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Concentration of some heavy metals (lead, arsenic & cadmium) in edible tissues of *Astacus leptodactylus* from Aras dam of Azerbaijan region, Iran

Marziye al-Sadat Hosseini[1], Zeynab Rahimi Afzal[2], Amin Khodadadi[3]*

[1] Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Razi, Kermanshah, Iran.
[2] Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
[3] Veterinarian and Specialist Health and Diseases in aquaculture, National Veterinary Organization, Tehran, Iran.
(*) Corresponding Author: aminkhodadadi@ymail.com

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Abstract

Heavy metals pose a significant risk to the fauna of aquatic animals due to exposure, bioaccumulation, and biomagnification. This study aimed to assess the levels of certain elements in the edible tissues of *Astacus leptodactylus* (*A. leptodactylus*) and determine the safety of this food product, as well as the pollution levels at the Aras dam sampling sites in northwest Iran during 2021. Freshwater crayfish *A. leptodactylus*, weighing between 50-60 g, were collected using specialized traps from three locations at the Aras dam during the spring. The content of heavy metals such as lead (Pb), arsenic (As), and cadmium (Cd) was analyzed using atomic absorption spectrophotometry. The findings indicated that the minimum concentrations in the crayfish meat were Pb (0.229 ± 0.021 ppm), Cd (0.046 ± 0.012 ppm), and As (0.218 ± 0.035 ppm). These results suggested that *A. leptodactylus* from the Aras reservoir is a viable food source due to the low levels of heavy metals in their soft tissues.

Introduction

Astacus leptodactylus (*A. leptodactylus*) typically attains a total length of about 150 mm, though males can grow up to 170 mm and exceptional individuals may reach 300 mm. They have a lifespan exceeding 10 years. Females achieve sexual maturity when they are between 3-5 years old and measure 75-83 mm. Their eggs range from 2.2-3.3 mm in diameter. This species is distinguished by its elongated, narrow claws, which have a rough upper surface and an underside that matches the body color. While their body shape and color can vary, they generally display shades of olive-green, yellowish to brown, often with a subtle red hue. The carapace, claws, and walking legs might show a mottled pattern. The underside of the body is white, and the carapace can be either wide or narrow with spiny sides, the number of which varies. There are two pairs of post-orbital ridges located behind the eyes on the carapace. The rostrum is notably long and pointed with toothed margins at its base. The thorax sides are textured roughly. In males, the chelae are typically longer than those of females, though shorter chelae have been noted in some males.

Originally, *A. leptodactylus* was found in regions including Turkey, Ukraine, Turkmenistan, southwestern Russia, and the Azerbaijan area of Iran, as well as Kazakhstan, Georgia, Belarus, Slovakia, Bulgaria, Romania, and Hungary. Its native range also encompassed the Caspian Sea, Black Sea, and the lower and middle sections of the Danube River, along with the lower stretches of the Don, Dniester, and Volga Rivers and their tributaries. Due to intentional relocation and natural dispersal, *A. leptodactylus* has broadened its geographic presence to the west and north, now inhabiting 29 countries, with introductions in 14 of these. It has been introduced to countries including the Czech Republic, Poland, Germany, Lithuania, Latvia, Finland, Denmark, the Netherlands, and England. Populations of this species are also maintained in France, Switzerland, Austria, Spain, and Italy (Kouba *et al*., 2015; Sprovieri, 2015).

The aquaculture of the narrow-clawed crayfish, *A. leptodactylus*, has garnered significant interest due to its high demand and market value. Turkey has been a longstanding exporter of live *A. leptodactylus*. This species is cultured extensively across Turkey and northwestern Iran and is also farmed in various Eastern European nations. Additionally, it is stocked in multiple locations within Belgium, Switzerland, and France. Farms in northern and central Italy also culture this species (Di Giulio and Hinton, 2008; Ariano *et al*., 2021).

Research on heavy metal concentrations in *A. leptodactylus* tissues reveals varying accumulation patterns across different elements and organs. Studies have found high levels of copper (Cu) and iron (Fe) in gills, zinc (Zn) in hepatopancreas and manganese (Mn) in exoskeleton (Naghshbandi *et al*., 2007). Temporal analysis of muscle tissue showed Zn and Cu as predominant elements, with concentrations generally below regulatory limits (Aksu *et al*., 2014). Tissue distribution studies identified strong correlations between metal accumulations, particularly in exoskeleton and gills (Tunca *et al*., 2016). Gender-specific differences were observed in accumulation trends for most examined elements, except aluminium (Al), with females showing lower nickel (Ni) and arsenic (As) concentrations in gill tissue compared to males (Tunca *et al*., 2016). Isotope fractionation was noted during heavy metal accumulation, particularly for chromium (Cr) and Cu isotopes (Tunca *et al*., 2016). These findings contribute to our understanding of heavy metal bioaccumulation in *A. leptodactylus* and its potential as a bioindicator for environmental pollution. Therefore, this study investigated to assess the levels of lead (Pb), cadmium (Cd), and As in the edible tissues of *A. leptodactylus* and determine the safety of this food product, as well as the pollution levels at the Aras dam sampling sites in northwest Iran.

Materials and Methods

Sampling

This research was carried out in the lake of the Aras dam in Iran with the geographical coordinates 39.103145 – 45.386229. For this study, specimens of the freshwater crayfish *A. leptodactylus*, weighing between 50-60 g, were collected using specialized traps from three locations around Aras dam during the spring season, 2021. These specimens were temporarily housed in an aerated water tank maintained at 10°C before being transported to a private laboratory in Urmia city, Iran. The edible portions of the crayfish were then excised and forwarded under controlled conditions to the Food and Drug Organization's laboratory at Urmia University for heavy metal analysis.

Metals analyses

In the analysis phase, crayfish tissues were first dried in Petri dishes at 105°C for 48 hours until all moisture was eliminated. The dried samples were then pulverized using a ceramic mortar and pestle. Approximately 0.5 g of this powdered tissue was placed into individual digestion flasks, to which a nitric-perchloric acid solution (2:1 ratio) was added. These flasks were gradually heated and maintained at 120°C for three hours to ensure complete dissolution of the samples, which were subsequently diluted with 0.4% nitric acid. All dissection tools and glassware used during this process were thoroughly cleaned with acid and rinsed with 0.4% nitric acid solution. Metal concentrations within these samples were quantified using an atomic absorption spectrophotometer.

Statistics

Statistical analyses were performed using SPSS software (version 21), employing t-tests and ANOVA, with a significance threshold set at $p < 0.05$.

Results

The results indicated that the concentrations of Pb, Cd, and As in the crayfish meat were 0.229 ± 0.021 ppm, 0.046 ± 0.012 ppm, and 0.218 ± 0.035 ppm, respectively. According to the INSO 12968 standard, these levels fall within permissible limits for heavy metals in food, suggesting that consumption of narrow-clawed crayfish from this region poses minimal health risks and indicates only slight contamination of these metals in the sampled areas.

Aquatic ecosystems are particularly susceptible to pollution due to their capacity to accumulate high concentrations of chemicals from terrestrial runoff and direct discharges, making them critical zones for monitoring environmental health. Fish and other aquatic organisms are pivotal in toxicological studies aimed at understanding both human and ecological impacts, serving as essential indicators that often bridge the gap between environmental and health sciences. This study aimed not only to evaluate potential public health risks from crayfish consumption but also to enhance understanding of heavy metal pollution in freshwater environments through the analysis of *A. leptodactylus*.

Discussion

Cd is recognized as toxic and serves no beneficial role in higher life forms. It is an environmental contaminant that poses significant health risks to organisms. When introduced into cellular environments, Cd induces oxidative stress and prompts cells to increase antioxidant production to shield against damage to macromolecules. Interestingly, some marine diatoms have evolved a Cd-dependent carbonic anhydrase, especially in Zn-deficient habitats where Cd substitutes for Zn in certain enzymatic reactions. This adaptation was identified using X-ray absorption near edge structure (XANES) spectroscopy. In humans, Cd is primarily accumulated in the kidneys. Throughout childhood and adolescence, individuals can inhale up to about 30 mg of Cd. Current research is investigating the potential links between Cd exposure and increased risks of cancer, cardiovascular diseases, and osteoporosis.

Pb is extremely toxic, affecting virtually every organ and system when ingested or inhaled. At concentrations of 100 mg/m^3 in air, it poses immediate life-threatening health risks. Once ingested, Pb predominantly enters the bloodstream and disrupts enzyme functions by binding to their sulfhydryl groups or by displacing other metal cofactors necessary for enzymatic activities. Pb exposure can severely impair kidney and brain function and can be fatal. Symptoms of Pb poisoning include nephropathy, abdominal pain resembling colic, and weakness in extremities. It may also cause slight increases in blood pressure, particularly in older adults, and contribute to anemia. Epidemiological studies have observed a correlation between elevated Pb exposure and reduced heart rate variability. High exposure during pregnancy can lead to miscarriage, while chronic exposure may decrease male fertility. In children, Pb exposure affects cerebral cortex synapse formation, neurochemical development, and ion channel organization, potentially leading to sleep disturbances and delayed puberty in girls.

Historically, As was utilized in various agricultural insecticides and poisons. Lead hydrogen arsenate, once a prevalent insecticide for fruit trees, has been associated with brain damage among agricultural workers. By the latter half of the 20th century, less toxic organic arsenicals like monosodium methyl arsenate (MSMA) and disodium methyl arsenate (DSMA) replaced lead arsenate. However, by 2013, the use of these organic arsenicals was discontinued in all agricultural applications except cotton farming (Goretti *et al*., 2016).

Negligible amounts of Pb, As, and Cd were detected across all samples of *A. leptodactylus*. As levels in the crayfish muscle were similar to those reported by Bellante *et al*. (2015) (0.537 μg/g w.w.), although higher concentrations were observed in the hepatopancreas compared to our study (1.128 μg/g w.w.). Comparable As levels in muscle tissue were also noted by Gedik *et al*. (2017) in crayfish from Louisiana, and Devesa *et al*. (2002), who found As concentrations ranging from 9.2 to 12 μg/g in muscle and 2.5 to 2.6 μg/g in hepatopancreas in crayfish from southern Spain, which were higher than those in our study. Conversely, studies by Mistri *et al*. (2020) and Tan *et al*. (2021) reported higher average As concentrations in both hepatopancreas and abdominal muscle than those found in our research. Additionally, a study by Naghshbandi *et al*. (2007) revealed the presence of heavy metals in Aras crayfish with the following order of concentration: Zn>Cu>Fe>Mn>Pb>Cd>Ni>Cr = Co. Their findings indicated elevated levels of Mn, Cu, Zn, Pb, and Fe in the exoskeleton and hepatopancreas, suggesting these tissues play a role in metal excretion.

It was concluded that *A. leptodactylus* from the Aras reservoir remains a suitable dietary choice due to its low heavy metal content in soft tissues. It is hoped that this project will be completed with the analysis of more heavy metals in the future.

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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