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ORIGINAL ARTICLE

Trace Element Content and Potential Human Health Risk from Consumption of the Deep-water Rose Shrimp *Parapenaeus longirostris* (Crustacea: Decapoda) from Pagasitikos Gulf, Greece

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KEYWORDS

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ABSTRACT: The content of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn as the whole-body burden and the content of Cd and Pb in muscle tissue of *Parapenaeus longirostris*, were evaluated in shrimp collected from the Pagasitikos Gulf, Greece. The whole-body burden of Fe was 102 ± 39, Mn 71 ± 23, As 64 ± 33, Zn 62 ± 8, Cu 34 ± 16, Ni 4.6 ± 2.9, Cr 1.01 ± 0.39, Cd 0.93 ± 0.33 and Pb 0.88 ± 0.47 ppm wet weight. Muscle tissue content for Cd was 0.47 ± 0.08 and for Pb 0.31 ± 0.06 ppm wet weight. The estimation of weekly intakes and target hazard quotients for the potentially toxic elements Cd and Pb revealed that rose shrimp from Pagasitikos Gulf could be considered safe for human consumption, probably with a general advisory to avoid the consumption of anything other than the muscle tissue.

INTRODUCTION

In the marine environment, elements pose a serious threat due to their toxic and non-biodegradable and long persistent properties [1] and they may further degrade the ecosystem [2, 3], thus accumulating in marine organisms and subsequently transferred to humans through the food chain, sometimes, in concentrations which are potentially toxic [4]. That potential toxicity can be of serious consequence, both for the environment and human health. High bioavailability of trace elements can, on one hand, have a direct toxic effect on the survival and growth of marine organisms, thus affecting community structure and production, while on the other hand; contaminated seafood might cause health implications when consumed by humans [5, 6].

Marine crustaceans and especially shrimps are high-value target species with great commercial importance and are widely consumed across the Mediterranean [7, 8]. In some cases, they tend to accumulate more trace elements than fish, as a result of differences in the evolutionary strategies adopted by the different phyla [9]. In the Eastern Mediterranean region, they constitute a major component of the so-called “Mediterranean diet” [10], thus, increasing the need to determine the content of trace elements in local populations and to assess the health risk their consumption poses.

The deepwater rose shrimp *Parapenaeus longirostris* is considered the most important species (in catchweight, landings, and value) among commercial crustaceans along with the coasts of Spain, France, Italy, and Greece [11-13]. Owning to its abundance and high economic importance, there are many studies regarding the aspects of ecology and biology of *P. longirostris* (e.g. [11, 14,

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and 15] and references within), contrary to very few studies focusing on trace element content [10, 16-21]. The present study aimed to provide new information regarding the content of several trace elements (namely As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn), in local deep-water rose shrimp stocks and, also, to assess the exposure to these potentially toxic elements from their consumption.

MATERIALS AND METHODS

Many studies on trace element contamination usually focus on muscle tissue since it is the obvious edible part. In Greece, however, it is a very common cooking practice in local restaurants to serve fried rose shrimp which are consumed as whole shrimps. It was, thus, decided to estimate the content of the 9 trace elements in the whole body of *P. longirostris*, but also estimate the Cd and Pb content in the muscle tissue in particular, since these two elements can have high nephrotoxic effects [22] and they are generally considered a hazard for human health. In fact, along with Hg, they are the only trace elements with regulations regarding their maximum concentration in seafood in the EU (EC Regulation No 1881/2006).

Rose shrimp samples were collected in June 2019, during an experimental trawl fishing expedition in the Pagasitikos Gulf (Figure 1). Shrimps below the minimum commercial size (carapace length < 20 mm), according to Greek fisheries regulation [12], were discarded and the rest of the catch was placed in polystyrene boxes, packed with ice flakes, and transported to the laboratory for further processing. Care was taken not to include shrimps with obvious signs of molting, to avoid misrepresentation of concentration levels of the elements, since crustacean exoskeleton may contain a significant amount of elements, but this may be reduced before molting [23]. In the laboratory, each rose shrimp was measured (Carapace Length, CL) to the nearest mm, for the estimation of size–frequency distribution of the sampled population. Also, they were weighed (Total Weight, TW) to the nearest 0.01 g, sexed, and finally, placed in individually labeled plastic bags and temporarily stored in a refrigerator (4°C), before sample preparation for the analytical procedures.

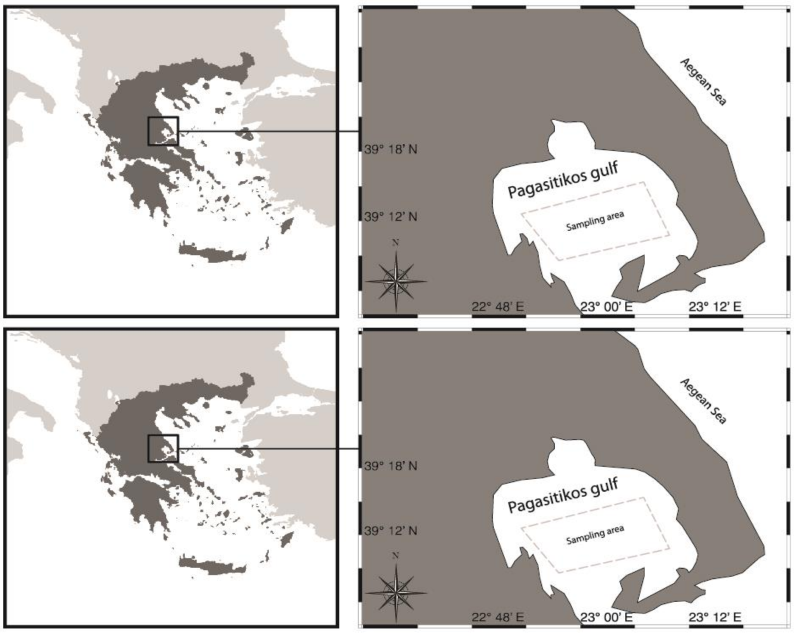

Figure 1. Map of the sampling area and the location of the Pagasitikos Gulf.

For the estimation of trace element content as whole-body burden 10 composite samples for each sex were prepared. Each sample contained 10 – 15 whole individuals of similar size, representing all the estimated CL size classes. For the estimation of Cd and Pb content in the muscle tissue of *P. longirostris,* another set of 10 composite samples for each sex were created, only this time each rose shrimp was carefully dissected for the separation of muscle tissue from the carapace, and the visceral package (Figure 2). All samples were stored at -

20°C, until further processed.

Before the detection analysis, the samples were removed from storage and thawed for 24 h at ambient room temperature and

Then, dried in an air-flow oven at 70°C, until constant weight. Each sample was homogenized and reduced to a fine powder, using mortar and pestle. Using a high precision analytical balance (MS104TS/A00, Mettler-Toledo) 0.5 ± 0.0001 g from each sample were added in a Teflon vessel, along with 9 ml of HNO_3, 2 ml H_2O_2, and 1 ml of ultra-pure water. The vessels were placed in a microwave wet-digestion system (Microwave 3000, Anton Paar) for 15 min, following the guidelines set by the EPA 3052 Method. After the conclusion of the digestion process, the vessels were cooled at ambient room temperature and their contents were transferred into a volumetric flask, filled up to 100 ml with ultra-pure water, and stored at -4°C, until the detection analyses.

For the detection of Cu, Fe, Mn, and Zn the samples were analyzed by Flame Atomic Absorption Spectrometry (FAAS), using an atomic absorption spectrometer (AAnalyst 400, PerkinElmer). For the detection of As, Cd, Cr, Ni, and Pb, Graphite Furnace Atomic Absorption Spectrometry (GFAAS) was used (HGA 900 graphite furnace, equipped with Zeeman effect background correction and an AS 800 autosampler, by PerkinElmer). All the analyses were made in the Laboratory of Marine Biology, Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly.

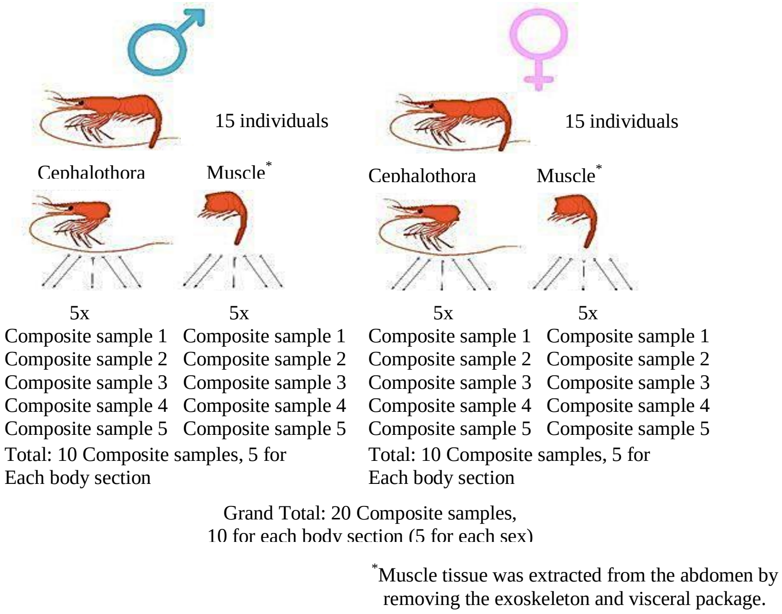

Figure 2. Composite sample creation process.

All samples were analyzed in triplicates and the concentration value for each element was represented by the median, rather than the mean of the results, because of their non-normal distribution [24]. The total content for each element was calculated by the WinLab32™ software (PerkinElmer) and expressed in ppm (μg g^{-1}) wet weight.

All chemicals were of analytical reagent grade (Sigma-Aldrich). The ultra-pure water was double-distilled, using a Millipore Direct-Q-UV water purifier. All glassware was cleaned before use by soaking in 10% HNO_3 for 48 h and rinsing with Milli-Q water. Certified reference materials (NIST 1577b, NIST 2976, and BCR 61) were used as samples for method validation, with a satisfactory recovery (92 – 103% for all measured elements).

The estimated weekly intakes (EWI) and target hazard quotient (THQ) of the trace elements Pb and Cd were calculated following the formulas described by Storelli [25] and Kalogeropoulos et al. [10], both for the whole body and the muscle tissue samples. The Provisional Tolerable Weekly Intakes (PTWI) of 2.5 μg kg^{-1} body weight for Cd and 25 μg kg^{-1} body weight for Pb were used [26]. The Total THQ (TTHQ), which is the sum of the respective THQs from Cd and Pb, was also calculated, as an indicator of the health risk from the consumption of *P. longirostris* [10, 25].

The null hypothesis of no significant differences in trace element content between the sexes was assessed using the student's t-test. Spearman's correlation coefficient was used to measure the strength of the potential associations between elements. All the statistical analyses were performed by the STATGRAPHICS Centurion software package (v.16.1.11) and values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The results from the estimation of the content of trace elements as a whole-body burden are presented in Table 1. The average content and the range of the trace elements were for Fe 102 (49 – 201) ppm, Mn 72 (23 – 138) ppm, As 64 (26 – 171) ppm, Zn 62 (46 – 80) ppm, Cu 35 (14 – 85) ppm, Ni 4.6 (0.62 – 12.1) ppm, Cr 1.01 (0.62 – 2.22) ppm, Cd 0.93 (0.61 – 1.88) ppm and Pb 0.88 (0.21 – 2.11) ppm wet weight. No significant differences were detected between the sexes and the ranking of elements, in terms of decreasing content, was Fe > Mn > As > Zn > Cu > Ni > Cr > Cd > Pb. (Figure 3).

Table 1. Mean concentration (ppm wet weight) ± Standard Deviation and range of individual values of the 9 trace elements in composite samples from the whole body of *P. longirostris*.

Element	Fe	Mn	As	Zn	Cu	Ni	Cr	Cd	Pb
Mean ± SD	102±39	71±23	64±33	62±8	34±16	4.6±2.9	1.01±0.39	0.93±0.33	0.88±0.47
Range	49-201	23-138	26-171	46-80	14-85	0.62-12.1	0.62-2.22	0.61-1.88	0.21-2.11

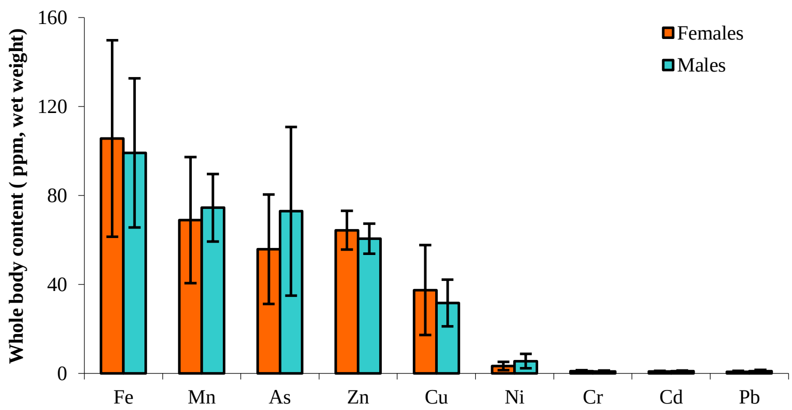

Figure 3. Average content (ppm, wet weight) of trace elements in the body of female and male *P. longirostris* shrimps (Bars represent standard deviations).

According to Spearman's correlation analysis, Fe had weak, but significant positive relationships with Mn and Zn, while arsenic had weak, but significant positive relationships with Mn and Cu (Table 2). Usually, a positive correlation between elements can be regarded as indicative of particular biochemical pathways [23] or it could suggest that these elements share common sources, have mutual dependence, or have identical behavior during transport [27].

Table 2. Spearman ranks correlations between each pair of trace elements, based on whole-body content.

	Mn	Zn	Ni	Cd	Cu	Pb	As	Cr
Fe	*0.427	*0.578	-0.119	-0.249	0.137	-0.004	0.136	0.280
Mn		-0.027	0.386	-0.181	0.122	-0.374	*0.457	0.079
Zn			-0.319	-0.196	0.186	0.352	-0.124	0.205
Ni				-0.282	0.068	-0.400	0.330	0.086
Cd					-0.188	-0.429	-0.228	0.280
Cu						-0.123	*0.422	0.231
Pb							0.242	0.039
As								0.167

*Indicates significant correlation, $p < 0.05$

Regarding the content of Cd and Pb in the muscle tissue of *P. longirostris*, no differences were detected between the sexes ($p > 0.05$) for either element. The average content of Cd in shrimp muscle tissue was 0.47±0.08 (0.36-0.61) and the respective content of Pb was 0.31±0.06 (0.20-0.39) ppm wet weight (Figure 4). The estimation results for all trace elements of the present study, along with the findings of similar studies, regarding *P. longirostris* and other shrimp species in the Eastern Mediterranean region, are presented in Table 3.

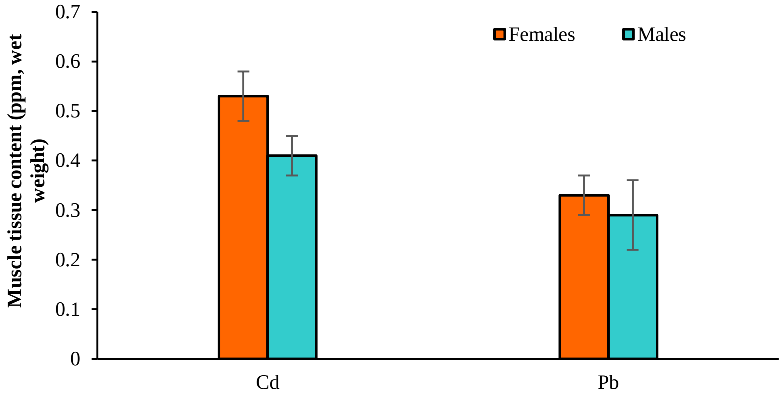

Figure 4. Average muscle tissue content (ppm, wet weight) of Cd and Pb in female and male *P. longirostris* shrimps (Bars represent standard deviations).

The estimated weekly intakes (EWI) and the target hazard quotients (THQ) for Cd and Pb, both from the whole body and muscle tissue of *P. longirostris* are shown in Table 4. Trace element intake through the consumption of whole individuals was estimated at 0.59 μg kg^{-1} body weight for Cd and 0.56 μg kg^{-1} body weight for Pb, representing 23.6% and 2.2% of the PTWI for each element. The respective intake from the consumption of muscle tissue was estimated at 0.30 μg kg^{-1} body weight for Cd and 0.20 μg kg^{-1} body weight for Pb, representing 11.9% and 0.8% of the PTWI for each element.

Many of the studied elements (Fe, Mn, Zn, Cu, and Cr) are essential for most animals because they play an important role in many physiological functions, from cell metabolism to growth, therefore their presence and content levels are to be expected. Furthermore, their high content scarcely poses a threat to human consumers [28] and this is, probably, why there are no Maximum Levels (MLS) established for these elements.

Conversely, Cd and Pb are both non-essential elements, which can have high nephrotoxic effects [22] and they are generally considered a hazard to human health. The EU has set a limit of 0.5 ppm for the concentration in crustaceans, for both elements (EC Regulation No 1881/2006). In the present study, the content of both elements in the whole body of *P. longirostris* individuals was relatively higher or in close agreement with similar studies in the Eastern Mediterranean region (Table 3) and higher than the EU limit.

Table 3. Concentrations of trace elements (ppm) in shrimps from different parts of the Eastern Mediterranean Sea.

	Species	Fe	Cu	Zn	Mn	Ni	Pb	Cr	Cd	As	Remarks
present study	*Parapenaeus longirostris*	102	35	62	72	4.6	0.88	1.01	0.93	64	Summer, wet weight, whole body
							0.31		0.47		Summer, wet weight, muscle
Olgunoğlu et al., 2015	*Aristaeomorpha foliacea*	1.09	2.71	11.16			0.43				Wet weight, muscle, Females
		24.21	144.12	34.79					2.5		Wet weight, liver, Females
		2.85	3.07	13.37			0.43				Wet weight, muscle, Males
		24.76	257.88	34.88			0.36		2.71		Wet weight, liver, Males
Olgunoğlu, 2015	*Aristeus antennatus*	1.609	2.099	9.953	0.698						Wet weight, Muscle
	Plesionika edwardsii	0.619	2.040	5.933							Wet weight, Muscle
	Plesionika martia	0.456	1.357	4.483							Wet weight, Muscle
Dökmeci et al., 2014	*Parapenaeus longirostris*		4.16	12.16		0.22	1.84	0.13	<0.02	0.86	Summer, wet weight, muscle
			25.48	22.42		2.23	<0.51	0.77	<0.05	2.33	Winter, wet weight, muscle
			3.98	16.17		19.25	2.12	0.37	0.1	9.93	Spring, wet weight, muscle
Kalogeropoulos et al., 2012	*Parapenaeus longirostris*	56	9.5	12			0.13	0.22	0.23		Wet Weight, muscle
Pastorelli et al., 2012	*Parapenaeus longirostris*						0.31		0.14		Wet weight, muscle
Turkmen, 2012	*Penaeus kerathurus*	32.47	28.47	63.45			0.81		0.22		Dry weight, Muscle, Males
		31.26	26.37	60.81			0.91		0.18		Dry weight, Muscle, Females
Çevik et al., 2008	*Parapenaeus longirostris*	20.31		42.93	2.39			0.28	0.09		Dry weight, muscle
Firat et al., 2008	*Penaeus semisulcatus*	18.69	32.24	27.75				60.38	16.72		Dry weight, muscle
Gokoglou et al., 2008	*Penaeus semisulcatus*	33.89	6.19	30.84	0.6				2.36		Wet weight, muscle
	Parapenaeus longirostris	11.81	1.33	14.57	1.52				0.23		Wet weight, muscle
Yilmaz and Yilmaz, 2007	*Penaeus semisulcatus*	5.9 – 33.1	17.2 – 41.0	6.0 – 10.2		0.6 – 3.4	0.3 – 0.6	6.8 – 13.1			Wet weight, muscle, males
		6.8 – 24.7	17.5 – 42.4	4.3 – 10.3		0.6 – 3.6	0.2 – 0.6	5.9 – 9.3			Wet weight, muscle, female
Kurun et al., 2007	*Palaemon adspersus*	32.6 – 64.7	30.2 – 45.3	25 – 70.1	2.9 -9.7	3.1 -6.4	2.6 – 5.9		0.10 – 0.98		Autumn, dry weight, muscle
	Palaemon serratus	10.5 – 38.4	31.3 -48.7	21.1 – 47.1	0.01 – 6.8	2.8 – 6.7	2.9 – 5.2		0.70 – 1.02		Autumn, dry weight, muscle
	Parapenaeus longirostris	7.1 – 88.8	19.4 – 27.9	28.9 – 50.8	0.01 – 8.8	1.4 – 7.5	2.6 – 7.5		0.53 – 1.22		Autumn, dry weight, muscle
Satsmadjis and Voutsinou-Taliadouri, 1983	*Parapenaeus longirostris*	7.6	6.5	13.3	0.62	0.06	0.28	0.05	0.05		Autumn, Wet Weight, muscle
Balkas et al., 1982	*Penaeus kerathurus*	3.1	7.4	13.2	0.2	1.4	0.34	0.14	0.03		Wet weight, muscle

These elevated levels could be attributed to the fact that the surface sediments of Pagasitikos Gulf have displayed elevated levels of Pb [29-31] and also, because whole-body samples include the hepatopancreas and viscera, where cadmium and lead burdens are, usually, greater [22, 23].

A key aspect in the assessment of the risks to human health from potentially harmful chemicals in food is the knowledge of the dietary intake of such substances, which must remain within determined safety margins [25]. In the present study, according to the EWI values, the dietary intake of Cd and Pb from *P. longirostris* seems to be higher when the whole body is consumed compared to the muscle tissue results. Still, in both cases, that intake represents the 11.9 – 23.6% and 0.8 – 2.2% of the PTWI for Cd and Pd, respectively, which is an indication that the health risk posed by the consumption of local *P. longirostris* shrimps can be considered insignificant. In addition to the interpretation of the weekly intake results, the estimated THQs were very low for both elements (Cd and Pb) and in both cases (whole body and muscle tissue). According to the guideline for interpreting hazard quotients (HQ) calculations, for HQ values lower than 0.1 no hazard exists, while for values between 0.1–1.0 the hazard is low [32]. Therefore, from the estimated TTHQ values of 0.058 and 0.050 for the whole body and muscle tissue, respectively, it could be assumed that the health risk from the consumption of *P. longirostris* shrimps from the Pagasitikos Gulf is minimal.

Table 4. Estimated weekly intakes (EWI, mg kg^{-1} body weight), percent coverage of provisional tolerable weekly intakes (PTWI), target hazard quotients (THQ), and total hazard quotient (TTHQ) for the trace elements Cd and Pb in the whole body and muscle tissue of *P. longirostris.*

	Whole Body		**Muscle Tissue**	
Element	Cd	Pb	Cd	Pb
EWI	0.59	0.56	0.30	0.20
PTWI (%)	23.6	2.2	11.9	0.8
THQ	0.046	0.013	0.043	0.007
TTHQ	0.058		0.050	

To conclude, it is worth noting that the findings of the present study were, in general, comparable to those obtained by other studies in the Eastern Mediterranean region, either in the same species or in other shrimps. In some cases, the high content values recoded, could be interpreted as signs of anthropogenic influence, but still, none of the elements, including the hazardous Cd and Pb, seem to pose a threat to human health. Thus, the deep-water rose shrimp from Pagasitikos Gulf could be considered safe for consumption, probably with a piece of general precautionary advice to the public to avoid consuming anything else than the muscle tissue, because the exposure to trace elements is the lowest, when compared with the whole body.

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Conflict of interests

We know of no conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

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