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Research Note

Levels of Cadmium in White and Brown Meat of Warty Crab (*Eriphia verrucosa*)

A. ARIANO,[1] A. LO VOI,[2] M. D'AMBOLA,[1] R. MARRONE,[1] D. CACACE,[2] AND L. SEVERINO[1]*

[1]*Department of Veterinary Medicine and Animal Production, Division of Toxicology, University of Naples Federico II, Via Delpino 1, 80137 Naples, Italy; and* [2]*Stazione Sperimentale per l'Industria delle Conserve Alimentari (SSICA), Via Nazionale 121, 84012 Angri, Salerno, Italy*

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ABSTRACT

European Union regulations that establish the maximum cadmium (Cd) levels for crab take into account only concentrations found in crab muscle (white meat), mainly from appendages; therefore, other organs and tissues (brown meat) are excluded. The objective of the present study was to evaluate Cd levels in both white and brown crab meat, in order to achieve a more complete assessment of health risk related to human consumption of warty crab. Microwave digestion and atomic absorption spectrometry were used to determine Cd concentrations in warty crab (*Eriphia verrucosa*) samples collected from the southern Tyrrhenian Sea in Italy. Cd concentrations in all samples of white crab meat were found to be very low (below the limit of quantification), although brown crab meat showed significantly higher Cd concentrations (up to 5.629 mg/kg wet weight; mean value, 1.465 mg/kg). Thus, the consumption of brown meat, common among certain populations of the Mediterranean region, where whole crustaceans are traditionally eaten, substantially increased Cd intake, resulting in alarmingly high estimated weekly intake values.

Seafood products are important components of the human diet and are a significant source of proteins, polyunsaturated fatty acids, and micronutrients. However, consumers could be exposed to various contaminants through the consumption of fish, shellfish, and crustaceans that have accumulated these contaminants while living in polluted waters.

Environmental pollution with heavy metals is ubiquitous and is owing both to the natural abundance of metals within the earth's crust and to human activities. Some of these metals are of great toxicological concern and have a wide range of toxic effects in both humans and animals *(17, 19)*.

The presence of cadmium (Cd) in the environment derives from sources both natural (volcanic activity and weathering of rocks) and anthropogenic (mining, metal production, combustion of fossil fuels, sewage sludge, and waste incineration). Several scientific reports indicate that freshwater and marine organisms *(6, 23)* and terrestrial plants and animals, at all levels of the food chain, can bioaccumulate high concentrations of Cd, depending on their biological characteristics *(1, 30),* the metal concentration, and bioavailability in different environmental media, such as sediments and soils *(32)*. In seafoods, high Cd concentrations can be found mainly in mollusks (e.g., bivalves and cephalopods) and crustaceans (e.g., oysters and crabs) *(10, 14)*.

Humans are exposed to Cd primarily through food, accounting for approximately 90% of the total intake in the nonsmoking general population *(30)*. Cd is a nonessential heavy metal; its role in promoting significant adverse health effects in humans and animals has been widely studied, especially in relation to high-level metal exposure *(1, 10, 13)*. Interest in recent years has focused on the possible implication of low-level, long-term Cd exposure in developmental diseases, highlighting the existence of vulnerable groups in the general population *(15)*. The adverse effects of long-term Cd exposure mainly target the kidneys *(14)*.

Cd toxicity was recently reassessed by the European Food Safety Authority (EFSA), and the provisional tolerable weekly intake established by the Joint FAO/WHO Expert Committee on Food Additives was changed to a tolerable weekly intake of 2.5 μg/kg of body weight *(11)*. To regulate human dietary exposure to Cd, the European Union (EU) established maximum levels (MLs) for this metal in different foods. For fish products, the EU set Cd MLs from 0.050 to 0.30 mg/kg, depending on the fish species. A Cd ML value of 1.0 mg/kg was fixed for bivalve mollusks and cephalopods and 0.50 mg/kg for crustaceans *(7, 8)*.

For crustaceans and, in particular, for crabs, such MLs refer only to the muscle meat of legs and claws (the appendages, also known as "white crab meat"), clearly excluding the cephalothorax (also known as "brown crab meat"). But note that bioaccumulation of Cd in crabs differs significantly in different organs and tissues. Several studies revealed that the cephalothorax is the main tissue involved in the metal bioaccumulation process *(3, 4, 24, 29)*. The cephalothorax, in fact, includes the digestive gland, well known to be the largest site for Cd storage and detoxification *(5)*.

* Author for correspondence. Tel: +390812536272; Fax: +390812536274; E-mail: lorella.severino@unina.it.

TABLE 1. *Concentrations of cadmium in warty crab samples*[a]

Tissue	Concn of cadmium (mg/kg)				
	Mean	SD	Median	Minimum	Maximum
White meat	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Brown meat	1.465	1.790	560.35	0.015	5.629

[a] $n = 40$ samples. LOQ, limit of quantification (0.550 μg/kg).

Among some populations in the Mediterranean, including Italy, the consumption of raw or cooked whole crabs, including the cephalothorax, abdomen, and gonads, is not infrequent, resulting in a public health concern. The concern is greatest for high consumers of crab because, in addition to the Cd exposure from crabs, the main food groups of this region, such as other seafood, cereals, and cereal products, are also major contributors to dietary Cd intake *(11, 16, 18, 21, 26, 27, 31)*.

Eriphia verrucosa, sometimes called the warty crab, is a species of crab found in the Mediterranean Sea, the Black Sea, and the eastern Atlantic Ocean, from Brittany to Mauritania and the Azores *(12)*. It is highly fecund and is reported to feed on bivalves, gastropods, and hermit crabs, or on mollusks and polychaetes. It is known to be a very popular food, especially in the traditional kitchen of certain Mediterranean regions *(25)*.

Therefore, the objectives of the present study were to evaluate Cd levels in samples of the warty crab collected from the southern Tyrrhenian Sea in Italy, to investigate the metal distribution in the white meat (muscle of claws and appendages) and brown meat (contents of the cephalothorax), and to assess the health risk related to human consumption of warty crab for the Mediterranean population that eats whole warty crabs.

MATERIALS AND METHODS

Biological material. Samples (40) of warty crab (*Eriphia verrucosa*) were caught from various locations along the northern coast of the Campania region in Italy. All samples were collected between May and July 2014. To minimize biological variation of metal concentration associated with gender, only males were taken in our sampling.

After capture, the crabs were weighed and the lengths and widths of their carapaces were measured (Absolute Digimatic caliper, Mitutoyo, Japan). Then they were immediately sealed in polyethylene bags, frozen at –20°C, and kept at the same temperature until delivery to the laboratory. The mean weights and carapace lengths and widths were 93.8 ± 27.6 g, 4.61 ± 0.81 cm, and 6.03 ± 0.80 cm, respectively.

In each sample, the muscle from claws and appendages (white meat) and the content of the cephalothorax (brown meat) were individually separated and weighed, and the relative percentages of white and brown meat were calculated (62% ± 12% and 38% ± 12%, respectively). Each tissue was subsequently homogenized by means of a laboratory mixer and was stored at –20°C until further analysis.

Chemical and instrumental analysis. Glassware and laboratory equipment were decontaminated before use with diluted ultrapure 65% HNO_3 (Romil UpA, Cambridge, UK) and were rinsed with Milli-Q water (Millipore Corp., Bedford, MA). Aliquots of each sample (0.50 ± 0.02 g) were digested in 5 ml of ultrapure 65% HNO_3 and 2 ml of 30% H_2O_2 (Romil UpA) in a microwave digestion system (Milestone, Bergamo, Italy). The final volume was obtained by adding Milli-Q water.

Cd concentrations in the digested samples were determined with an atomic absorption spectrometer (Aanalyst 600, Perkin-Elmer, Bonenseewerk, Germany) equipped with a graphite furnace and a L'vov platform and were expressed as milligrams per kilogram of wet weight.

The equipment was calibrated using standard solutions of Cd (prepared from certified stock solutions) and matrix modifier solutions of palladium and Mg $(NO_3)_2$ (atomic spectroscopy standard, Perkin-Elmer); each calibration curve consisted of four concentration levels for Cd. Concentrations for each sample were determined in the medium range of the calibration curve.

Recovery of the metal was determined by adding known amounts of Cd to metal-free samples, which were then subjected to the same digestion procedure. The resulting solutions were analyzed for metal concentrations. Recovery of Cd from spiked samples ranged from 85 to 120%.

The limit of detection and the limit of quantification (LOQ) were calculated by determining the standard deviation of 10 independent blanks and the slope in a calibration curve in the range 1, 2, 4, and 8 mg/kg for Cd. The limit of detection was 0.165 μg/kg wet weight (wet wt), and the LOQ was 0.550 μg/kg wet wt.

The performance of the method was assessed through participation in interlaboratory studies organized by the Food Analysis Performance Assessment Scheme (Sand Hutton, UK); the studies were conducted with fish tissue.

Statistical analysis. All data are expressed as mean and standard deviation of at least three measurements. The Statgraphic Centurion XV statistical package, version 15 (StatPoint Technologies, Inc., Warrenton, VA) was used to determine significant differences among means. The comparison was done with multiple range tests.

RESULTS AND DISCUSSION

Cd concentration in crab. Cd concentrations in white and brown crab meat samples are summarized in Table 1. Data are expressed as the mean concentration of Cd with associated standard deviations and minimum and maximum values for all analyzed samples.

Cd concentrations in white meat were below the LOQ in all tested samples and were largely below the maximum concentration level. Therefore, the consumption of muscle of appendages of such crab species does not constitute a health hazard associated with Cd content *(2, 9)*.

Cd in brown meat was quantified in all samples; 47.5% exceeded 0.50 mg/kg, above the EU ML for muscle meat, which confirms the data reported by the EU Commission

TABLE 2. *Concentrations of cadmium in brown meat of warty crab samples by weight groups*

Wt groups	*n*	Concn of cadmium (mg/kg)		
		Mean	SD	Median
Wt $\leq$ 75 g	9	1.431	2.360	385.97
75 $<$ wt $\leq$ 90 g	12	0.640	1.178	115.03
90 $<$ wt $\leq$ 105 g	9	1.409	1.460	750.95
Wt $>$ 105 g	10	2.536	1.781	2,566.63

TABLE 3. *Concentrations of cadmium in brown meat of warty crab samples by carapace width groups*[a]

Cw groups	*n*	Concn of cadmium (mg/kg)		
		Mean	SD	Median
A (cw $\leq$ 6.0 cm)	21	0.322	0.412	161.49
B (cw $>$ 6.0 cm)	19	2.729	1.884	2,677.47

[a] Cw, carapace width.

note of 2011. As confirmation of this, the EU Commission, in the 2011 information note, reported that a EU monitoring exercise in 2009 and 2010 found a mean value of 8 mg/kg Cd (well above the ML) in brown crab meat and a mean value of 0.08 mg/kg Cd in white crab meat (well below the ML). In the same note, the EU Commission pointed out that, although only white crab meat is generally consumed, in some countries brown crab meat is also eaten by some consumers, who are then exposed to unusually high, unacceptable levels of Cd intake *(9)*.

The content of Cd in brown meat was related to some physical parameters of the samples. No significant differences ($P > 0.05$) in Cd concentrations were found between samples of different weight (Table 2). Significant differences ($P < 0.05$) in Cd concentrations in brown crab meat were found between samples of different carapace width; this is explained by the fact that the size of a crab, as opposed to its weight, is strictly related to the age of the crab and, thus, to metal bioaccumulation *(28)*. Based on this, the data found for Cd content in brown crab meat was split into two groups by carapace width (cw): group A (cw $\leq$ 6.0 cm) and group B (cw $>$ 6.0 cm) (Table 3).

Occurrence of Cd has been widely explored in crab species; however, few studies have dealt with Cd contamination in crab species in Italy and, to our knowledge, none have examined Cd contamination in warty crab. A study by Angeletti et al. *(2)* of Cd concentration in Mediterranean spider crab (*Maya squinado*) in the Adriatic Sea found a very low concentration of Cd in muscle from appendages, confirming our data; however, they observed a lower Cd concentration in the cephalothorax of Mediterranean spider crab. Negligible concentrations of Cd were found in muscle from spider crab appendages (*Maya brachydactyla*) in the Atlantic Ocean, whereas the Cd concentration in the hepatopancreas was 2 mg/kg. These results were approximately comparable to our data *(20)*. In 2011, Mutlu et al. *(22)* detected Cd from 0.03 to 0.08 mg/g wet wt and from 0.04 to 0.1 mg/g wet wt, respectively, for muscle tissue and gill of blue crab (*Callinectes sapidus*) from Mediterranean lagoons.

The Cd concentrations in white and brown warty crab meat reported in the present article were comparable with those previously reported by other authors for other species of crab; that is, a higher level of Cd was found in brown meat than in white meat, confirming the primary role of the hepatopancreas in the bioaccumulation and detoxification processes of Cd in specific organisms such as crustaceans *(3–5, 24, 29)*.

Estimate of Cd intake. An exposure assessment was carried out. Estimated weekly intake (EWI) values were calculated for consumption of white meat alone and for consumption of whole crab (both white and brown meat) so as to take into consideration the food habits of specific population groups. The EWI values for whole crab were determined taking into account the consumption of whole crab, the consumption of only group A whole crab (cw $\leq$ 6.0 cm), and the consumption of group B whole crab (cw $>$ 6.0 cm).

To establish possible human health implications related to consumption of both white and brown meat of warty crab, the Cd EWIs were subsequently compared with the tolerable weekly intake of 2.5 μg/kg of body weight recently fixed by EFSA *(10, 11)*.

For weekly consumption of 100 g of warty crab meat (62% white meat and 38% brown meat), EWI values were found to be 55.78 μg/week for the consumption of whole crab, 6.99 μg/week for group A whole crab, and 103.70 μg/week for the consumption of group B whole crab. These values accounted, respectively, for 32, 7, and 55% of the tolerable weekly intake set by EFSA.

Considering the negligible level of Cd in white meat, its contribution to the metal exposure did not increase the EWIs. In contrast, the consumption of whole crab (both white and brown meat) substantially increased Cd intake, reaching high EWI values that almost reached the limit set by EFSA (2.5 μg/kg) when the other main contributors to dietary Cd intake, i.e., fish and seafood products, cereals and cereal products, tubercles, and vegetables, were included in the exposure assessment *(11, 16, 18, 21, 26, 27, 31)*.

In conclusion, the observed results confirm the EU Commission note of 2011 *(9)* and highlight that the consumption of brown crab meat (abdomen, gonads, and, in particular, digestive gland) could substantially increase Cd intake to high EWI values. This is useful information mainly for heavy consumers of whole crabs because they could be exposed to high levels of Cd, considering the contributions of other foods to dietary Cd intake.

Future health risk assessment studies for heavy consumers of whole warty crab should consider all edible parts, with the aim of suggesting that targeted consumers limit their consumption of brown crab meat, especially from larger crabs, taking into account realistic background levels of Cd in this species.

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REFERENCES

1. Agency for Toxic Substances and Disease Registry. 2008. Draft toxicological profile for Cd. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta.
2. Angeletti, R., G. Binato, M. Guidotti, S. Morelli, A. A. Pastorelli, E. Sagratella, and P. Stacchini. 2014. Cd bioaccumulation in Mediterranean spider crab (*Maya squinado*): human consumption and health implications for exposure in Italian population. *Chemosphere* 100:83–88.
3. Barrento, S., A. Marques, B. Teixeira, M. L. Carvalho, P. Vaz-Pires, and M. L. Nunes. 2009. Influence of season and sex on the contents of minerals and trace elements in brown crab (*Cancer pagurus,* Linnaeus, 1758). *J. Agric. Food Chem.* 57:3253–3260.
4. Barrento, S., A. Marques, B. Teixeira, M. L. Carvalho, P. Vaz-Pires, and M. L. Nunes. 2009. Accumulation of elements (S, As, Br, Sr, Cd, Hg, Pb) in two populations of *Cancer pagurus*: ecological implications to human consumption. *Food Chem. Toxicol.* 47:150–156.
5. Chavez-Crooker, P., P. Pozo, H. Castro, M. S. Dice, I. Boutet, A. Tanguy, D. Moraga, and G. A. Ahearn. 2003. Cellular localization of calcium, heavy metals, and metallothionein in lobster (*Homarus americanus*) hepatopancreas. *Comp. Biochem. Phys. C* 136:213–224.
6. Copat, C., G. Arena, M. Fiore, C. Ledda, R. Fallico, S. Sciacca, and M. Ferrante. 2013. Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: consumption advisories. *Food Chem. Toxicol.* 53:33–37.
7. European Commission. 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* L 364:5–24.
8. European Commission. 2011. Commission Regulation (EU) No 2011/420 of 29 April 2011. Amending regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* L 111:3–6.
9. European Commission. 2011. Information Note on consumption of brown crab meat. Health and Consumers Directorate-General. Available at: http://ec.europa.eu/food/food/chemicalsafety/contaminants/information_note_cons_brown_crab_en.pdf.html. Accessed 18 September 2014.
10. European Food Safety Authority. 2009. Scientific opinion of the panel on contaminants in the food chain. Cd in food. *EFSA J.* 980:1–139.
11. European Food Safety Authority. 2012. Cd dietary exposure in the European population. *EFSA J.* 10:2551.
12. Forskål, P. 1775. Descriptiones animalium, avium, amphibiorum, piscium, insectorum, vermium; quæ in itinere Orientali observavit Petrus Forskål. Mölleri, Hauniae, Copenhagen.
13. International Agency for Research on Cancer. 1993. Beryllium, Cd, mercury and exposures in the glass manufacturing industry, p. 444. *In* IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 58. International Agency for Research on Cancer, Lyon, France.
14. Järup, L., and A. Åkesson. 2009. Current status of Cd as an environmental health problem. *Toxicol. Appl. Pharmacol.* 238:201–208.
15. Järup, L., M. Berglund, C. G. Elinder, G. Nordberg, and M. Vahter. 1998. Health effects of Cd exposure—a review of the literature and a risk estimate. *Scand. J. Work Environ. Health* 24:1–52.
16. Karavoltsos, S., A. Sakellari, and M. Scoullos. 2003. Cd exposure of the Greek population. *Bull. Environ. Contam. Toxicol.* 71:1108–1115.
17. Langner, A. N., A. Manu, and M. A. Tabatabai. 2011. Heavy metals distribution in an Iowa suburban landscape. *J. Environ. Qual.* 40:83–89.
18. Llobet, J. M., G. Falcó, C. Casas, A. Teixidó, and J. L. Domingo. 2003. Concentrations of arsenic, Cd, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *J. Agric. Food Chem.* 51:838–842.
19. Markert, B., S. Wuenschmann, S. Fraenzle, A. M. Graciana Figueiredo, A. P. Ribeiro, and M. Wang. 2011. Bioindication of atmospheric trace metals with special references to megacities. *Environ. Pollut.* 159:1991–1995.
20. Marques, A., B. Teixeira, S. Barrento, P. Anacleto, M. L. Carvalho, and M. L. Nunes. 2010. Chemical composition of Atlantic spider crab *Maja brachydactyla*: human health implications. *J. Food Compos. Anal.* 23:230–237.
21. Martorell, I., G. Perelló, R. Martí-Cid, J. M. Llobet, V. Castell, and J. L. Domingo. 2001. Human exposure to arsenic, Cd, mercury, and lead from foods in Catalonia, Spain: temporal trend. *Biol. Trace Elem. Res.* 142:309–322.
22. Mutlu, C., M. Türkmen, A. Türkmen, and T. Tepe. 2011. Comparison of metal concentrations in tissues of blue crab, *Callinectes sapidus* from Mediterranean lagoons. *Bull. Environ. Contam. Toxicol.* 87:282–286.
23. Olmedo, P., A. Pla, A. F. Hernández, F. Barbier, L. Ayouni, and F. Gil. 2013. Determination of toxic elements (mercury, Cd, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. *Environ. Int.* 59:63–72.
24. Pinheiro, M. A. A., P. P. Gandara e Silva, L. F. De Almeida Duarte, A. A. Almeida, and F. Pinheiro Zanotto. 2012. Accumulation of six metals in the mangrove crab *Ucides cordatus* (Crustacea: Ucididae) and its food source, the red mangrove *Rhizophora mangle* (Angiosperma: Rhizophoraceae). *Ecotoxicol. Environ. Saf.* 81:114–121.
25. Rossi, C., and V. Parisi. 1973. Experimental studies of predation by the crab *Eriphia verrucosa* on both snail and hermit crabs occupants of conspecific gastropod shells. *Boll. Zool.* 40:117–135.
26. Rubio, C., A. Hardisson, J. I. Reguera, C. Revert, M. A. Lafuente, and T. Gonzalez-Iglesias. 2006. Cd dietary intake in the Canary Islands, Spain. *Environ. Res.* 100:123–129.
27. Sand, S., and W. Becker. 2012. Assessment of dietary Cd exposure in Sweden and population health concern including scenario analysis. *Food Chem. Toxicol.* 50:536–544.
28. Swaileh, K. M., and D. Adelung. 1995. Effect of body size and season on the concentrations of Cu, Cd, Pb and Zn, in *Diastylis rathkei* from Keil Bay, Western Baltic. *Mar. Pollut. Bull.* 31:103–107.
29. Turoczy, N. J., B. D. Mitchell, A. H. Levings, and V. S. Rajendram. 2001. Cd, copper, mercury, and zinc concentrations in tissues of the King Crab (*Pseudocarcinus gigas*) from southeast Australian waters. *Environ. Int.* 27:327–334.
30. United Nations Environment Programme. 2008. Draft final review of scientific information on Cd. United Nations Environment Programme, Geneva.
31. Vromman, V., N. Waegeneers, C. Cornelis, I. De Boosere, M. Van Holderbeke, C. Vinkx, E. Smolders, A. Huyghebaert, and L. Pussemier. 2010. Dietary Cd intake by the Belgian adult population. *Food Addit. Contam. A* 27:1665–1673.
32. World Health Organization. 2006. Elemental speciation in human health risk assessment. Environmental Health Criteria 234. Available at: http://www.inchem.org/documents/ehc/ehc/ehc234.pdf. Accessed 18 September 2014.