SF) was used for the assay just after defrosting and the second batch (SFR) was used for the assay after defrosting at +4°C for 5 days. A third batch (SR) was assayed after being refrigerated at +4°C for 5 days. TVB-N was determined on edible parts of fresh shrimps of the day (SD). Before each experiment, shrimp samples were cut into small pieces and homogenised with a stainless steel blender cup. Priorto use, all plastic and glassware were cleaned by soaking in diluted HNO3 (10% v/v) and rinsed with distilled water.

Sample preparation by organic matter digestion

Samples mineralization was done according to European Standard EN 13805 (2002). One g of lyophilized shrimp's samples was weighed and placed a digestion vessel. The mixtures were mixed with 10 mL 3:2 (HNO_3, 67%v/v, VWR chemicals Prolabo) and left to digest over night at room temperature. Later, they were heated for 3 h in a water bath at 70ºC with swirling at 30 min intervals to ensure complete digestion. After cooling, the digest matter was transferredinto 20 mL standard asks, rinsing with de-ionized water and made up to the mark. Prepared sample solutions were kept in acid-leached and hermetically sealed polyethylene bottles at room temperature until metal analyses. A blank solution has been performed using all reagents instead of the sample (lyophilized shrimps).The element standard solutions used for calibration were prepared by diluting stock Heavy-metal levels in examined tissues were measured according to the method described by Finerty et al. (1990).

Trace metal elements measurement

Atomic Absorption Spectrometry (AAS; UNICAM 929) was used to determine trace elements concentration. The element standard solutions used for calibration were prepared by diluting stock. Trace metal elements levels in examined tissues were measured according to the method described by Finerty et al. (1990). Aspiration of the digested samples was performed using an air acetylene flame. The concentrations of trace metal elements were determined with the support of calibration curves relying on Beer Lambert's law(Skoog *et al.*, 2005). Calibrations by consecutive dilutions were achieved using standard solutions according to the manufacturer's protocol. Determination was based on average values of

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No.2 (2024)
http://www.veterinaria.org
Received: 11/06/2024 Accepted: 16/10/2024 Published: 22/11/2024

triplicates for each sample. Pb and Cd in samples were determined by graphite furnace using argon as inert gas. Cu and Zn measurements were carried out in an air/acetylene flame. The measurements were carried out with temperature programmed at 100°C, 800°C and 900°C for Cd, Pb, Cu and Zn. For each trace metal elements, there was a specific hollow cathode lamp and the machine was set at a particular absorption wavelength, 228.0 nm, 217.0 nm, 213.9 nm and 324.7 nm for determination of Cd, Pb, Zn, and Cu respectively. The content of metals trace elements in the assessed tissues was expressed in mg/kg of fresh mass and concentrations below the limit were termed as BDL (Below detectable limit).

TVB-N measurement

For the biochemical evaluation of the shelf-life, TVB-N test was used. TVB-N was performed following the method of Regulation EC N. 2074 (2005) with certain modifications made by Altissimo et al., (2017)[15]. Five grams of homogenized sample were transferred into a test tube with 70 mL of double-distilled water, some drops of silicone as anti-foam and few pieces of pumice stones were added. After a maceration phase of 30 minutes, a receiver flask containing 25 mL of boric acid at 2% and three drops of Tashiro indicator was prepared. Let macerate and add 1-2 g of magnesium oxide to the test tube containing the sample. The receiver flask and the tube containing the sample were then arranged into the distillation unit's positions and the instrument was started. At the end of distillation, the solution turned to an emerald green colour, titrated with 0.1N sulphuric acid until the colour turned to violet. The calculation of TVB-N was done with the following formula:

$$\text{TVB-N (mg/100 g)} = \frac{[(V1 - V0) \times 14 \times 0.1 \times 100]}{M}$$

Where V1 is the volume in mL of sulphuric acid used for sample titration; V0 is the volume in mL of sulphuric acid used for blank titration; M is the sample weight; 14 is the molecular weight of nitrogen; 0.1 is the normality of sulphuric acid. Each experiment was replicated three times for every sample and a parallel blank solution was performed using all reagents instead of the sample.

Statistical analysis

All values were expressed as means ± standard error of the means (SEM). The statistical analysis was performed by using one-way analysis of variance for comparison between unpeeled and peeled shrimps' groups. TVB-N parameter was examined by ANOVA for repeated-measures general linear model where storage time was the repeated measure, and post-hoc analyses were carried out using Bonferroni test. Results were expressed as mean±standard error of mean (SEM). All P values less than 0.05 were considered statistically significant. Calculations were made using the SPSS 20.0 (SPSS Inc., Chicago, IL, US). Differences were considered significant at p<0.05.

Results

Trace metal elements concentrations

Table 1:*Tracemetal elements (Cd, Pb, Cu and Zn) concentrations in unpeeled and peeled Aristeusantennatusshrimp'spurchased at different days in Algiers' fishing ports*

Characters	Dry digested shrimp			AAS Detection limits
	Unpeeled shrimp	Peeled shrimp	*P value*	
Cadmium (Cd) (mg/kg)	BDL	BDL	/	0.01
Lead (Pb) (mg/kg)	0.0724±0.0042	0.0482±0.006	0.005	0.01
Copper (Cu) (mg/kg)	0.0193±0.0037	0.0118±0.0039	NS	0.01
Zinc (Zn) (mg/kg)	0.0919±0.0038	0.0729±0.0044	0.005	0.01

Data are expressed as mean ± standard deviation (SEM); (n = 10);BDL: Below Detectable Limit

Table 1 shows the concentrations of Cd, Pb, Cu and Zn obtained in all shrimps' *Aristeus antennatus* samples. The results revealed that Cd concentrations were below the detectable limit (BDL) in both unpeeled and peeled shrimps. However, the concentrations of Pb and Zn were significantly (P<0.05) higher in unpeeled shrimps compared to peeled ones, 0.0724±0.0042 *vs* 0.0482±0.006 mg/kg respectively in the case of lead and 0.0919±0.0038 *vs* 0.0729±0.0044 mg/kg respectively for zinc. One can notice that the concentrations of Cu,0.0118±0.0039 *vs* 0.0193±0.0037 mg/kg, show no significant difference between peeled and unpeeled shrimps. However, we note that the maximum values obtained for the four trace metal elements and reported on Table 1**,** were far below the maximum values recommended by WHO and FAO(2018).

TVB-N levels

The results of TBV-N levels' determination in shrimps *Aristeus antennatus* with different types of storage are shown on Figure 1. These results showed that in fresh shrimps (SD) TBV-N level is 5.644 mg/100g. In the shrimps frozen at -18°C (SF), the level becomes 17.952 mg/100g; whereas in those frozen at -18°C and left in the refrigerator at 4°C for 5 days

(SFR), the level is 23.998 mg/100g. The shrimps refrigerated at 4°C for 5 days (SR) give the highest value of 42.647 mg/100g. Statistical analysis by ANOVA showed significant differences between the different batches.

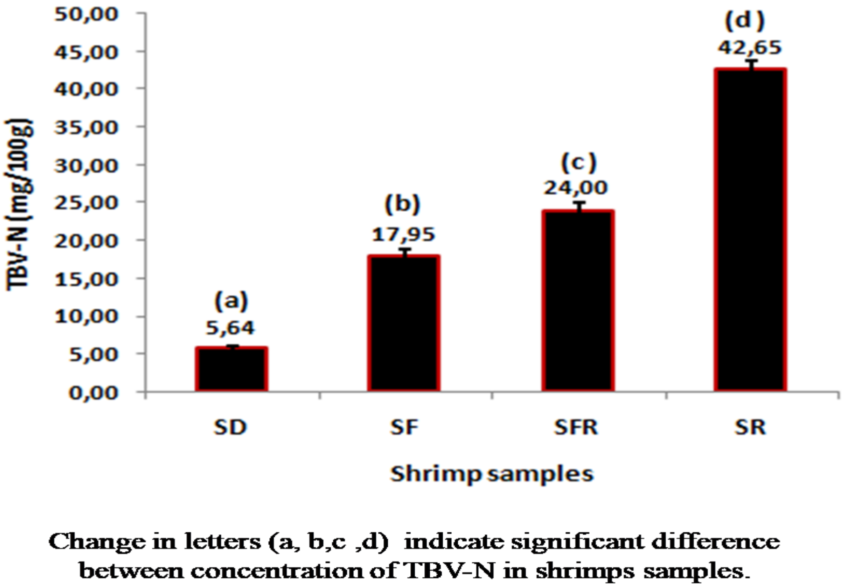

Figure 1: Average TVB-N concentration (mg/100g) of fresh and preserved shrimps'edible part at different storage conditions

***SD:** fresh shrimps of the day, **SF:** shrimps frozen at -18°C and just after defrosting, **SFR:**shrimps frozen at -18°C and defrosting at +4°C for 5 days, **SR:** refrigerated at +4°C for 5 days.*

Discussion

Shrimp quality involves several factors, which for the consumer include safety, nutritional quality, availability, convenience, integrity and freshness when eating. For this purpose, this study was carried out in order to determine, in fresh shrimp's *Aristeus antennatus* samples purchased at different days in Algier's fishing ports, the concentrations of some toxic heavy metals (trace metal elements) such as Cd and Pb, along with essential heavy metals such as Cu and Zn. At the same time, we tried to determine the state of freshness of these samples after different types of conservation used by the households. For this we controlled the rates of TBV-N and assessed the probabilistic human health impacts upon consumption.

Trace metal elements concentrations

Trace metal elements are serious pollutants because they are stable and not easily removed by oxidation, precipitation or any other natural process. Lower concentrations of metal trace elements can also kill organisms. The magnitude of these effects depends on the inherent toxicity of the metal trace element, its concentration, chemical form and affected species. Cumulative effects of trace metal elements or chronic poisoning may occur as a result of long-term exposure, even at low concentrations (Jarup, 2003;Mehouel *et al*., 2019; Yousif *et al*., 2021). This suggests health risks from daily consumption (Ortiz-Moriano et al., 2024).

Trace metal concentrations vary between unshelled and shelled *Aristeus antennatus* shrimp purchased on different days in the fishing ports of Algiers. Higher levels were found in unshelled shrimp. This may be explained by the fact that trace metal elements were bioaccumulated in the shell of shrimp more than in the edible part of the shrimp, because the shell is in direct contact with the marine environment. However, levels of Cd were below the detection limit in both unshelled and shelled shrimp samples. The average Pb contamination in unshelled shrimp was higher than that in shelled shrimp, while the Cu value in unshelled shrimp was close to that of shelled shrimp. Zn levels were higher in unshelled shrimp. In addition, the maximum value obtained for these trace metal elements was below the WHO and FAO recommended maximum limit values for human consumption in both shell and unshell shrimp samples (Soultani *et al*., 2019; Biswas *et al*., 2021). Heavy metal concentrations can vary by sex and organ. Indeed, they are higher in the liver of male and female shrimp than those of muscle. In addition, they are slightly higher in the liver and muscle of male shrimp than those of female shrimp (Olgunoğlu *et al*., 2023). While these concentrations do not appear to be related to the breeding cycle in red shrimp *Aristeus antennatus* (Dravaa *et al*., 2004). Similarly, Yılmaz *et al*. (2017) showed high levels of heavy metals in metabolic tissues such as the liver and gonads, while the lowest levels were found in edible tissues (muscles) of species. Cd, Pd, Cu and Zn were lower than shrimp results obtained by Abdennour et al. (2000) in northeastern Algeria, by Hossain and Khan, (2001) of the Gulf of Bengal, by Soegiantoa (2007) of coastal waters of Gresik, Indonesia, by Traoré et al. (2015) in shrimp (*Macrobrachium vollenhovenii*) in the Aghien and Potou lagoons in southeastern Ivory Coast, by

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No.2 (2024)
http://www.veterinaria.org
Received: 11/06/2024 Accepted: 16/10/2024 Published: 22/11/2024

Erdem*et al*. (2015) on grey shrimp in Turkey, Nhu *et al*. (2021) on the Red River coastal zone in Vietnam and Biswas *et al*. (2021) Farmed shrimp (Penaeus monodon) from Khulna, Bangladesh. However, Baki *et al*. (2018) reported higher levels of Cd but lower levels of Pb in shrimp from St. Martin Island, Bangladesh. Additionally, Soultani *et al.* (2019) found lower levels of lead and cadmium in the muscle and cephalothorax of Mediterranean shrimp (*Parapenaeus longirostris*) and northern shrimp (*Pandalus borealis*). Lead and cadmium are toxic heavy metals that occupy the top positions in all lists of toxic substances (Storelli *et al*., 2005). When consumed in quantities above the safe threshold, they can have serious adverse effects on human health, such as liver and kidney damage, nervous system disorders, cardiovascular disease, hematological effects, developmental abnormalities and reproductive effects (Varol *et al*., 2017). For example, Cd causes cancer and Pb leads to behavioural deficits and reduced survival, growth and learning. In addition, the toxicity and devastating effects of Cd have been amply demonstrated by itai-itai disease in humans (Tuzen, 2003). Lead transfer can occur at low concentrations across different trophic levels, but not in shrimp due to their detoxification mechanisms, reducing its bioavailability in water (Benítez-Fernández *et al*., 2023).

Cu and Zn are essential heavy metals due to their important function in biological systems. These are low levels of essential trace metal elements, but toxic at higher levels (Hossain and Khan, 2001; Jarup, 2003). Despite higher levels of zinc found in shrimp from the west coast of India, they remain within regulatory limits indicating that they pose no threat to consumers (Rao *et al*., 2016).

The cumulative effect of these metal trace elements following long-term exposure, even at low concentrations, can lead to chronic poisoning (Hossain and Khan, 2001; Jarup, 2003). Zn is one of the essential elements for human and plant functions. Zn is known to be a cofactor of more than 300 enzymes involved in RNA and DNA metabolism, and it is also important for the structural stabilization of many proteins. When Zn exceeds safe levels, it becomes toxic (Hossain and Khan, 2001; Irkin *et al*., 2021), but a deficiency of Zn can lead to several disorders such as cardiovascular disease (Afridi et al. 2011) and cancer (Kazi *et al*., 2010). Cu is an essential heavy metal involved in the activity of certain enzymes and necessary for the synthesis of hemoglobin. Poor distribution of Cu can result in decreased cupro-enzymatic activity and affect the skeletal and vascular systems (Prohaska, 2006). It can also cause anemia, neutropenia and osteoporosis (Angelova et al., 2011). Excessive consumption of Cu can lead to kidney damage and even death (Yusif *et al*., 2021).

From a health perspective, it is recommended to consume these seafood products in balanced and limited quantities, especially in developing countries and especially by children, pregnant women and people with weakened immune systems. In addition, it is essential that these seafood products come from clean waters and are stored and processed under appropriate conditions (Gencer and Kocatepe, 2024).

TVB-N levels

In terms of consumer demand, *Aristeus antennatus* shrimp are sold both fresh and frozen. TVB-N is often used as a biomarker for protein and amine degradation. In our study, the mean level of NBVT in fresh (SD) shrimp samples (5.65 mg/100g) is almost equal to the method's limit of quantification. With different conservation modes, TVB-N showed different average levels (Figure 1). The highest average level, 42.65 mg/100g, was obtained in shrimp preserved at +4°C for five days (SR). Our results were lower than those recorded by Chakrabortty *et al*. (2017) in fresh water shrimp (Galda) from the Bagerhat region, and lower than those recorded by Altissimi et al. (2017) in fresh fresh pink prawns and thawed Argentine red shrimps. NBVT is considered an important characteristic for the assessment of seafood quality, and appears as the most common chemical indicator of marine fish deterioration (Altissimi *et al*., 2017). The TVB-N is a useful indicator for assessing quality and quality variations not only for crustaceans but also for all aquatic products (So-Hyun *et al*.,2020).

Shrimp are highly susceptible to microbiological and chemical deterioration due to their high water content, neutral pH, relatively large amounts of free amino acids, and the natural presence of autolytic enzymes (Attala, 2012). The deterioration in quality is species-specific and highly dependent on temperature and time. Storage, freezing and cooking can affect the freshness and quality of products. According to Khodanazary (2019), The shelf life of Metapenaeus affinis stored in ice is, according to bacteriological and physico-chemical results, 9 days, during which time it is ready for consumption.

During storage, the action of endogenous microorganisms and enzymes causes changes in chemical composition. Sensory evaluation of most shrimp is not sufficient to detect quality. Altissimi *et al*. (2017) reported that the acceptability limits of TBV-N for crustaceans were poorly known. But, it remains the most sensitive to detect quality variations than the sensory method (CONDURSO et al., 2016). Other authors also report that TBV-N could be a good quality indicator for crustaceans. This is the case in Australia and Japan, where it is commonly used as a commercial shrimp quality index with an acceptability limit of 30 mg/100 g (Smaldone*et al*., 2011).

Conclusion

This study found that the concentrations of Cd, Pb, Cu and Zn in *Aristeus antennatus* shrimp from the Mediterranean coast of Algiers were significantly below the recommended maximum limits, making the risk to the consumer very low. Given the short life cycle of shrimp and their role as a link in the fish food chain, the risk of bioaccumulation of heavy metals is high, especially for long-lived marine species. In addition, the TVB-N test showed good quality of these shrimps even after various conservation treatments. Fresh or frozen shrimp are recommended.

Acknowledgments
The authors thank Dr Djamila Ait Aissa and Dr Selma Akcha for their kind efforts in revising the manuscript. The authors declare no competing interest.

References
1. **Abdennour, C., Smith, B. D., Boulakoud, M. S., Samraoui,B. & Rainbow P. S., 2000.**Trace metals in marine, brackish and freshwaterprawns(Crustacea, Decapoda) fromnortheastAlgeria.*Hydrobiologia*432: 217–227.
2. **Afridi, H.I., Kazi, T.G., Kazi, N., Kandhro, G.A., Baig, J.A., Jamali, M.K., Arain, M.B., Shah, A.Q., 2011.** Interactions between cadmium and zinc in the biological samples of Pakistani smokers and non-smokers cardiovascular disease patients. *Biol. Trace Elem. Res.*, 139 (3): 257-268. https://doi.org/10.1007/s12011-009-8607-3
3. **Altissimi, S., Mercuri, M.L., Framboas, M., Tommasino, M., Pelli, S., Benedetti, F., Bella, S.D., Haouet, N., 2017.**Indicators of protein spoilage in fresh and defrosted crustaceans and cephalopods stored in domestic condition. *Ital. J. Food Saf.*, 6 (4): 6921. https://doi.org/10.4081/ijfs.2017.6921
4. **Andayesh, S., Hadiani, M.R., Mousavi, Z., Shoeibi, S., 2015.** Lead, cadmium, arsenic and mercury in canned tuna fish marketed in Tehran, Iran. *Food Addi.t Contam. Part B Surveill*, 8 (2): 93-98. https://doi.org/10.1080/19393210.2014.993430
5. **Angelova, M., Asenova, S., Nedkova, V., Koleva-Kolarova, R., 2011.** Copper in the human organism: Mini-Review. *Trakia Journal of Sciences*, 9 (1): 88-98,
6. **Attala, O.A., 2012.** Impact of Using Organic Acids and Sodium Sulfite on the Quality of Unpeeled Shrimp. *World Journal of Fish and Marine Sciences*, 4 (3): 284-289. https://doi.org/10.5829/idosi.wjfms.2012.04.03.6356
7. **Baki, M.A., Hossain, M.M., Akter, J., Quraishi, S.B., HaqueShojib, M.F., AtiqueUllah, A.K.M., Khan, M.F., 2018.** Concentration of heavy metals in seafood (fishes, shrimp, lobster and crabs) and human health assessment in Saint Martin Island, Bangladesh. *Ecotoxicol.Environ.Saf.*, 159: 153-163. https://doi.org/10.1016/j.ecoenv.2018.04.035
8. **Benítez-Fernández, N.C., Pérez-Legaspi, I.A. and Rubio-Franchini, I., 2023.** Transfer of lead throughdifferenttrophiclevels:Microalgae (*Nannochloropsisoculata*), Rotifer (*Brachionusplicatilis*), and Prawn (*Macrobrachiumrosenbergii*).*Rev. Int.Contam. Ambie*. 39, 405-416.https://doi.org/10.20937/RICA.54233
9. **Biswas, C., Soma, S.S., Rohani, M.F., Rahman, M.H., Bashar, A., Hossain, M.S., 2021.**Assessment of heavy metals in farmed shrimp, Penaeus monodon sampled from Khulna, Bangladesh: An inimical to food safety aspects. *Heliyon*, 7 (3): e06587. https://doi.org/10.1016/j.heliyon.2021.e06587
10. **Bryan, G.W., Langston, W.J., 1992.** Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a review. *Environmental Pollution* (76): 89-131. https://doi.org/10.1016/0269-7491(92) 90099-V
11. **Chakrabortty, A., Aziz, M.A., Rashad, M.A., 2017.** Study on protein losses and total volatile base nitrogen (TVBN) of fresh water prawn (Galda) in the shrimp value chain of bagerhat region, Bangladesh for the development of sea food quality. *Journal of Engineering Science*, 08 (1): 79-82.
12. **Condurso,C., Tripodi, G., Cincotta, F., Lanza, C. M., Mazzaglia, A. and Verzera, A., 2016.** Quality assessment of mediterranean shrimps during frozen storage. Ital. J. Food Sci., vol 28: 497-509.
13. **Davydova, S., 2005.** Heavy metals as toxicants in big cities. *Microchemical Journal*, 79 (1-2): 133-136. https://doi.org/10.1016/j.microc.2004.06.010
14. **Dravaa, G.,Capellia, R., Mingantia, V., De Pellegrinia, R., Relinib, L.O., Ivaldi, M., 2004.**Trace elements in the muscle of redshrimp*Aristeusantennatus*(Risso, 1816) (Crustacea, Decapoda) fromLiguriansea (NWMediterranean): variations related to the reproductive cycle. Science of the Total Environment 321 : 87-92.
15. **E.C., (European commission)2074 EaEN:** Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004.
16. **Erdem, M.E., Turan, H., Kaya, Y., 2015.** Mineral and trace element contents of warty crab (*Eriphiaverrucosa*) and brown shrimp (*Crangoncrangon*). *Istanbul University Journal of Fisheries & Aquatic Sciences*, 2 (30): 26-31. https://doi.org/10.18864/iujfas.82582
17. **Fallah, F., Ebrahimnezhad, Y., Maheri-Sis, N., Ghasemi-Sadabadi, M., 2016.** The effect of different levels of diet total volatile nitrogen on performance, carcass characteristics and meat total volatile nitrogen in broiler chickens. *Archives Animal Breeding*, 59 (2): 191-199. https://doi.org/10.5194/aab-59-191-2016
18. **FAO., 2018.** The State of World Fisheries and Aquaculture, Food and Agriculture Organization of the United Nations, Rome, Italy.

19. **FAO/WHO., 1984.** List of maximum levels recommended for contaminants by the joint FAO/WHO Codex alimentarius commission, second series. CAC/FAL, Rome, in: Food and Drugs, 3, Malaysia Law Publisher, KualaLumpur, pp. 1-8.
20. **Finerty, M.W., Madden, J.D., Feagleyt, S.E., Grodner, R.M., 1990.** Effect of Environs and Seasonality on Metal Residues in Tissues of Wild and Pond-raised Crayfish in Southern Louisiana. *Arch.Environ.Contam.Toxicol.*(19): 94-100.
https://doi.org/10.1007/BF01059817
21. **Gencer, D.C., Kocatepe, D., 2024.**Mineral content of somecrustaceans and molluscs in Turkiyé and riskassesments for public health. Scientific Study&ResearchChemistry& Chemical Engineering, Biotechnology, Food Industry, 25 (1) : 019 - 034.
22. **Hoenig, M., De Kersabiec, A., 1989.**Atomisationélectrothermiqueenspectrométried'absorptionatomique.Ed. Masson, Paris, 296 p.
23. **Hosomi, R., Yoshida, M., Fukunaga, K., 2012.** Seafood consumption and components for health. *Glob J Health Sci*, 4 (3): 72-86. https://doi.org/10.5539/gjhs.v4n3p72
24. **Hossain, M.S., Ahmed Khan, Y.S., 2001.** Trace metals in Penaeid shrimp and Spiny lobster from the Bay of Bengal. *Science Asia* (27): 165-168.
25. **Irkin, L.C., 2021.** The Effects of Shellfish Consumption Frequency for Human Health. In, Mukherjee SRaS (Ed): Update on Malacologyed., IntechOpen,
26. **Jarup, L., 2003.** Hazards of heavy metal contamination. *Br Med Bull*, 68: 167-182. https://doi.org/10.1093/bmb/ldg032
27. **Kazi, T.G., Wadhwa, S.K., Afridi, H.I., Kazi, N., Kandhro, G.A., Baig, J.A., Shah, A.Q., Kolachi, N.Fé, Arain, M.B., 2010.** Interaction of cadmium and zinc in biological samples of smokers and chewing tobacco female mouth cancer patients. *J Hazard Mater*, 176 (1-3): 985-991. https://doi.org/10.1016/j.jhazmat.2009.11.139
28. **Khodanazary, A. (2019).**Freshness assessment of shrimp *Metapenaeusaffinis*by quality index method and estimation of its shelf life, International Journal of Food Properties, 22:1, 309-319, DOI: 10.1080/10942912.2019.1580719
29. **Mehouel, F., Bouayad, L., Berber, A., Van Hauteghem, I., Van de Wiele, M., 2019.** Analysis and risk assessment of arsenic, cadmium and lead in two fish species (*Sardinapilchardus* and *Xiphias gladius*) from Algerian coastal water. *Food Addit.Contam.Part A Chem. Anal. Control Expo. Risk Assess,* 36 (10): 1515-1521. https://doi.org/10.1080/19440049.2019.1634840
30. **Nhu, D.L., Thi, T.H.H., Vu Phong, P., Thi Lien, N., ThiThuy, D., Le Minh, D., Thi Mai, H.P., Thi Xuan, B.P., Tien Dat, N., ThanhNghi, D., Thi My, H.L., Phuong, T.L., Thi Phuong, Q.L., 2021.** Trace Metal Element Analysis in Some Seafood in the Coastal Zone of the Red River (Ba Lat Estuary, Vietnam) by Green Sample Preparation and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). *Journal of Analytical Methods in Chemistry*: 1-14. https://doi.org/10.1155/2021/6649362
31. **Prohaska, J.R., Broderius, M., 2006.** Plasma peptidylglycine alpha-amidating monooxygenase (PAM) and ceruloplasmin are affected by age and copper status in rats and mice. *Comp BiochemPhysiol B BiochemMolBiol*, 143 (3): 360-366. https://doi.org/10.1016/j.cbpb.2005.12.010
32. **Olgunoğlu, M.P., Olgunoğlu, İ.A. and Bayhan, Y.K., 2023.**Analysis of Heavy Metal Concentrations (Cd, Pb, Cu,Zn, Fe) in Giant RedShrimp (*Aristaeomorphafoliacea*Risso 1827) from the MediterraneanSea.In Turkmen M., Challenging Issues on Environment and Earth Science Vol. 6. Ed. B.P. International : 79-86.DOI: 10.9734/bpi/ciees/v6/1735C
33. **Ortiz-Moriano,M.P., Machado-Schiaffino,G., Garcia-Vazquez,E., Ardura A., 2024.**Traceability challenges and heavy metal risks in commercial shrimp and prawn.*Food Control* 157 : 1-14.
34. **Quratulan, A., Semra, B., Naeema, E., Qadeer, M.A., 2015.** Concentrations of Heavy Metals in *Sardinellagibbosa*(Bleeker, 1849) from the Coast of Balochistan, akistan. *Bull Environ ContamToxicol* (95): 221–225. https://doi.org/10.1007/s00128-015-1572-y
35. **Rao, K. R., Viji, P., Sreeramulu,K. and Sreedhar, U., 2016.**Proximate Composition and Heavy Metal Accumulation inDeep-SeaCrustaceansfromSelected Stations in the IndianExclusive Economic Zone (EEZ).FisheryTechnology, 53 : 155 – 161.
36. **Skoog, D.A., Holler, F.J., Crouch, S.R., West, D.M., 2014.** Fundamentals of analytical chemistry. 9th ed., Thomson-Brooks/Cole, Belmont, Calif,
37. **Smaldone, G., Marrone, R., Vollano, L., Chirollo, C., Pellicane, A., Palma, G., 2011.** Shelf life of thawed crustaceans treated with sulphites. *Italian Journal of Food Safety* (1):https://doi.org/10.4081/ijfs.2011.758
38. **Soegianto, A., 2007.** Trace Metal Concentrations in Shrimp and Fish Collected from Gresik Coastal Waters, Indonesia. *Science Asia*, 33 (2): 235-238. https://doi.org/10.2306/scienceasia1513-1874.2007.33.235
39. **So-Hyun, K., Eun-Ju, J., Dong-Lee, H., Seung-Eun, L., Yang-Bong, L., Sueng-mok, Ch. And Seon-Bong, K., 2020.** Quality assessment and acceptability of whiteleg shrimp (*Litopenaeusvannamei*) using biochemical parameters. Fisheries and Aquatic Sciences, 23 :21, 1-10.

40. **Soultani, G.,Sinanoglou, V. J.,Stathopoulou, E., Rasmussen, R.R.,Lacobsen, C.,Komaitis, M.,Sloth, J.J.,2019.**Evaluation of lead, mercury, cadmium and arsenic accumulation, and fattyacids' profile in muscle and cephalothorax of *Parapenaeuslongirostris*(Mediterraneanshrimp) and of *Pandalusborealis* (northernshrimp). *International Food Research Journal*, 26 (1) : 175-185.
41. **Storelli, M.M., Marcotrigiano, G.O., 2005.**Bioindicator organisms: heavy metal pollution evaluation in the Ionian Sea (Mediterranean Sea--Italy). *Environ Monit Assess*, 102 (1-3): 159-166. https://doi.org/10.1007/s10661-005-6018-2
42. **Traore, A., Ake-Assi, Y., AhoussiKouassi, E., Soro, N., 2015.** Evaluation de la concentration des éléments traces (Pb, Cu, Zn, Fe, Cd et Hg) dans les crevettes (*Macrobrachiumvollenhovenii*) des lagunesAghien et Potou (sud-est de la Côte D'Ivoire). *Larhyss Journal* (24): 129-142.
43. **Tüzen, M., 2003.** Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chemistry* (80): 119–123. https://doi.org/10.1016/s0308-8146(02)00264-9
44. **Varol, M., Kaya, G.K., Alp, A., 2017.** Heavy metal and arsenic concentrations in rainbow trout (Oncorhynchus mykiss) farmed in a dam reservoir on the Firat (Euphrates) River: Risk-based consumption advisories. *Sci Total Environ*, 599-600: 1288-1296. https://doi.org/10.1016/j.scitotenv.2017.05.052
45. **Yılmaz A.B., YanarA. and Alkan E.N., 2017.**Review of heavymetal accumulation on aquaticenvironment in Northern East MediterreneanSea part I:some essential metals. Reviews on EnvironmentalHealth :1-45.DOI 10.1515/reveh-2016-0065
46. **Yousif, R., Choudhary, M.I., Ahmed, S., Ahmed, Q., 2021.** Review: Bioaccumulation of heavy metals in fish and other aquatic organisms from Karachi Coast, Pakistan. *Nusantara Bioscience,* 13 (1). https://doi.org/10.13057/nusbiosci/n130111

Table. Cd, Pb, Cu and Zn maximum levels in unpeeled and peeled Aristeus antennatus* shrimp's *samples and maximum recommended limits for human consumption defined by WHO and FAO. (2018)

Characters	Unpeeled shrimp maximum obtained values (mg/kg)	Peeled shrimp maximum obtained values (mg/kg)	Maximum recommended limits for human consumption defined by WHO and FAO (mg/kg per day)
Cadmium (Cd)	*BDL*	*BDL*	1
Lead (Pb)	0.092	0.082	2
Copper (Cu)	0.197	0.132	0.5
Zinc (Zn)	0.274	0.247	100

Data are expressed as mean ± standard deviation (SEM); (n = 10); BDL: Below Detectable Limit