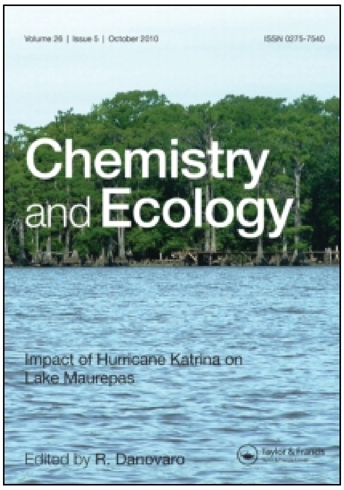

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Mohammed A. Abd-Allah[a]; Maha A. Abdallah[b]
[a] Food Inspection lab. Alexandria, Animal Health Research Institute, Giza. [b] National Institute of Oceanography and Fisheries, Alexandria, Egypt

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Effect of cooking on metal content of freshwater crayfish *Procambarus clarkii*

MOHAMMED A. ABD-ALLAH† and MAHA A. ABDALLAH*‡

†Food Inspection lab. Alexandria, Animal Health Research Institute, Dokki, Giza.
‡National Institute of Oceanography and Fisheries, Kayet Bay, Alexandria, Egypt

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Zinc, copper, lead, chromium, and cadmium levels were measured using an atomic absorption spectrophotometer in abdominal muscle of freshwater crayfish (*Procambarus clarkii*). The metal concentrations could be arranged in descending order according to their concentrations in raw muscles: zinc, copper, lead, chromium, and cadmium. After cooking, metals were found in the same order as in raw tissues, except that chromium preceded lead. Zinc concentration was significantly higher than copper, lead, chromium, and cadmium in both raw and cooked muscles. On the other hand, copper concentration was significantly higher than lead, chromium, and cadmium. A significant positive correlation was found between raw and cooked muscle elements. Generally, zinc, chromium concentrations were within the allowable limits in both cooked and raw muscles, but cadmium and lead levels exceeded the maximum permissible limits for human consumption in both cooked and raw muscles. Copper concentrations of cooked muscles exceeded the maximum permissible limits for human.

Keywords: Freshwater crayfish; *Procambarus clarkii*; Heavy metals

1. Introduction

Freshwater crayfish (*Procambarus clarkii*) are small freshwater crustaceans, distributed extensively all over the world. Crayfish is consumed all over the world, and it is known for its delicate meat [1]. *Procambarus clarkii* and *P. zonangulus* have been found in Egypt during the early 1980s. A survey for newly introduced crayfishes was carried out for the first time during the period from September 1993 to September 1994. Collection stations were chosen along the River Nile and its main tributaries at 35 sites; from Qena (500 km south Cairo) to the outlets of the Nile Delta. The two species coexist in mixed populations throughout most of the examined localities, though *P. clarkii* indicated remarkable predominance over *P. zonangulus.* However, they have established viable populations in the aquatic ecosystems of Giza, Cairo, and some Nile Delta governorates. *P. clarkii* populations in Cairo and Qalyoubiya governorates have an annual life cycle with two nearly separate breeding stocks. Egg laying occurred in mid-spring

*Corresponding author. Present address: 15 Mohamed Hussein, St., Sidi-Bisher, Alexandria, Egypt. Email: mahaahmed72002@yahoo.com

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and late fall [2]. Crayfish causes an ecological problem, due to its feeding on newly hatched fishes, frog eggs, and tadpoles. In addition to its habit of digging burrows in clays for hiding and lay eggs, this may threaten the irrigation system. The temperate climate and abundant organic matter in the river favoured the massive growth and reproduction of these animals.

One of the suggested solutions in Egypt to eradicate these wild animals is to eat it. According to a food microbiological study, 20% of samples of *P. clarkii* from the Nile River are safe for human consumption, while 33.33% are marginally acceptable [3].

P. clarkii has different feeding habits and habitats. Food and water are important pathways for heavy-metal accumulation in freshwater crayfish *P. clarkii* [4–6]. Zinc is accumulated in small amounts via food relative to that accumulated from water, but the former have a much longer retention time than the latter, which is lost rapidly [7]. Hepatopancreatic tissue, alimentary tract, and exoskeleton show higher concentrations of lead, mercury, cadmium, and aluminium than the abdominal muscles [8, 9]. So, crayfish could be biological sentinels of the environmental pollution level in the sampled area [6], as accumulation of non-essential metals in crayfish tissues reflects the concentrations of metals in the sediment [10]. The aim of the present study was to determine the levels of certain heavy metals in the abdominal muscles of *P. clarkii* before and after cooking.

2. Material and methods

Thirty random samples of freshwater crayfish (*P. clarkii*) were collected from different locations at rural areas around Alexandria city. Samples were taken in sterile polyethylene bags and transferred directly in the same day to the laboratory where they were analysed. The average weight of the animals was 58 ± 0.38 g, the average length was 15 ± 0.41 cm, and the male:female ratio was 1.73. The peeled tail of the same animal was divided into two parts longitudinally as to raw and boiling treatment (the commonly used local cooking method) by boiling tissues at 100 °C, for 15 min. Samples were digested according to the UNEP/FAO/IAEA/IOC method [11]. Heavy metals were analysed using an atomic absorption spectrophotometer (Varian Techtron-Model 1250), and the analytical procedure was checked using reference material (DORM1, Institute of Environmental Chemistry, NRC Canada). Heavy-metal concentrations are expressed as $\mu g\ g^{-1}$ wet mass. Statistical analysis of the results was carried out using a one-way ANOVA followed by a Tukey–Kramer comparison of means. Differences between level means in raw and cooked muscles were treated using Student's *t*-test.

3. Results and discussion

3.1 *Metal concentrations in raw tissues*

Heavy metals in raw muscles of *P. clarkii* were arranged according to their concentrations in descending order as zinc, copper, lead, chromium, and cadmium (figure 1). This arrangement may relate to tissue capacity for accumulating heavy metals. The significant increase in zinc concentration in tissues of *P. clarkii* in comparison with other measured metals (tables 1 and 2) could be attributed to the high Zn concentration in the surrounding environment of the animal (irrigating canals and drainage streams) due to the heavy usage of herbicide and fungicide as well as superphosphate (fertilizer) that contains high concentrations of Zn and other metals as impurities [12]. Similarly, Mathis and Cummins [13] found a significantly higher concentration of zinc in omnivorous fish. However, the zinc concentration was below permissible limits ($1000\ \mu g\ g^{-1}$) suggested in a previous publication [14].

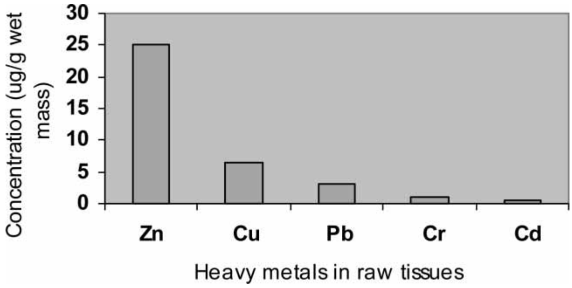

Figure 1. Heavy-metal concentrations in raw tissues of *P. clarkii*.

Table 1. Mean differences between heavy-metal concentrations (μg g^{-1} wet mass) in raw peeled tail of *P. clarkii*.

	Cu	Pb	Cr	Cd
Zn	18.623*	21.980*	24.161*	24.773*
Cu	–	3.358	5.538	6.150
Pb	–	–	2.181	2.793
Cr	–	–	–	0.612

*Significant at $P \leq 0.01$ using the Tukey–Kramer test, $Q_{(5,14)} = 5.64$.

Copper is an essential element in the metabolism of the crayfish; it is a constituent of several enzymes and is essential for normal growth and development [15]. The concentration of Cu in the present study (figure 1 and table 3) was below the maximum permissible limits (10 μg g^{-1} wet mass) previously reported [14]. Also, it was higher than the concentration reported previously [8] in abdominal muscles of *P. clarkii* (<3μg g^{-1}). The use of copper sulphate in agriculture as a fungicide could increase the level of this metal in soil, irrigation canals, and drainage streams where crayfish lives [16].

Lead was noted for its specific accumulation in gill tissue of *P. clarkii* [17]. Lead levels in the muscle (figure 1 and table 3) were higher than the concentration of this metal found in the abdominal muscles of *P. clarkii* [8]. Also, lead concentrations exceed the maximum legal limit for human consumption in fish tissue (0.2–0.4 μg g^{-1} wet mass) proposed in an EC study [18].

For chromium, an essential trace element which is not particularly abundant in fish tissue, there are virtually no toxicity standards. At high environmental concentrations, chromium is a mutagen, teratogen, and carcinogen; no biomagnifications have been observed in food chains for this metal. The average chromium levels in our results for both raw and cooked tissue

Table 2. Mean differences between heavy-metal concentrations (μg g^{-1} wet mass) in cooked peeled tail of *P. clarkii*.

	Cu	Cr	Pb	Cd
Zn	12.835*	20.394*	19.836*	16.428*
Cu	–	7.559*	7.731*	6.725*
Cr	–	–	0.602	1.011
Pb	–	–	–	0.511

*Significant at $P \leq 0.01$ using the Tukey–Kramer test, $Q_{(5,16)} = 5.5$.

Table 3. Heavy-metal concentrations in raw and cooked peeled tail of *P. clarkii* and the permissible limits ($\mu g\ g^{-1}$ wet mass).

Heavy metal	Raw	Cooked	Allowable limits
Zn	25.1616 ± 2.627	$33.4676 \pm 2.577^{*}$	1000 [14]
Cu	6.5432 ± 0.493	$13.8684 \pm 0.957^{***}$	10 [14]
Pb	3.1857 ± 1.454	$1.3525 \pm 0.384^{**}$	0.2–0.4 [18]
Cr	1.0048 ± 0.427	2.3264 ± 1.295	12 [21]
Cd	0.393 ± 0.205	0.284 ± 0.081	0.05–0.1 [18]

Mean ± SE *significant at $P \leq 0.05$ using the t-test; **significant at $P \leq 0.01$ using the t-test; ***significant at $P \leq 0.001$ using the t-test.

were well below the levels reported previously [19–21] (figure 1 and table 3). Additionally, our results disagree with Eisler [20], who reported that chromium seldom exceeds 0.3 $\mu g\ g^{-1}$ fresh weight in edible crustacean tissues. On the other hand, chromium concentrations in *P. clarkii* were below the action level (12 ppm) suggested previously [21, 22] to control the level of chromium in human food, particularly crustacean.

Cadmium levels (table 3) in the raw and cooked abdominal muscles of *P. clarkii* exceeded the maximum permissible limits (0.05–0.1 $\mu g\ g^{-1}$ wet mass) suggested in an EC study [18]. However, in the present study, zinc and copper levels are within the permissible limits, except that cadmium and lead levels exceeded the maximum permissible limits for human consumption.

3.2 *Metal concentrations in cooked tissues*

Cooking of *P. clarkii* by boiling at 100 °C for 15 min had a detrimental effect on its metal content. Figure 2 and table 3 show that after cooking, the concentrations of zinc, copper, and chromium increased, while the levels of lead and cadmium decreased. Statistical analysis for concentration means revealed that zinc was significantly higher than copper, lead, chromium, and cadmium. Also, copper was significantly higher than lead, chromium, and cadmium (table 2).

A comparison of the means of metals of raw tissues with those for cooked tissues (table 3) revealed that zinc, copper, and lead concentrations became significantly higher in cooked tissues than in raw tissues. Chromium showed an increase, but cadmium showed a slight decrease, and in both cases the changes were non-significant. Increasing the metal concentration after boiling may be due to loss of moisture and fat as mentioned previously [23, 24] in studying the effect of cooking practices on total mercury concentration in fish. Elmossalami and Emara [3] showed that *P. clarkii* has 82.19% and 0.59% as moisture and fat percentages, respectively.

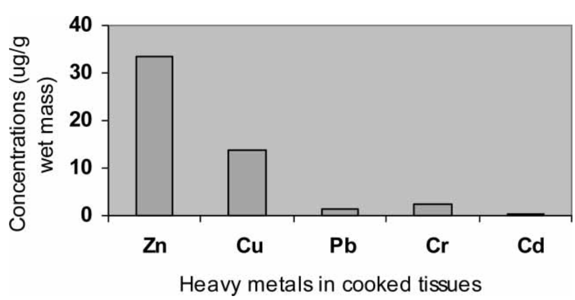

Figure 2. Heavy-metal concentrations in cooked tissues of *P. clarkii*.

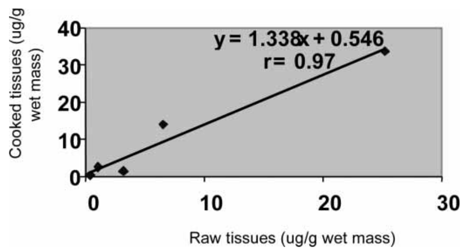

Figure 3. Correlation between raw and cooked tissues metals in *P. clarkii*.

Del Ramo *et al.* [25] reported that cadmium is accumulated in the low-molecular-weight protein fractions in *P. clarkii*. So, the decrease in cadmium concentration after cooking may be related to the protein molecule to which the metal was binding.

A significant positive correlation was recognized between raw and cooked tissue metals (figure 3). This relation could be used to predict heavy-metal concentrations in crayfish muscles after cooking, particularly for the heavy metals under investigation.

In general, the heavy-metal content of cooked muscles would be significantly higher than that in raw muscles. So, consumption of these animals may have a dangerous effect on human health, since excessive intake of copper causes toxic symptoms such as nausea, vomiting, headache, and jaundice [26]. Exposure to lead is a major worry because the metal damages the brain. Even trace amounts can diminish intelligence, particularly among children, in whom it causes lowered IQs [27]. Cadmium is a suspected carcinogen that also causes kidney damage [28]. Humans need small amounts of certain metals, such as zinc, to maintain good health; excessive intake of zinc (50–150 mg) produces toxicity symptoms such as impaired copper absorption, vomiting and intestinal irritation, impairment of immune response, lowered HDL cholesterol, and anaemia [29].

4. Conclusion

In the present study, cooking by boiling leads to an increase in concentrations of essential elements (Zn, Cu, and Cr) but a decrease in non-essential elements (Pb and Cd). Further studies should be carried out on cooking by boiling at different temperatures and times to reduce the dangerous effect of non-essential elements, since this is the local cooking method.

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