Heliyon 7 (2021) e07194

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Human health risk assessment of selected metal(loid)s via crayfish (*Faxonius virilis; Procambarus acutus acutus*) consumption in Missouri

Abua Ikem [a,b,*], Olukayode James Ayodeji [c], James Wetzel [a,b]

[a] Department of Agriculture and Environmental Sciences, Lincoln University, Jefferson City, Missouri 65101, United States

[b] Cooperative Research Programs, Lincoln University, Jefferson City, Missouri 65101, United States

[c] Department of Environmental Toxicology, Texas Tech University, Lubbock, TX 79416, United States

HIGHLIGHTS

- Accumulation of metal(loid)s in two crayfish species from aquaculture.
- Levels of metal(loid)s in muscle differed ($p < 0.05$) across the study sites.
- EWI values were below the provisional tolerable weekly intakes.
- Consumption of muscle posed no probabilistic health risk (THQ <1; TTHQ <1).
- As and Ni in muscle posed cancer risks relative to the benchmark (10^{-5}).

ARTICLE INFO

Keywords:
Crayfish species
Aquaculture
Faxonius virilis
Procambarus acutus acutus
Metal(loid)s
Bioaccumulation
Health risks
Missouri

ABSTRACT

Farmed crustaceans are an important component in addressing the rising animal protein demand. The present study determined the concentrations of fourteen elements (Ag, As, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Sn, Pb, and Zn) in the edible abdominal muscle of cultured freshwater crayfish species (*Faxonius virilis; Procambarus acutus acutus*) from Missouri. Also, this paper describes the dietary intake and the human health risks from the consumption of crayfish muscle in the adult population. Overall, 172 animals were captured between February 2017 and January 2018 for assessment. Concentrations of metals (Ag, Be, Cd, Cu, Co, Cr, Fe, Mn, Ni, Pb, Sn, Mo, and Zn) and metalloid (As) in the muscle tissue were determined after microwave-assisted acid digestion by ICP - OES. Health indices (EDI/EWI: estimated daily/weekly intakes; THQ: target hazard quotient; TTHQ: total target hazard quotient; ILCR: incremental lifetime cancer risk; and ∑ILCR: cumulative lifetime cancer risk) were calculated and compared to thresholds. Of all samples, the highest concentrations (mg kg^{-1} wet weight) of metal(loid)s in muscle were Ag (0.11), As (3.15), Be (0.21), Cd (0.11), Co (0.32), Cr (1.22), Cu (107), Fe (23.0), Mn (8.54), Mo (0.62), Ni (2.65), Pb (1.76), Sn (5.91), and Zn (19.2). In both species, the average As, Cd, and Zn concentrations were below the legal limits. However, the levels of Cu, Pb, and As, in some samples, were in exceedance of the maximum levels. In both species, a significant correlation ($p < 0.05$) was observed between the carapace length (CL) and animal body weight (BW). In *P. acutus*, CL, BW, and animal total length were homogenous ($p > 0.05$) among the sexes. Non-parametric Kruskal–Wallis test results indicated significant differences ($p < 0.05$) in the levels of As, Be, and Zn in *F. virilis*, and Be and Cr in *P. a. acutus* among the genders. Significant inter-species differences ($p < 0.05$) were observed in the levels of Be, Ni, and Pb and the growth factors. The EDI/EWI values were below the permissible limits. THQ and TTHQ values, being below 1.0, indicated no probabilistic health risk. Regarding carcinogenic risk, only As and Ni indicated cancer risk (ILCR $>10^{-5}$ and ∑ILCR $>10^{-5}$) to the adult population. High metals/metalloid exposure from crayfish muscle consumption posed potential health hazards to the adult population.

* Corresponding author.
E-mail address: ikema@lincolnu.edu (A. Ikem).

https://doi.org/10.1016/j.heliyon.2021.e07194
Received 8 September 2020; Received in revised form 2 February 2021; Accepted 28 May 2021

1. Introduction

Crayfish species are keystone benthic invertebrates with over 640 species spread over three families (*Astacidae*, *Cambaridae*, and *Parastacidae*) of decapod crustaceans (Crandall and Buhay, 2008; Huner, 2019). Apart from their ecological roles, they are an excellent source of proteins and other essential nutrients (Alipour et al., 2021; FAO, 2020; Schmidt et al., 2021), which promote improved well-being and the reduction of some disease conditions. Adults are recommended to eat 2 or 3 servings (1 serving = 4 ounces) a week from the "Best Choices" list and children (at age 2) are limited to a serving (1 ounce) (USDHHS/USDA, 2015). Despite the nutritional benefits from crayfish, they accumulate essential and non-essential elements and organic pollutants (Mancinelli et al., 2018; Tavoloni et al., 2021). Consequently, seafood presents potential risks to consumers since the dietary pathway is the major exposure route.

Metal(loid)s are present in the natural environment but their anthropogenic sources which include domestic, industrial, and agricultural wastes have exacerbated their levels in aquatic systems. Heavy metals are of great concern and a growing problem worldwide, contributing to 9 million excess deaths (Heidari et al., 2021; Dippong et al., 2017) and could cause health problems (Dippong et al., 2020). Sources of heavy metals in aquatic systems include leaching of rocks especially by rainwater, atmospheric deposition of airborne dust or industrial emissions, forest fires, and vegetation (Dippong et al., 2017), and fertilizers, animal agriculture wastes, etc. Metal(loid)s are defined as essential or non-essential elements, depending on their biochemical roles in humans (Sharafi et al., 2019a) and organisms. The essential elements such as Cu, Fe, Mn, and Zn play several roles in physiological and biochemical processes in humans. Notwithstanding, they can also be harmful (Zhou et al., 2021) above certain thresholds. The non-essential elements such as Pb, As, Hg, and Cd are toxic, persistent, and not easily biodegradable in the environment (ATSDR, 2021). According to the Agency for Toxic Substances and Disease Registry (ATSDR), arsenic (As), Cd, Pb, and Hg are ranked 1st, 7th, 2nd, and 3rd, respectively on the substance priority list (ATSDR, 2019) due to their toxicities. These toxic elements, even at low amounts, cause severe effects such as renal, cardiovascular, neurological, and bone diseases (Shalini et al., 2020), and brain disorders, gastrointestinal problems, leukemia, thrombotic diseases, and a variety of cancers (Sharafi et al., 2019a). Specifically, Hg is neurotoxic, nephrotoxic, and inactivates enzymes leading to hepatoxicity (Renu et al., 2021). Pb is a possible carcinogen and affects cognitive development in children while inorganic arsenic (iAs; the most toxic form of As) is carcinogenic, and damage macromolecules such as DNA, lipids, and proteins. The accumulation of Cd can cause hepatic lipid peroxidation, mitochondrial lipid peroxidation, and depletion of glutathione in humans (Renu et al., 2021; ATSDR, 2021). Heavy metals such as Cd, Pb, As, and Hg may affect critical processes of adult neurogenesis and impair cognitive function and olfaction (Wang and Matsushita, 2021). Zn dyshomeostasis can result in neurodegenerative disorders, including Parkinson's disease. Mn accumulation in humans can cause central nervous system disorder. Fe is implicated in reactive oxygen species generation, causing cell dysfunction and finally cell death (Ullah et al., 2021). Cr exerts carcinogenic effects through mutagenesis (Wallace and Djordjevic, 2020).

As microbenthic organisms, crayfish species are in constant contact with bottom sediments (detritus) (Śmietana et al., 2020). Consequently, they accumulate metals in their habitat through food ingestion, absorption, and ion exchange across the membrane. The contaminants may cause cellular or whole-body level damage and mortalities. The higher trophic level interactions of crayfish facilitate metal biomagnification (Gunderson et al., 2021) with the possible transfer of metals to wildlife and humans. Previous studies found toxic elements in crustaceans from natural (Suárez-Serrano et al., 2010; Allert et al., 2013; Varol and Sünbül, 2018) and cultured (Gedik et al., 2017; Xiong et al., 2020) systems with varying levels in the tissues and organs (Shalini et al., 2020; Zhau et al.,

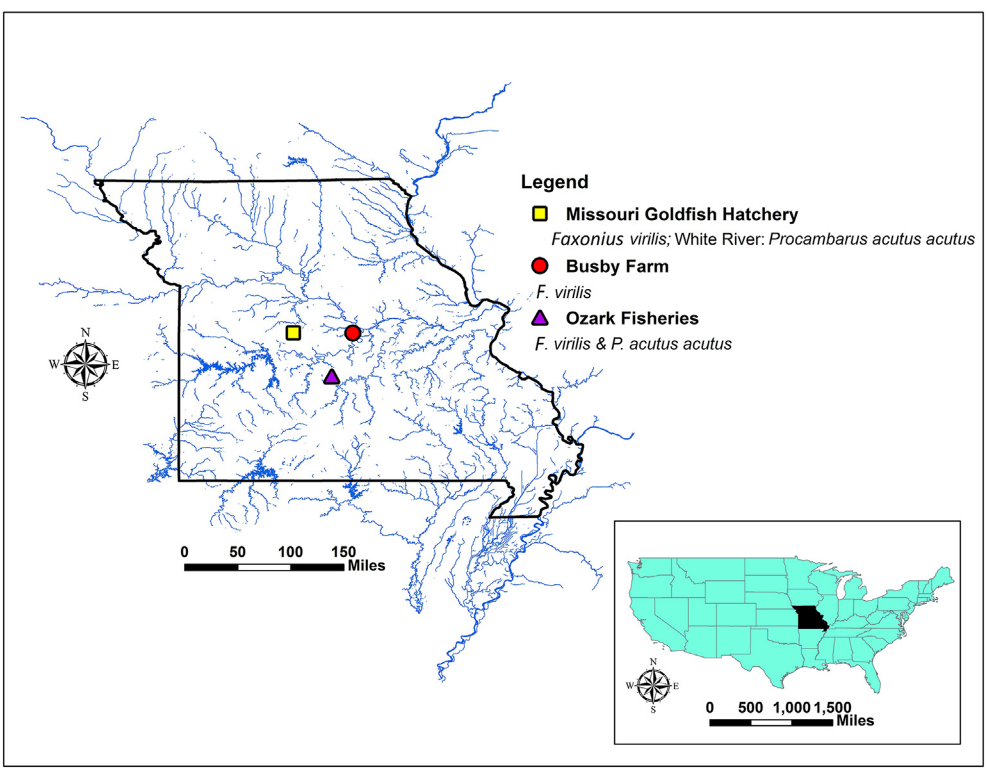

Figure 1. Map showing the state of Missouri, the aquaculture farms, and the crayfish species collected. Square indicates Missouri Goldfish Hatchery (*Faxonius virilis;* and White River: *Procambarus acutus acutus);* the circle indicates Busby farm (*F. virilis),* and the triangle indicates Ozark Fisheries (*F. virilis*; and *P. a. acutus)*. Sources for map preparation*: United States Geological Survey (USGS) "National Hydrology Dataset" (NHD), 2017 and ESRI "States" Shapefile for the United States of America.*

Table 1. Habitat, feeding pattern, total length (mm), and body weight (g) of crayfish species (*F. virilis; P. a. acutus*) from aquaculture production systems.

Study site[a]	Crayfish species	Total length ±SD	Body weight ±SD	Habitat	Feeding pattern
Missouri Goldfish Hatchery	*F. virilis* (n = 54)	108 ± 24.4 (71.2–155)	19.0 ± 11.7 (5.7–45.1)	Benthic: Found in ponds, marshes, lakes, rivers, and streams – hide under rocks, logs or from predators.	Aquatic plants, vegetation snails, insects, tadpoles and small fish, and scavenging dead animals.
	P. acutus acutus (n =18)	117 ± 15.4 (98.7–144)	21.7 ± 5.8 (14.0–30.6)		
Ozark Fisheries	*F. virilis* (n = 36)	82.4 ± 15.6 (63.8–155)	9.2 ± 6.9 (4.7–45.3)		
	P. acutus acutus (n = 40)	111 ± 20.6 (79.2–149)	17.1 ± 8.4 (7.6–45.7)		
Busby farm	*F. virilis* (n = 24)	61.1 ± 6.0 (48.5–71.5)	3.5 ± 1.0 (2.2–5.7)		

[a] Aquaculture production system; ± SD = Standard deviation; Ranges are in parenthesis.

2021). Crayfish is widely consumed and metal transfers in organisms occur from the water column, sediment, and biota (Shalini et al., 2020). Concerning cultured systems, contaminants in crayfish may also emanate from algaecides application, fertilizers, fish feeds (Zhang et al., 2020), and other sources. Thus, there is the potential for human exposure to metals through crayfish consumption.

About heavy metals and their toxicological effects, the United States Environmental Protection Agency (US EPA), the European Commission (EC), the World Health Organization/Food and Agriculture Organization (WHO/FAO), and others have established legislations, maximum levels, and health-based guidelines for seafood (WHO/FAO, 2015; US EPA, 2019a; JECFA, 1989; 2000; 2011; EFSA, 2014; 2015; Official Journal of the European Union, 2008; 2011; MAFF, 1998; FSANZ, 2013). Further, hazard identification and exposure assessments are key steps (US EPA, 1991) towards the management of risks in the population. Risk assessment ascertains the hazard associated with the intake or absorption of pollutants at different levels within exposed areas (Neris et al., 2021). Estimation of health risks through dietary exposure may be performed using the deterministic and probabilistic approaches (Meerpoel et al., 2021). Some of the influencing factors in risk estimation include ingestion rate, frequency of consumption, duration of food consumption, the weight of people consuming food (vary from place to place; Sharafi et al., 2019b), and the average lifetime of the risk group. Acute or chronic metal exposures can cause severe disorders and extreme damage due to oxidative stress (Nain and Kumar, 2020). Numerous studies (Traina et al., 2019; Anandkumar et al., 2020; Alipour et al., 2021; Peng et al., 2016; Sharafi et al., 2019b) evaluated the human health risks from the consumption of various foods to inform the public, support policy changes, and improve food quality. Generally, human risk assessments alleviate public health concerns, promote the seafood industry (Suami et al., 2019), and support reduced disease burdens (US EPA, 2012).

Faxonius virilis (formerly known as *Orconectes virilis*), and *Procambarus acutus acutus* belong to the genus *Faxonius* and *Procambarus*, respectively. *F. virilis* are native to Missouri's prairie regions (Skalicky, 2018), and abundant in the northern United States and southern Canada (Green and Storey, 2016). *P. a. acutus* is an opportunistic and generalist feeder (Eversole et al., 1999) and known as the eastern White River crayfish (Mazlum and Eversole, 2004). Generally, the presence of trace elements in crayfish can imply contamination. To our knowledge, there are no previous human health risk assessments in this region on metals and metalloid concentrations in muscle of cultured *F. virilis* and *P. a. acutus*. Given the importance of seafood in the human diet and the increasing contribution of aquaculture towards global food security, more studies are, therefore necessary to assess cultured crayfish metal body burdens and the potential health risks. Thus, this study highlights the elemental content of two North American crayfish species and estimates the dietary exposure to metals through crayfish consumption by the Missouri adult population. The objectives of the current study were to (1) determine the concentrations of metals [silver (Ag), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), tin (Sn), lead (Pb), and zinc (Zn)] and metalloid [arsenic (As)] in the edible abdominal muscle of cultured

Figure 2. a) Male forms of *F. virilis*, and b) *P. a. acutus*.

freshwater crayfish species (*F. virilis;* and *P. a. acutus*) from Missouri; (2) estimate the dietary intake of metal(loid)s from crayfish abdominal muscle; and (3) assess the non-carcinogenic and carcinogenic human health risks from the consumption of muscle.

2. Materials and methods

2.1. Sampling of crayfish individuals (F. virilis and P. a. acutus)

Two crayfish species (*F. virilis*, and *P. a. acutus*) were collected from aquaculture farms (Missouri Goldfish Hatchery, Ozark Fisheries, and Busby farm/aquaculture pond) in Missouri between February 2017 and January 2018 by seine (Larson and Olden, 2017). The locations of the farms (Figure 1) were (i) Alan T. Busby farm (38.505862° N, -92.246250° W; Lincoln University, Jefferson City, MO 65101); (ii) Ozark Fisheries (37.913109° N, -92.533045° W; Ozark Fisheries Road, Stoutland, MO 65567); and (iii) Missouri Goldfish Hatchery (38.507064° N, -93.057675° W; Hatchery Road, Stover, MO 65078). The Alan T. Busby aquaculture pond is located eight miles from the Lincoln University campus. The number of samples collected, as summarized in Table 1, were (i) Missouri Goldfish Hatchery: *F. virilis* (n = 54), and *P. a. acutus* (n = 18; low abundance); (ii) Ozark Fisheries: *F. virilis* (n = 36), and *P. a. acutus* (n = 40); and (iii) Busby farm: *F. virilis (*n = 24). Table 1 also presents the crayfish collection sites, species, animal total length (TL), animal body weight (BW), habitat, and feeding pattern. During sampling, we noticed that *P. a. acutus* species was not abundant for collection at Busby farm. The study ponds were fed as follows: watershed, rainfall, and runoff (Busby farm); and largely spring-dominated, rainfall, and a small amount of surface runoff (Ozark Fisheries and Missouri Goldfish Hatchery). Crayfish identification was conducted using the guides (Pflieger, 1996; DiStefano et al., 2008). Figure 2a and b present the male forms of *F. virilis* and *P. a. acutus,* respectively.

After crayfish collection, we placed the animals in containers with water from their natural environment and transported them to our laboratory. On reaching our laboratory we excluded animals that were unhealthy or with defects and sorted the specimens according to species and gender. Finally, all samples were frozen at – 40 °C in labeled zip-lock bags until analysis.

2.2. Reagents and standards

The reagents used were analytical or trace metal grades. Ultrapure water (resistivity of 18.2 MΩ cm) prepared by a Milli-Q® Integral 5 water purification system (Millipore Corporation, USA) was used for rinses, dilutions, and preparation of blanks. Hydrogen peroxide (certified ACS grade; 30%, v/v), concentrated nitric acid (trace metal grade; 65%, v/v), and yttrium (Y) stock standard (1000 mg L^{-1}) as internal standard, were purchased from Fisher Scientific (Hanover Park, Illinois, USA). Working standards were prepared from a multi-element standard stock solution (100 mg L^{-1}) acquired from SPEX CertiPrep (Metuchen, NJ, USA). Independent calibration verification (ICV; multi-element suite) and QCS-26 (quality control standard) solutions used for quality assurance purposes were purchased from High Purity Standards (Charleston, SC, USA). The tuning of the Agilent 5110 inductively coupled plasma-optical emission spectrometer (ICP–OES) was performed using a diluted internal calibration stock solution (Agilent Technologies, Santa Clara, CA, USA). Certified reference material (SRM 1640a: trace element in natural water) used for instrument readiness check was obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA). Method accuracy was evaluated using a certified reference material (TORT–2: lobster hepatopancreas reference material for trace metals) acquired from the National Research Council (NRC, Ontario, Canada). Ultrapure argon gas (99.995% purity) used for the generation of the ICP plasma and sample aspiration was supplied by Airgas Mid-America (Holts Summit, MO, USA).

2.3. Sample digestion

The growth factors (CL, TL, and BW) of each specimen were measured and recorded before dissection. Subsamples (0.2–0.5 g wet weight (ww)) of muscle tissue were weighed into acid-cleaned microwave digestion vessels (TFM™ PTFE) and mineralized with a mixture of concentrated nitric acid and hydrogen peroxide (2.5: 1) in a high-pressure Ethos EZ microwave digester (Milestone Inc., Shelton, CT, USA). The digestion conditions earlier described by Ikem et al. (2015) was followed: Step 1: 1000 W (maximum power) at 100 °C for 3 min and hold for 4 min; Step 2: 1000 W at 150 °C for 3 min and hold for 4 min; Step 3: 1000 W at 180 °C for 3 min and hold for 4 min; and Step 4: the digests were cooled to room temperature before depressurization and opening of the vessels. The digested samples were quantitatively transferred into 25 mL standard flasks and then brought to volume with ultrapure water. Finally, the digested samples were stored in pre-cleaned 60 mL polyethylene bottles and refrigerated at 4 °C until ICP analysis. Each sample was digested in triplicate along with a blank and the certified reference material (TORT–2).

2.4. Elemental analysis and quality assurance

An inductively coupled plasma-optical emission spectrometer (Agilent 5110 synchronous vertical dual view (SVDV) ICP–OES; Agilent Technologies, Inc., USA) was used for the quantitative determination of fourteen elements (As, Ag, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Sn, Pb, and Zn) in crayfish muscle samples. Elements were measured in compliance with the International guideline, EN ISO/IEC 17025:2017 (ISO, 2017) and according to US EPA Method 200.7 (US EPA, 2001) with modifications (Cauduro and Ryan, 2017). Additionally, the protocols and standards followed the Standard Methods for the Examination of Water and Wastewater (ICP Method 3120B; APHA, 2005). The instrument parameters and operational conditions were as follows: power (1.20 KW); radiofrequency generator (27 MHz); detector: Vistachip II charge-coupled device (CCD), stabilization time (15 s); nebulizer flow (0.7 L min^{-1}); plasma flow (12 L min^{-1}); auxiliary flow (1 L min^{-1}); makeup flow (1 L min^{-1}); multiple conditions (SVDV); viewing height (8 mm); SPS 4 autosampler rinse pump (control speed: fast); replicate read time (3 s); pump speed (12 rpm); sample uptake delay (15 s; fast pump); rinse time (30 s, fast pump); and read time (5 s). For the analysis, an internal standard, Y (0.4 mg L^{-1}), was added to sample solutions (ratio 1: 1). The elements were measured using their characteristic atomic emission lines. The wavelengths (nm) of measurements were Ag: 328.068; As: 188.980; Be: 313.042; Cd: 214.439; Co: 238.892; Cr: 267.716; Cu: 327.395; Fe: 238.204; Mn: 257.610; Mo: 202.032; Ni: 231.604; Pb: 220.353; Sn: 189.925; and Zn: 213.857. All bottles and flasks were washed with metal-free detergent, rinsed many times with ultrapure water, and acid-washed in 30% nitric acid. Finally, all containers were rinsed thoroughly with ultrapure water to avoid contamination. Blanks, and ICV, QCS-26, and SRM 1640a solutions were analyzed along with each batch of 15 samples. Samples were analyzed axially and the ICP equipment was optimized daily for maximum sensitivity. External calibrations were performed using working calibration standards prepared from dilution of a multi-element stock standard (100 mg L^{-1}; SPEX CertiPrep, NJ, USA). The linear curves of the calibration lines produced R^2 values greater than 0.995. The ICP was programmed to recalibrate automatically after every 10 samples in a sequence. Background correction was performed through the fast-automated curve fitting technique (FACT) to achieve the detection limits. The analytical concentrations were calculated using ICP Expert software (Version 7.4.1. 10449; Agilent Technologies).

The validation of the analytical procedure for analyzed elements in crayfish muscle was performed by evaluation of the limit of detection (LOD), limit of quantitation (LOQ), trueness, and precision following EURACHEM criteria (EURACHEM, 2014). The LOD and LOQ values were calculated as three times the standard deviation (3.3σ) and ten times the standard deviation (10σ) of results (EURACHEM, 2014; Lo Turco et al., 2020) obtained from the analysis of twenty-five blanks. The LOD values (μg L^{-1}) were Ag (1.6), As (37.6), Be (2.1), Cd (1.6), Co (2.5), Cr (2.4), Cu (1.7), Fe (1.7), Mn (2.0), Mo (2.1), Ni (3.4), Pb (9.3), Sn (8.3), and Zn (11.5).

Table 2 presents the instrument validation and method accuracy from the analysis of certified reference materials, SRM 1640a and TORT-2, respectively (ISO 5725-2 guide: ISO, 2019). Multi-element standard solutions (ICV and QCS-26) at 1 mg L^{-1} each, were used for additional quality assurance. The recovery rates of elements from SRM 1640a were ±5% of certified values while those from TORT-2 were within ±16% of the certified values in most cases (Table 2). The recovery results from the analysis of ICV and QCS-26 were within ±5% of certified values (Table 3). All samples were analyzed in triplicate and the analytical results are expressed in mg kg^{-1} ww.

2.5. Calculations of recovery rates and analyte concentrations

The recovery rates of analyzed elements from three solutions (ICV, QCS-26, and SRM 1640a), and TORT–2 certified reference was calculated according to Eq. (1):

$$ARR = (MV/CV) * 100 \quad (1)$$

Where ARR is the analyte recovery rate (%), MV is the measured element concentration in the sample, and CV is the certified value provided for the element (Table 2; NIST: www.nist.gov; NRC). Concentrations of

Table 2. Element, view mode[a], wavelength, limit of detection (LOD[b]; n = 25; μg L^{-1}), limit of quantification (LOQ; n = 25; μg kg^{-1}), and found values for SRM 1640a–Trace elements in natural water (μg L^{-1}; n = 46) and TORT-2 (Lobster hepatopancreas reference material for trace metals; mg kg^{-1}; n = 12) using ICP – OES.

Element	Wavelength (nm)	LOD[b]	LOQ	SRM 1640a			TORT-2		
				Measured	Certified	% Rec.	Measured	Certified	% Rec.
Ag	328.068	1.6	4.8	7.02 ± 0.71	8.08 ± 0.046	86.9	-	-	-
As	188.98	37.6	114	7.91 ± 10.2	8.08 ± 0.070	98.0	17.8 ± 1.6	21.6 ± 1.8	82.4
Be	313.042	2.1	6.5	2.70 ± 0.9	3.03 ± 0.028	89.1	-	-	-
Cd	214.439	1.6	4.9	3.96 ± 0.36	3.99 ± 0.074	99.9	22.9 ± 1.5	26.7 ± 0.6	85.6
Co	238.892	2.5	7.5	20.1 ± 1.29	20.2 ± 0.24	99.4	0.40 ± 0.002	0.51 ± 0.09	78.2
Cr	267.716	2.4	7.1	40.3 ± 1.7	40.5 ± 0.30	99.4	0.77 ± 0.25	0.77 ± 0.15	99.9
Cu	327.395	1.7	5.2	87.2 ± 3.62	85.8 ± 0.51	102	93.9 ± 25.9	106 ± 10	88.6
Fe	238.204	1.7	5.0	38.4 ± 1.9	36.8 ± 1.8	105	94.1 ± 8.68	105 ± 13.0	89.6
Mn	257.61	2.0	6.1	39.9 ± 1.6	40.4 ± 0.36	99.6	11.8 ± 0.74	13.6 ± 1.2	86.8
Mo	202.032	2.1	6.4	43.6 ± 3.1	45.6 ± 0.61	95.6	-	-	-
Ni	231.604	3.4	10.3	25.9 ± 1.3	25.3 ± 0.14	102	2.29 ± 0.22	2.5 ± 0.19	91.5
Pb	220.353	9.3	28.0	13.6 ± 4.1	12.1 ± 0.05	113	0.37 ± 0.14	0.35 ± 0.13	105
Sn	189.925	8.3	25.2	0.98*	1.00**	98.0	-	(0.04)**	-
Zn	213.857	11.5	34.8	54.2 ± 2.99	55.6 ± 0.35	98.2	151 ± 10.3	180 ± 6.0	84

[a] View mode: All samples were determined under the axial mode.
[b] LOD was calculated from analysis of 50 μg L^{-1} spiked solution, Rec. = Recovery.
* Spiked solution (1 mg L^{-1}) analyzed in triplicate.
** Not certified value, Errors are expressed as standard deviation.

Table 3. Element, view mode*, wavelength, and found values for independent calibration verification (ICV; mg L^{-1}; n = 69) and QCS-26 (quality control sample; mg L^{-1}; n = 120) solutions using ICP – OES.

Element	Wavelength (nm)	ICV[a]			QCS-26[a]		
		Measured	Cert.	% Rec.	Measured	Cert.	% Rec.
Ag	328.068	1.00 ± 0.11	1.00	100	0.98 ± 0.09	1.00	98.2
As	188.98	0.97 ± 0.04	1.00	97.3	0.96 ± 0.05	1.00	96.4
Be	313.042	0.99 ± 0.04	1.00	98.6	0.98 ± 0.05	1.00	98.2
Cd	214.439	0.99 ± 0.04	1.00	98.7	0.98 ± 0.05	1.00	97.7
Co	238.892	0.97 ± 0.04	1.00	97.1	0.96 ± 0.05	1.00	96.2
Cr	267.716	0.98 ± 0.04	1.00	98.1	0.97 ± 0.05	1.00	97.4
Cu	327.395	0.99 ± 0.04	1.00	98.6	0.98 ± 0.05	1.00	98.0
Fe	238.204	0.98 ± 0.04	1.00	97.5	0.97 ± 0.05	1.00	96.8
Mn	257.61	0.97 ± 0.03	1.00	97.1	0.97 ± 0.05	1.00	96.6
Mo	202.032	0.99 ± 0.04	1.00	99.0	0.98 ± 0.05	1.00	98.3
Ni	231.604	0.97 ± 0.04	1.00	97.3	0.97 ± 0.05	1.00	96.6
Pb	220.353	0.96 ± 0.04	1.00	96.2	0.96 ± 0.05	1.00	95.6
Sn	189.925	0.99 ± 0.03	1.00	99.2	-	-	-
Zn	213.857	0.98 ± 0.04	1.00	98.3	0.97 ± 0.05	1.00	97.2

ICV = Independent calibration verification; Cert. = Certified value; Rec. = Recovery; the recovery rate of Sn was ascertained from the analysis of 1 mg L^{-1} spiked solution in triplicate.
[a] Unit in mg L^{-1}.
* View mode = axial.

elements in crayfish muscle, expressed as mg kg^{-1} ww was calculated using Eq. (2):

$$C = [(AC/SW) * VD * DF] \quad (2)$$

Where C is the element concentration in crayfish muscle (μg g^{-1}), AC = analytical concentration result (μg mL^{-1}), SW = sample weight (g), VD = volume of digested sample (mL), and DF = dilution factor.

2.6. Assessment of human health risks

The metal(loid)s content in crayfish muscle were compared with the European maximum metal levels in crustaceans (Official Journal of the European Union, 2008, 2011); the Food Standards Australia and New Zealand (FSANZ, 2013); Ministry of Agriculture, Fisheries and Food (MAFF, 1998); and the Codex Alimentarius Commission (WHO/FAO, 2015; FAO, 1983) limits. Moreover, the daily/weekly intake (EDI/EWI) values (this study) were compared to the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (JECFA, 1989; 2000; FAO/WHO, 2005; WHO, 1993); and the EC (EFSA, 2009, 2014, 2015) permitted limits. Finally, we compared the calculated non-carcinogenic and carcinogenic effects to reference and benchmark values, respectively.

2.6.1. Estimated weekly intake (EWI) of metals/metalloid from crayfish consumption

The risk to human health associated with the consumption of crayfish muscle was through the estimated daily/weekly intake (EDI/EWI) values of As, Pb, and other elements (Milenkovic et al., 2019). The EWI expressed as μg kg^{-1} body weight per day was according to Eq. (3):

$$EWI = [(AC * DIR * F)/(BWt)] \quad (3)$$

Where AC = analyte concentration (μg g^{-1}) in crayfish muscle, DIR = daily ingestion rate of muscle (crustaceans: 4.9 g/person/day; FAO, 2005; Sioen et al., 2009), F = frequency of ingestion (7 days), and BWt = body weight (70 kg adult assumed for the USA population; US EPA, 1989). The EDI/EWI values (this study) were compared with the provisional tolerable weekly intakes (PTWIs; JECFA, 1989, 2000, 2011), oral reference dose (RfD_o; US EPA, 2019a), and other regulatory thresholds (EFSA, 2014, 2015).

2.6.2. Non-carcinogenic risk from crayfish muscle consumption

The target hazard quotient (THQ) is the ratio of the exposure dose to the RfD_o (Traina et al., 2019), which indicates the non-carcinogenic risk from metals/metalloid via crayfish muscle consumption. THQ <1 (i.e., the exposure level is below the RfD_o) implies that there are significant health benefits and consumers are safe from the consumption of crayfish muscle, whereas THQ >1 (i.e., the exposure level is greater than the RfD_o) indicates adverse effects from metal exposure (US EPA, 1989). The US EPA described THQ in the guidelines for human exposure (US EPA, 1989), and the calculation followed Eq. (4):

$$THQ = [(EFr * ED * IR * C)]/[(RfD_o * BWt * AET)] * [10^{-3}] \quad (4)$$

Where EFr is the exposure frequency (365 days per year); ED is the exposure duration (70 years, assumed as the average lifetime in the United States; US EPA, 2009); IR is the crayfish ingestion rate (crustaceans: 4.9 g person $^{-1}$ per day assumed for adults in the United States; FAO, 2005; Sioen et al., 2009); C is the average metal/metalloid concentration found in crayfish muscle (μg g^{-1} ww); RfD_o is the oral reference dose in mg kg^{-1} per day (US EPA, 1991); BWt is the average body weight for an adult (70 kg) in the United States (US EPA, 1991), and AET is the average exposure time for non-carcinogens (365 days year $^{-1}$ * ED = 25, 550 days; 70 years assumed in this study; US EPA, 2009). To estimate THQ, the US EPA assumed that the ingested dose is equal to the absorbed contaminant dose (US EPA, 1989).

Exposure to more than one contaminant from food will probably produce associated combined or interactive effects (Li et al., 2013).

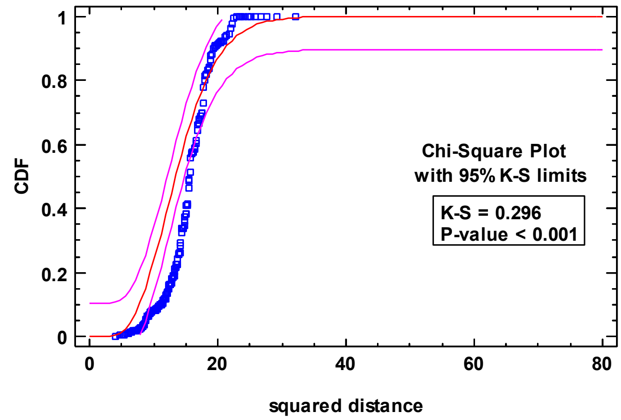

Figure 3. Multivariate normality test of metals/metalloid concentrations found in crayfish muscle across the collection sites (n = 172; conditions of the test: no transformation, probability band = 95%, goodness-of-fit test: modified Kolmogorov-Smirnov; CDF = cumulative distance function).

Consequently, total THQ (TTHQ; i.e., the sum of the individual THQ for the metals/metalloid) from exposure through crayfish muscle consumption was estimated according to Eq. (5):

$$TTHQ_{Crayfish\ muscle} = THQ_{(As)} + THQ_{(Be)} + THQ_{(Cd)} + \text{—} + THQ_{(Sn)} + THQ_{(Zn)} \quad (5)$$

Where TTHQ ≤1.0 suggests a chance of no adverse non-cancer effects, TTHQ >1.0 implies an adverse effect on the target population (Sharafi et al., 2019a; Dippong et al., 2019), and TTHQ >10.0 indicates a chronic toxic effect (Wei and Cen, 2020). THQ_{As} values were calculated using the RfD_o for iAs, which represents the most toxic form (US EPA, 2019a).

2.6.3. Carcinogenic risk from crayfish muscle consumption

The incremental lifetime cancer risk (ILCR) was calculated using the EWI values (this study) and the provided cancer slope factors (CSF; mg kg^{-1} per day) for iAs, Cr, Ni, and Pb (US EPA, 2019a) according to Eq. (6):

$$ILCR = [(EFr * ED * IR * C * CSF)]/[(BWt * AET)] * [10^{-3}] \quad (6)$$

Where CSF is a plausible upper-bound estimate of the probability of a response per unit intake of a chemical over a lifetime (US EPA, 2019b). The CSF for iAs, Cr (VI), Ni (nickel subsulfide), and Pb (subacetate) used in the calculation were 1.5, 0.5, 1.7, and 0.0085, respectively (US EPA, 2019a). The remaining parameters were described earlier. The CSF values for Ag, Be, Cd, Co, Cu, Fe, Mn, Mo, Sn, and Zn were not provided by the US EPA. For regulatory purposes, the US EPA acceptable cancer risk range is 10^{-6} (i.e., the risk of developing cancer is 1 in 1,000,000) to 10^{-4} (i.e., the risk of developing cancer is 1 in 10,000) (US EPA, 1991). An ILCR value $<10^{-6}$ implies negligible cancer risk while an ILCR value $>10^{-4}$ signifies potential cancer risk (US EPA, 1991) from the consumption of crayfish muscle. In the current work, we also estimated the cumulative cancer risk index (ΣILCR, i.e., the sum of the individual heavy metal cancer risk) from potential exposure to multiple carcinogenic heavy metals through crayfish consumption.

2.7. Data treatment and statistical analyses

ICP Expert software (Agilent Technologies, Inc.) calculated the elemental concentrations found in crayfish muscle from the ICP – OES external calibrations. Microsoft® Excel 2016 (Microsoft Corporation, USA) was utilized for the descriptive statistics, expressed as a range, average, and standard error (±SE). Other statistical analyses were conducted using Statgraphics Centurion 18-X64 version 17.1.04 (Statpoint Technologies, USA). The normality test performed using Shapiro

Table 4. Range, average elemental concentrations (mg kg^{-1} ww), and limits of variation (±SE) for crayfish abdominal muscle (*Faxonius virilis;* and *Procambarus acutus acutus*) from aquaculture.

Element	Missouri Goldfish Hatchery (*F. virilis*, n = 54)	Missouri Goldfish Hatchery (*P. acutus acutus,* n = 18)	Ozark Fisheries (*P. acutus acutus*, n = 40)	Ozark Fisheries (*F. virilis*, n = 36)	Busby Farm (*F. virilis*, n = 24)
Ag	< LOD – 0.103	< LOD	< LOD – 0.097	< LOD – 0.11	< LOD
	0.002 ± 0.002	< LOD	0.002 ± 0.002	0.006 ± 0.004	< LOD
As	0.10–2.56	0.10–0.91	< LOD – 2.55	0.10–3.15	0.30–1.75
	0.93 ± 0.09	0.51 ± 0.06	0.89 ± 0.11	1.23 ± 0.12	1.09 ± 0.10
Be	< LOD – 0.21	< LOD – 0.10	< LOD – 0.20	< LOD – 0.21	< LOD – 0.10
	0.04 ± 0.01	0.03 ± 0.01	0.09 ± 0.01	0.05 ± 0.01	0.01 ± 0.010
Cd	< LOD – 0.11	< LOD – 0.10	< LOD – 0.10	< LOD – 0.11	< LOD – 0.10
	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
Co	< LOD – 0.30	< LOD – 0.20	< LOD – 0.30	< LOD – 0.32	0.096–0.103
	0.11 ± 0.01	0.04 ± 0.02	0.11 ± 0.01	0.13 ± 0.01	0.10 ± 0.01
Cr	< LOD – 0.69	< LOD – 0.20	< LOD – 1.22	< LOD – 0.83	< LOD – 0.099
	0.18 ± 0.02	0.10 ± 0.01	0.23 ± 0.03	0.23 ± 0.04	0.01 ± 0.01
Cu	1.03–21.2	3.85–7.39	4.41–35.6	5.69–107	0.30–3.66
	8.97 ± 0.59	6.22 ± 0.30	13.4 ± 0.89	27.3 ± 3.05	2.26 ± 0.15
Fe	2.00–23.0	1.76–3.34	2.20–19.5	2.66–10.3	0.41–3.83
	9.04 ± 2.44	2.63 ± 0.13	4.28 ± 0.53	4.82 ± 0.30	1.39 ± 0.15
Mn	0.20–8.54	0.29–0.61	0.20–1.96	0.20–2.40	0.79–4.94
	1.31 ± 0.22	0.43 ± 0.03	0.71 ± 0.07	0.42 ± 0.07	1.82 ± 0.18
Mo	< LOD – 0.41	< LOD – 0.20	< LOD – 0.31	< LOD – 0.61	< LOD – 0.62
	0.15 ± 0.01	0.12 ± 0.02	0.13 ± 0.02	0.15 ± 0.02	0.17 ± 0.04
Ni	< LOD – 2.65	< LOD – 0.61	< LOD – 2.02	< LOD – 0.62	< LOD – 0.69
	0.31 ± 0.06	0.24 ± 0.05	0.70 ± 0.09	0.21 ± 0.03	0.12 ± 0.03
Pb	< LOD – 1.76	0.10–1.42	< LOD – 1.69	< LOD – 1.56	< LOD – 0.71
	0.59 ± 0.06	0.99 ± 0.10	0.71 ± 0.06	0.35 ± 0.06	0.40 ± 0.04
Sn	1.32–5.91	1.42–3.25	0.97–3.97	1.65–5.26	< LOD – 2.18
	2.72 ± 0.11	2.36 ± 0.14	3.06 ± 0.10	3.45 ± 0.13	0.66 ± 0.16
Zn	9.48–19.2	9.89–12.3	7.69–16.0	6.31–17.8	1.78–2.96
	12.4 ± 0.24	10.6 ± 0.18	11.4 ± 0.33	9.99 ± 0.35	2.03 ± 0.05

n = the number of specimens; LOD = limit of detection; SE = standard error.

Wilk W indicated a non-Gaussian distribution of our dataset. Royston's test combined the Shapiro-Wilk W statistics for the separate variables and compared the result to a Chi-square distribution (Royston, 1983). Accordingly, the non-parametric Kruskal – Wallis ANOVA was conducted to compare the metal(loid)s concentrations and the growth factors (TL, CL, and BW) across the collection sites. Further, the ANOVA procedure tested both the inter-species and gender differences in the dataset. Figure 3 shows the Chi-square plot of the squared distances of each observation from the sample centroid. Finally, Spearman's rank correlation analysis was performed to understand the degree of

Table 5. 95.0% confidence intervals for the means and standard deviations (elements in mg kg^{-1} ww) of the variables (*F. virilis*; n = 114). Total length (TL; (mm)); body weight (BW; (g; ww)); carapace length (CL; (mm)); and ± *Stnd. error* = Standard error.

Element	*Mean*	*Stnd. error*	*Lower limit*	*Upper limit*	*Sigma*	*Lower limit*	*Upper limit*
Ag	0.003	0.002	0.00	0.006	0.017	0.015	0.019
As	1.06	0.06	0.93	1.18	0.67	0.59	0.77
Be	0.037	0.006	0.025	0.049	0.063	0.055	0.072
BW	12.6	1.02	10.6	14.7	10.9	9.67	12.6
Cd	0.032	0.005	0.023	0.041	0.048	0.042	0.055
CL	11.8	0.29	11.3	12.4	3.04	2.69	3.50
Co	0.11	0.007	0.098	0.13	0.08	0.067	0.088
Cr	0.16	0.016	0.13	0.19	0.17	0.15	0.19
Cu	13.3	1.36	10.7	16.0	14.5	12.8	16.6
Fe	4.63	0.31	4.01	5.25	3.34	2.95	3.84
Mn	1.14	0.12	0.89	1.38	1.30	1.15	1.50
Mo	0.16	0.013	0.13	0.18	0.14	0.12	0.16
Ni	0.24	0.032	0.18	0.30	0.34	0.30	0.39
Pb	0.48	0.038	0.40	0.55	0.41	0.36	0.47
Sn	2.52	0.12	2.28	2.76	1.29	1.14	1.48
TL	90.1	2.50	85.2	95.1	26.7	23.7	30.7
Zn	9.46	0.41	8.66	10.3	4.34	3.84	4.99

Table 6. 95.0% confidence intervals for the means and standard deviations of the variables (*P. a. acutus*; n = 58). Total length (TL; (mm)); body weight (BW; (g; ww)); carapace length (CL; (mm)); and ± *Stnd. error* = Standard error.

Element	*Mean*	*Stnd. error*	*Lower limit*	*Upper limit*	*Sigma*	*Lower limit*	*Upper limit*
Ag	0.002	0.002	0.00	0.005	0.013	0.011	0.016
As	0.78	0.083	0.61	0.95	0.63	0.54	0.78
Be	0.07	0.009	0.05	0.088	0.071	0.06	0.087
BW	18.5	1.04	16.4	20.6	7.90	6.68	9.67
Cd	0.017	0.005	0.007	0.027	0.038	0.032	0.046
CL	15.2	0.30	14.6	15.8	2.30	1.95	2.82
Co	0.09	0.012	0.069	0.12	0.09	0.077	0.11
Cr	0.19	0.025	0.14	0.24	0.19	0.16	0.24
Cu	11.2	0.76	9.66	12.7	5.78	4.89	7.08
Fe	3.77	0.38	3.00	4.53	2.91	2.46	3.57
Mn	0.62	0.051	0.52	0.73	0.39	0.33	0.48
Mo	0.13	0.012	0.10	0.15	0.094	0.08	0.12
Ni	0.56	0.067	0.42	0.69	0.51	0.43	0.62
Pb	0.80	0.057	0.68	0.91	0.43	0.36	0.53
Sn	2.84	0.090	2.66	3.02	0.69	0.58	0.84
TL	113	2.53	108	118	19.2	16.3	23.6
Zn	11.1	0.24	10.7	11.6	1.83	1.55	2.24

associations among the variables. For all statistical tests, $P < 0.05$ was accepted as statistically significant.

3. Results and discussion

3.1. Certified reference materials and other standards

Table 2 presents the recovery rates of analyzed elements in SRM 1640a and TORT–2 reference standards. The results (78%–105%) were within the acceptance range. Repeatability results from the analysis of QCS-26 and ICV solutions expressed as relative standard deviations (RSDs), ranged from 4.12% to 4.68% and 0.54%–1.24%, respectively. The recovery rates of elements from the ICV and QCS-26 solutions (Table 3) were within the ranges of 96%–101% and 90%–103%, respectively. Moreover, the recovery rates of the internal standard (Y) ranged from 97% to 111%.

3.2. Metal(loid)s concentrations in crayfish muscle in comparison with published values

Table 4 presents the metal(loids) content in crayfish muscle expressed as a range, average, and standard error (±SE), considering the species and collection sites. Additional summary statistics for each species are reported in Table 5 (*F. virilis*) and Table 6 (*P. a. acutus*).

The average TL (113 ± 2.5 mm) achieved in *P. a. acutus* was higher than the corresponding average in *F. virilis* (90.1 ± 2.5 mm; SE) (Tables 5 and 6). Similarly, the mean BW in *P. a. acutus* (18.5 ± 1.0 g ww) was higher than the average recorded for *F. virilis* (12.6 ± 1.0 g ww).

Considering all collection sites and species, the mean levels of metal(loid)s in muscle ranged from <LOD – 107 mg kg^{-1} ww, with Cu the most abundant metal followed by Fe and Zn (Table 4). Of all samples, the rank of the highest concentrations (mg kg^{-1} wet weight) of metals/metalloid in muscle was Cu (107) > Fe (23.0) > Zn (19.2) > Mn (8.54) > Sn (5.91) > As (3.15) > Ni (2.65) > Pb (1.76) > Cr (1.22) > Mo (0.62) > Co (0.32) > Be (0.21), Cd (0.11) ~ Ag (0.11). Copper was the highest in muscle of *F. virilis* collected from Ozark Fisheries while those of Fe and Zn were in samples from Missouri Goldfish Hatchery.

Published data for the crayfish species studied was not available and here we compare our values with those found in other crustaceans. Accordingly, the summary statistics of metal(loid)s (this study) in comparison with published values and existing standards are presented in Table 7. Yet, the regulatory agencies have not provided the maximum allowable levels (MALs) for Ag, Be, Co, Cr, Fe, Mn, Mo, Ni, and Sn in crayfish.

3.2.1. Arsenic

Arsenic is present in foods, animal feeds additives, water, pharmaceutical products, and widely distributed in soils and sediments. Arsenic being a non-essential element, is not required in animal metabolism. Arsenic-induced toxicity in humans is due to iAs [As (III), and As (V)] which can cause various cancers, low intelligence quotient (IQ) scores in children (ATSDR, 2021), increased oxidative stress, and the facilitation of DNA damage (Wallace and Djordjevic, 2020). According to Rainbow and Luoma (2011), the build-up of metal in the metabolically available form occurs when the uptake rate exceeds the excretion/detoxification rate. Arsenic was detectable in all samples except for two samples (*P. a. acutus;* Ozark Fisheries; Table 4). The highest As concentration (3.15 mg kg^{-1} ww) was achieved in *F. virilis* from Ozark Fisheries.

In this study, the Food Standards Australia and New Zealand maximum limit for iAs (2 mg kg^{-1}; FSANZ, 2013) was exceeded in 11% and 14% of *F. virilis* samples from Missouri Goldfish Hatchery and Ozark Fisheries, respectively. In *P. a. acutus*, approximately 10% of samples from Ozark Fisheries also exceeded the 2-ppm limit. The mean As level attained in both species (Table 7) was consistently lower than the concentration in the crustacean, *Squilla mantis* (Bonsignore et al., 2018). Similarly, the mean As level (mg kg^{-1} ww) found in *F. virilis* (1.06), and *P. a. acutus* (0.78) were lower than the average levels (34.6 ± 12.2 mg kg^{-1}) achieved in the crustacean *Parapenaeus longirostris* (Traina et al., 2019) and shrimp, *Penaeus semisulcatus* muscle (1.40 μg g^{-1}; Shalini et al., 2020), and two crayfish species (2.6–13.9 μg g^{-1}; Hull et al., 2021). Nevertheless, the As content in the species (this study) were more than ten-fold lower than the maximal found in *Astacus leptodactylus* (Varol and Sünbül, 2017), the average concentration reported for *P. longirostris* (Traina et al., 2019), and below the iAs limit (2 ppm; FSANZ, 2013).

3.2.2. Beryllium

Beryllium is found naturally in rocks, soil, and volcanic rocks, and weapons, and other applications (ATSDR, 2021). Be concentrations found in muscle were negligible and values ranged from <LOD – 0.21 mg kg^{-1} ww (Table 4). Be was detectable in 8.3%, 36%, and 33% of *F. virilis* samples from Busby farm, Ozark Fisheries, and Missouri Goldfish Hatchery, respectively. In *P. a. acutus*, the detection of Be was 65% (Ozark Fisheries) and 33% (Missouri Goldfish Hatchery) of samples. However, Be was not detectable in crayfish tail samples from Northern

California watersheds (Hothem et al., 2007). According to the International Agency for Research on Cancer, Be and the compounds are human carcinogens (WHO, 1980).

3.2.3. Cadmium

Cadmium is a non-essential element and involved in energy-dependent routes for calcium uptake (Rainbow, 1995) considering other factors such as molting. Physicochemical and physiological factors influence the uptake rate of Cd by crustaceans. Furthermore, exposure to sub-lethal doses of Cu, Zn, Cd, and Hg results in the synthesis of metal-proteins involved in homeostatic metabolism (Taylor et al., 1995). Cd was detectable in 27% of samples across the sites with ranges from <LOD to 0.11 mg kg^{-1} ww (Table 4). Dietary intake is the main source of Cd exposure in humans. Cd is toxic even at low concentrations and is considered a probable carcinogen. Cd binds to small metallothionein proteins and accumulates in the kidneys and liver (Alipour et al., 2021) and bone (ATSDR, 2021).

Our average Cd level (all samples) was lower than the concentration (2.2 mg kg^{-1}) found in the crayfish *P. clarkia* (Goretti et al., 2016, Table 7), and shrimp *P. semisulcatus* muscle (0.072 μg g^{-1}; Shalini et al., 2020) but comparable to the level in crayfish tail muscle (Hothem et al., 2007) and farmed *P. clarkia* (Gedik et al., 2017). In contrast, our average Cd content was higher than the level in *A. leptodactylus* (Varol and Sünbül, 2017). Regardless of the species and collection sites, Cd content in muscle was lower than the acceptable level (0.5 mg kg^{-1} ww) set by the European Commission Regulation No. 629/2008 (Official Journal of the European Union, 2008).

3.2.4. Cobalt

Exposure to Co in humans is through inhalation of ambient air, occupational exposure, and from industrial land medical applications (ATSDR, 2021). Cobalt is an important component of vitamin B_{12}. Co was undetectable in 26% of samples and the highest level (0.13 mg kg^{-1} ww) was attained in *F. virilis* samples from Ozark Fisheries. The low levels of Co in the abdominal muscle were probably due to the inhibition of bio-accumulation in the presence of other heavy metals, most importantly Ni, Cu, Zn, and Mn (Norwood et al., 2007 cited by Tunca et al., 2013). Co is known to have adverse effects on the heart in humans (EGV, 2003).

Table 7. Summary of metals/metalloid concentrations (mg kg^{-1} ww) in comparison with regulatory thresholds and literature values for crustaceans.

Crayfish species	Statistics	Carapace length (mm)	Total length (mm)	Total body weight (g)	Ag	As	Be	Cd	Co	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sn	Zn
This Study; *F. virilis*[a] (n = 114)	Mean	11.8	90.1	12.6	0.00	1.06	0.04	0.03	0.11	0.16	13.3	4.63	1.14	0.16	0.24	0.48	2.52	9.46
	SE	0.29	2.50	1.02	0.00	0.06	0.01	0.00	0.01	0.02	1.36	0.31	0.12	0.01	0.03	0.04	0.12	0.41
	Min	7.02	48.5	2.16	0.00	0.10	0.00	0.00	0.00	0.00	0.30	0.41	0.20	0.00	0.00	0.00	0.00	1.78
	Max	19.9	155	45.3	0.11	3.15	0.21	0.11	0.32	0.83	107	23.0	8.54	0.62	2.65	1.76	5.91	19.2
This Study; *P. acutus acutus*[b] (n = 58)	Mean	15.2	113	18.5	0.00	0.78	0.07	0.02	0.09	0.19	11.2	3.77	0.62	0.13	0.56	0.80	2.84	11.1
	SE	0.30	2.53	1.04	0.00	0.08	0.01	0.00	0.01	0.03	0.76	0.38	0.05	0.01	0.07	0.06	0.09	0.24
	Min	10.8	79.2	7.57	0.00	0.00	0.00	0.00	0.00	0.00	3.85	1.76	0.20	0.00	0.00	0.00	0.97	7.69
	Max	20.7	149	45.7	0.10	2.55	0.20	0.10	0.30	1.22	35.6	19.5	1.96	0.31	2.02	1.69	3.97	16.0
Crustacean[c]; *Squilla mantis* (Livorno; Tuscany Coast, Italy)			133		-	19.1	-	0.21	-	0.05	12.1				0.21	0.03	-	20.9
Crayfish *Procambarus clarkia*[d]; Lake Trasimeno (n = 10); Italy								2.2			27					2.0		98
Crayfish *P. longirostris* [e]; Porticello, Sicilian coast, Italy						50.4		0.04								0.09		
Crayfish *Astacus leptodactylus* (Maximum level), Keban Dam Reservoir, Turkey (metal values in μg kg^{-1} ww; Varol and Sünbül, 2017).						3.78		4.1								42.3		
Legislations for metals in crayfish																		
European legislation - Maximum metal levels in Crustaceans								0.50[f]								0.5[g]		
Food Standards Australia New Zealand[h]						2[i]		-								0.5		
Codex Alimentarius commission[j]						-										0.3[k]		30[l]
Ministry of Agriculture, Fisheries and Food (MAFF)											20[m]							

SE = Standard error; Min = Minimum; Max = Maximum.

[a] All *F. virilis* samples from the study sites (Missouri Gold Hatchery; Ozark Fisheries; and Busby farm).

[b] All *P. acutus acutus* samples from the study sites (Missouri Gold Hatchery; and Ozark Fisheries).

[c] Crustacean *Squilla mantis* (Bonsignore et al., 2018).

[d] Crayfish *Procambarus clarkia* (Goretti et al., 2016).

[e] Crayfish *P. longirostris* (Traina et al., 2019).

[f] Official Journal of the European Union (2008).

[g] Official Journal of the European Union (2011).

[h] FSANZ, 2013.

[i] Inorganic arsenic.

[J] WHO/FAO (World Health Organization/Food and Agriculture Organization), 2015.

[k] Fish.

[l] FAO, 1983.

[m] MAFF, 1998.

3.2.5. Chromium

The sources of Cr include smelting and mining activities and machinery and the electroplating industry (Dippong et al., 2020). Cr is an essential element in organisms and humans. Cr (III) potentiates insulin action (control of carbohydrate, lipid, and protein metabolism). Chromium was detectable in most samples except in two samples of *F. virilis* from Busby farm. Cr (III) is a ubiquitous inorganic substance that is common in foods. Cr levels ranged from <LOD to 1.22 mg kg^{-1} ww (Table 4) with the highest average (0.23 mg kg^{-1} ww) achieved in both species (Ozark Fisheries). Our Cr values were over ten-fold lower than the average concentration reported for cultured red swamp crayfish, *P. clarkia* muscle (Xiong et al., 2020). Cr (VI) is mutagenic and carcinogenic. Additionally, chronic exposure to Cr (VI) potentially can induce renal failure, anemia, hemolysis, and liver failure (EGV, 2003; US EPA, 2020). According to Bollinger et al. (1997), bioaccumulation of Cr was not significant in the muscle of *P. clarkii* since muscle levels are similar to those found in the hemolymph.

3.2.6. Copper

Copper is an essential element, occurs as metalloproteins, and functions as enzymes connected to cell respiration and energy utilization. Sources of Cu are from the machinery and electroplating industry and mining/smelting activities (Dippong et al., 2020). In aquaculture, the sources of Cu include commercial algaecides (copper II sulfate as an active ingredient), antifungal, and anti-parasitic agents (Lahman et al., 2015; Zhao et al., 2019). Cu was detected in all samples and the levels ranged from 0.3 to 107.1 mg kg^{-1} ww (Table 4). Cu concentration was highest (107 mg kg^{-1} ww) in *F. virilis* from the Ozark Fisheries while the least average (3.66 mg kg^{-1} ww) was achieved in *F. virilis* from the Busby site. To our knowledge, algaecides were not applied to the Busby pond.

Regarding Cu, approximately 39% (*F. virilis*) and 3% (*P. a. acutus*) samples were in exceedance of the FAO 30 mg kg^{-1} Cu limit for fish (FAO, 1983). Likewise, the MAFF legal limit (20 mg kg^{-1}; MAFF, 1998) was surpassed in 61% of *F. virilis* and 10% of *P. a. acutus* samples from the Ozark Fisheries. The average Cu level (Table 7) for *F. virilis* (13.3 mg kg^{-1} ww) and *P. a. acutus* (11.2 mg kg^{-1} ww) were consistently lower than those (μg g^{-1} dry weight (dw)) in *P. clarkii* abdominal muscle (industrial site: 187 ± 75, Goretti et al., 2016), and in crayfish from a reference site (47.0 mg kg^{-1}; Rowe et al., 2001) but higher than the concentration in *P. clarkii* muscle (44.6 μg g^{-1} dw; Madden et al., 1991 cited by Anandkumar et al., 2020). All the same, the average levels in this study were comparable to the mean concentration (12.3 mg kg^{-1} ww) in cultured *P. clarkia* (Xiong et al., 2020). Cu toxicity includes severe intravascular hemolysis and proteinuria (WHO, 1996). Also, Cu exposure may catalyze the production of hydroxyl radical ($OH^{\cdot}$), which reacts with macromolecules to cause enzyme inactivation, DNA damage, and lipid peroxidation (Wei and Yang, 2016).

Crustaceans accumulate Cu when the uptake rate outweighs the release rate, but toxic threshold bioavailability varies between organisms and metals (Rainbow et al., 2007; Rainbow and Luoma, 2011). Previous work found that *Palaemonetes varians* fed with diets of polychaetes *Nereis diversicolor* accumulated increased Cu concentration (but not Zn) which caused the induction of metallothionein-like (MTLP) proteins in the hepatopancreas of the decapod crustacean. Nonetheless, Cu in the non-detoxified subcellular component also increased over time in the hepatopancreas with potentially sublethal toxic effects (Rainbow and Smith, 2013). Generally, metal accumulation in crustaceans is highest in the hepatopancreas than in the muscle tissue.

3.2.7. Iron

Iron, a transition metal, is ubiquitous in the environment (EGV, 2003). Iron has an essential role as a constituent of cytochrome, catalase, and oxygen-transporting proteins and important in living organisms. Fe was detectable in all samples. The highest Fe was achieved in *F. virilis* (23.0 mg kg^{-1} ww; Missouri Goldfish Hatchery) and *P. a. acutus* (19.5 mg kg^{-1} ww; Ozark Fisheries). High cellular Fe concentrations can lead to oxidative cellular damage (Vuori, 1995). In humans, acute Fe poisoning is associated with severe gastrointestinal damage including hemorrhagic gastroenteritis. The mean Fe in cultured *P. clarkia* muscle (78.5 mg kg^{-1} ww) (Xiong et al., 2020) was higher than the concentrations reported in the present study (Table 7).

3.2.8. Manganese

Manganese occurs naturally and from contamination of soils, sediments, and water (EGV, 2003). Mn is a constituent of several enzymes such as hydrolases, kinases, decarboxylases, and transferases. Deficiencies include impaired growth, skeletal abnormalities, depressed reproductive function, and defects in lipid and carbohydrate metabolism (WHO, 1996). Mn was detected in all samples with concentrations in the range from 0.20 to 8.54 mg kg^{-1} ww (Table 7). The highest average (1.82 mg kg^{-1} ww; Table 4) was attained in *F. virilis* (Busby farm). The level found in cultured *P. clarkia* muscle (6.21 mg kg^{-1} ww; Xiong et al., 2020) was higher than our average values for the species. In contrast, our average levels in both species were lower than the levels (16 mg kg^{-1} dw) in *P. clarkii* muscle (Anandkumar et al., 2020).

3.2.9. Molybdenum

Molybdenum is found extensively in nature and plays an important function as a micronutrient in plants and animals, including humans. Mo is involved in several enzyme processes. Nonetheless, Mo status influences susceptibility to certain types of cancer (WHO, 1996). Mo was undetectable in 22% of samples, and the concentration was in the range from <LOD to 0.62 mg kg^{-1} ww, with average values comparable across the sites.

3.2.10. Nickel

Nickel occurs with other elements in the earth's crust and other sources include mining, coal-burning power plants, waste incinerators, and others (ATSDR, 2021). Ni influences Fe absorption and metabolism and probably significant in the hemopoietic process in humans (EGV, 2003). Ni was undetectable in 15% of all samples and the levels ranged from <LOD to 2.65 mg kg^{-1} ww. The highest average Ni concentration was observed in *P. a. acutus* from Ozark Fisheries. Ni and the compounds are human carcinogens causing cancers of the lung, nasal cavity, and paranasal sinuses after inhalation (EFSA, 2015). Our average values of Ni recorded in *F. virilis* samples (Ozark Fisheries and Busby farm; Table 4) were comparable to those reported for wild and farmed crayfish (Zhou et al., 2021) but higher in *P. a. acutus* from Ozark Fisheries. Average Ni levels (this study) were below those in crayfish tail muscle (Hothem et al., 2007). Crustaceans may be sensitive to nickel exposure (Gissi et al., 2016). In a study, the most sensitive taxa were crustaceans, snail, anemone, and polychaete based on acute endpoints. The LC50 (50% lethality) values ranged from 7 – 150 μg Ni L^{-1}, following acute 24–96-h exposures (Lussier et al., 1999; and Asadpour et al., 2013 cited by Gissi et al., 2016).

3.2.11. Silver

Silver exists with other elements and often a by-product during the retrieval of Cu, Pb, Zn, and Au ores. Moreover, Ag is used in making jewelry, equipment, and dental fillings (ATSDR, 2021). Silver concentrations in muscle across the collection sites varied from <LOD to 110 μg kg^{-1} ww (Table 4). Anyhow, Ag was undetectable in *P. a. acutus* samples from Missouri Goldfish Hatchery and Busby farm except for one sample from Ozark Fisheries. In *F. virilis,* Ag was detectable in one and two samples from Missouri Goldfish Hatchery and Ozark Fisheries, respectively. Ag is directly involved in the transcription of metallothioneins instead of displacing Zn from pre-existing Zn-metallothioneins (Plessl et al., 2019). Human exposures to Ag at certain levels can create a con-

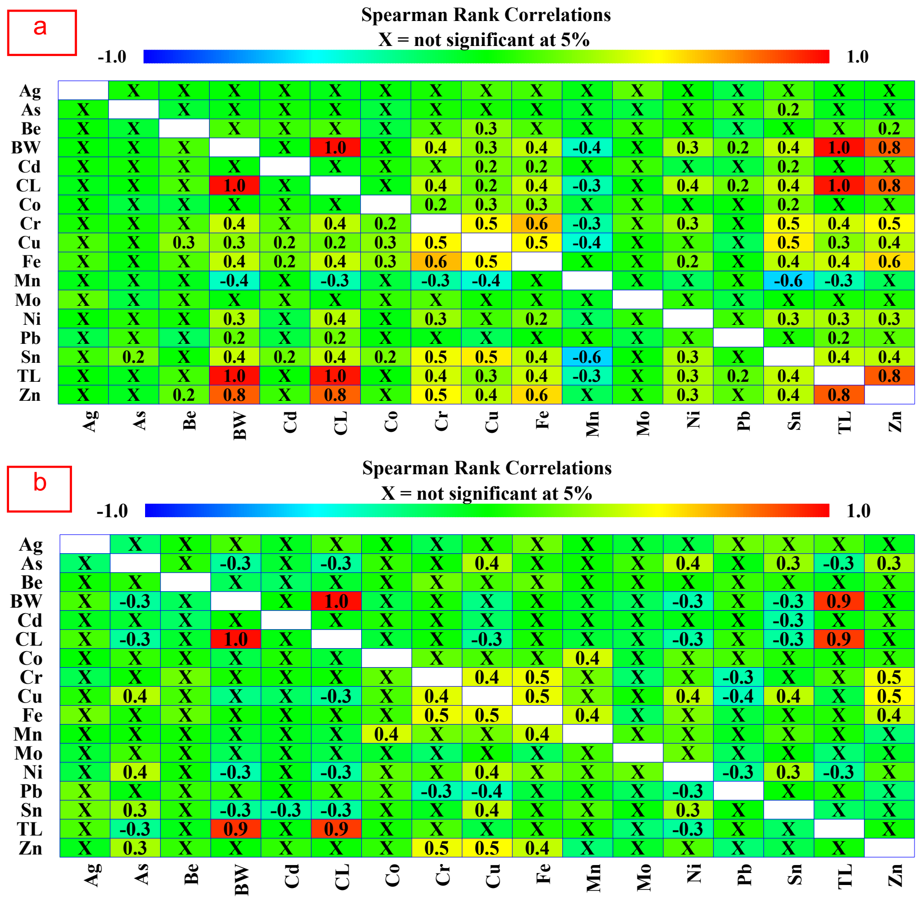

Figure 4. Spearman's rank coefficients (r) for correlations among the growth factors and metals/metalloid concentrations in a) *F. virilis*, and b) *P. a. acutus* species. The values shown are statistically significant at P < 0.05.

dition known as argyria (ATSDR, 2021).

3.2.12. Tin

Tin is present in brass and soldering materials. Besides, Sn is used in the lining of cans for food, beverages, and aerosols (ATSDR, 2021). Tin was undetectable in one *F. virilis* sample from Busby farm. The highest mean (3.45 mg kg^{-1} ww) was recorded in *F. virilis* (Ozark Fisheries). Sn probably may contribute to macromolecular structure and function at the active site of metalloenzymes (EGV, 2003). Organotin is particularly toxic and attacks the central nervous system leading to ataxia (WHO, 1996).

3.2.13. Lead

Lead is ubiquitous in the environment and is characterized as a neurotoxin and a possible carcinogen (Wallace and Djordjevic, 2020). The sources of Pb include the burning of fossil fuels, mining, and manufacturing (ATSDR, 2021). Pb was undetectable in 7.5% of all samples and found concentrations in muscle ranged from <LOD to 1.73 mg kg^{-1} ww (Table 4). The highest average Pb (0.99 mg kg^{-1} ww) was achieved in *P. a. acutus* (Missouri Goldfish Hatchery). Chronic exposure to Pb can cause kidney damage, mental retardation, and high blood pressure. Moreover, it affects the nervous system of infants and children and causes IQ decrements (WHO, 1996). Crustaceans build up Pb and other contaminants from their environment. Anderson et al. (1997) reported that the accumulation of Pb in the abdominal muscle is time- and dose-dependent.

Our average Pb levels across the sites (Table 4) were higher than the concentration (0.042 μg g^{-1}) found in the shrimp *P. semisulcatus* muscle (Shalini et al., 2020). Howbeit, the mean Pb in cultured *P. clarkia* (1.33 mg kg^{-1} ww; Xiong et al., 2020) was higher than our average Pb (Table 7). A low average Pb (<0.04 mg kg^{-1} ww) was found in *P. longirostris* (Traina et al., 2019) and *A. leptodactylus* (Varol and Sünbül, 2017). The Pb limit (0.5 mg kg^{-1}) provided by the WHO/FAO and European Commission (WHO/FAO, 2015; Official Journal of the European Union, 2008) was exceeded by 56%, 28%, and 42% of samples from the Missouri Goldfish Hatchery, Ozark Fisheries, and Busby farm sites, respectively. Furthermore, Pb concentrations in 69% of all samples exceeded the Codex Alimentarius Commission (FAO, 1983) legal limit (0.3 mg kg^{-1}) for fish. The mean Pb levels in *F. virilis* (0.48 mg kg^{-1} ww), and *P. a. acutus* (0.80 mg kg^{-1} ww) were higher than the concentration (0.03 mg kg^{-1}) found in *S. mantis* but lower in *P. clarkia* (Goretti et al., 2016).

3.2.14. Zinc

Zinc is ubiquitous, being present in soils, plants, and biota. Sources of Zn include disposal of fertilizers, coal combustion, and waste incineration, and so on (Desaulty and Petelet-Giraud, 2020). Zinc plays an essential role in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids (WHO, 1996). Previous research highlighted the influence of metallothioneins in Cu and Zn uptake and detoxification (Amiard et al., 2006) and animal tolerance may play a role in metal uptake (Rainbow et al., 2000). According to Gunderson et al. (2021), glutathione S-transferase activity did not change with Zn exposure, which implied less sensitivity of crayfish to Zn than Hg. A previous study highlighted that the detoxification of hepatopancreas Zn involved both MTLP and metal-rich granules (Rainbow and Smith, 2013). Zn was detectable in all samples analyzed. In *F. virilis*, the highest Zn level (19.2 mg kg^{-1} ww; Table 4) was recorded in a sample from Missouri Goldfish Hatchery. In *P. a. acutus*, the highest Zn concentration (16.0 mg kg^{-1} ww) was found in a sample from Ozark Fisheries. Symptoms of excess Zn

Table 8. Estimated daily (EDI; μg per day) and weekly (EWI; μg kg^{-1} body weight) intakes of metals/metalloid through the consumption of crayfish muscle.

Element	PTWI[a]	RfDo[b]	Missouri Goldfish Hatchery (*F. virilis*; n = 54)		Missouri Goldfish Hatchery (*P. acutus acutus*; n = 18)		Ozark Fisheries (*P. acutus acutus*; n = 43)		Ozark Fisheries (*F. virilis*; n = 36)		Busby Farm (*F. virilis*; n = 24)	
			EDI**	EWI	EDI**	EWI	EDI**	EWI	EDI**	EWI	EDI**	EWI
Ag		5	0.01	0	0	0	0.01	0	0.03	0	0	0
As	15[d,e]	0.3	4.55	0.45	2.6	0.26	4.36	0.44	6.04	0.6	5.32	0.53
Be		2	0.18	0.02	0.17	0.02	0.42	0.04	0.27	0.03	0.04	0
Cd	25[f] (2.5)[k]	1	0.15	0.01	0.11	0.01	0.07	0.01	0.21	0.02	0.1	0.01
Co		0.3	0.54	0.05	0.22	0.02	0.56	0.06	0.62	0.06	0.49	0.05
Cr	300[g]	1500	0.87	0.09	0.49	0.05	1.11	0.11	1.12	0.11	0.04	0
Cu III*	3500[h]	40	44	4.4	30.5	3.05	65.7	6.57	134	13.4	11.1	1.11
Fe	5600[h]	700	29.1	2.91	12.9	1.29	21	2.1	23.6	2.36	6.82	0.68
Mn	980	140	6.4	0.64	2.11	0.21	3.48	0.35	2.07	0.21	8.91	0.89
Mo		5	0.75	0.07	0.59	0.06	0.64	0.06	0.76	0.08	0.86	0.09
Ni	35[h] (2.8)[i]	20	1.52	0.15	4.83	0.48	3.43	0.34	1.04	0.1	0.57	0.06
Pb	25[j]	3.571[c]	2.9	0.29	11.6	1.16	3.48	0.35	1.72	0.17	1.95	0.2
Sn	14000[d]	600	13.4	1.33	51.8	5.18	15	1.5	16.9	1.69	3.21	0.32
Zn	7000[d]	300	60.8	6.08	1.19	0.12	55.8	5.58	49	4.9	9.94	0.99

* Only Cu (III) was considered in this study.

** EDI (μg per day).

[a] PTWI is the provisional tolerable weekly intake (μg kg^{-1} body weight per week).

[b] RfD_o is the United States Environmental Protection Agency oral reference dose (μg kg^{-1} per day); US EPA, 2019a.

[c] Estimated from PTWI value.

[d] JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1989.

[e] Arsenic (Inorganic).

[f] JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2011. Permissible tolerable monthly intake (PTMI) of Cd is 25 μg kg^{-1} body weight per month.

[g] Tolerable daily intake for Cr (III); EFSA: European Food Safety Authority (2014).

[h] WHO (World Health Organization), 1993.

[i] 2.8 μg Ni kg^{-1} body weight per day recommended; EFSA: European Food Safety Authority (2015).

[j] JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000.

[k] 2.5 μg Cd kg^{-1} body weight per week recommended; EFSA, 2009.

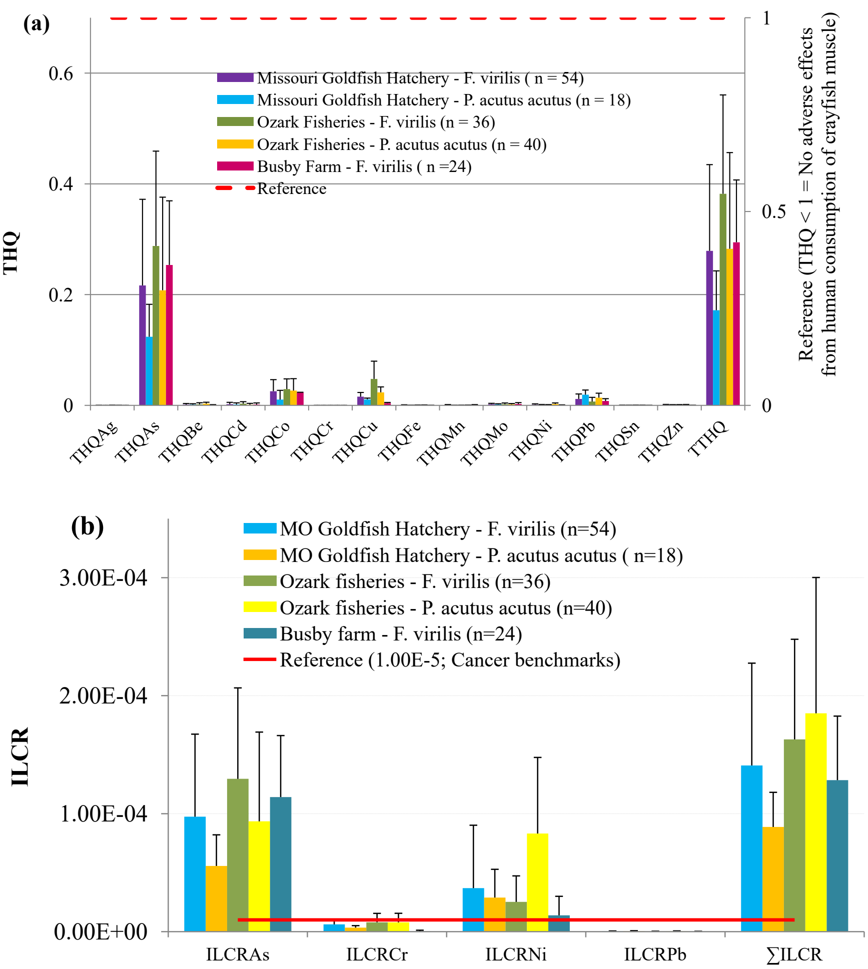

Figure 5. a) Target hazard quotient (THQ) for individual metals/metalloid and total target hazard quotient (TTHQ), and b) incremental lifetime cancer risk (ILCR) of exposure to As, Cr, Ni, and Pb via the consumption of crayfish muscle. The dotted red line is the non-cancer benchmark (1.0), the red line indicates the cancer risk benchmark (1.00 x 10^{-5}), and ∑ILCR is the sum of individual cancer risk of exposure to As, Cr, Ni, and Pb via the consumption of crayfish muscle.

exposure in crustaceans include gill histopathology, retardation of limb generation, decreased glycogen, etc. (Eisler, 1993 cited by Gunderson et al., 2021).

Among the species and collection sites, Zn concentrations were below the FAO (1983) recommended limit (30 mg kg^{-1}). Likewise, our average Zn levels in *F. virilis* (9.46 mg kg^{-1}) and *P. a. acutus* (11.1 mg kg^{-1} ww), were consistently below the mean concentrations reported for *P. clarkia* (98 mg kg^{-1}; Goretti et al., 2016), farmed *P. clarkii* (61.6 mg kg^{-1} dw; Gedik et al., 2017), cultured *P. clarkia* muscle (21.1 mg kg^{-1} ww), tail muscle (Hothem et al., 2007), and the 30-ppm legal limit for fish (FAO, 1983). However, our average Zn contents were higher than the mean level for *P. clarkii* muscle from the USA (Anandkumar et al., 2020).

Summarizing, the levels of metals/metalloid in crayfish muscle indicated their assimilation from food, habitat such as sediment/substrate-mud, and aquaculture practices (e.g., application of algaecides and/or insecticides, and feeds/fertilizers). Besides, metal accumulations in crayfish are also influenced by the water chemistry (dependent on the local geology and runoffs). The degree of metal accumulation in crustaceans includes the external concentration of the metal, the metal type and bioavailability, uptake/release rates, population tolerance, competition for metal absorption sites, environmental conditions (Rainbow and Luoma, 2011; Soedarini et al., 2012; Rainbow et al., 2000), the species (Mazzei et al., 2014), etc. Generally, trace elements such as Cr, Cu, Fe, Mn, Mo, Ni, and Zn play beneficial roles in humans (WHO, 1996) and organisms (Shalini et al., 2020). Notwithstanding, trace elements have potential adverse effects over certain thresholds (US EPA, 2020).

3.3. Non-parametric Kruskal-Wallis analysis

In *F. virilis*, significant differences ($p < 0.05$) were observed in the levels of Co, Cr, Cu, Fe, Mn, Ni, Pb, Pb, Sn, and Zn and the growth factors (TL, CL, and BW) across the collection sites. The highest TL and BW were attained in *P. a. acutus* (Missouri Goldfish Hatchery; Table 1). Fletcher et al. (2020) reported differences in Zn accumulation in the crayfish *Cambarus latimanus* across the collection sites. Concerning *F. virilis*, significant differences were observed between the concentrations of As ($p = 0.039$), Be ($p = 0.013$), and Zn ($p = 0.004$) across the sexes (males: n = 52; females: n = 62). Tunca et al. (2013) found substantial differences in metal/metalloid accretion between the sexes of freshwater crayfish *A. leptodactylus*.

For *P. a. acutus*, the concentrations of Co, Cr, Cu, Fe, Mn, Ni, Pb, and Sn in muscle differed ($p < 0.05$) across the sites (Ozark Fisheries and Missouri Goldfish Hatchery). Furthermore, the mean TL, CL, and BW values recorded in *P. a. acutus* from the Missouri Goldfish Hatchery were significantly ($p < 0.05$) higher than the corresponding average for samples from the Ozark Fisheries. Differences were observed in the concentrations of Be ($p = 0.0066$), and Cr ($p = 0.0099$) across the genders in *P. a. acutus*. In contrast, TL (test statistic = 3.48765; p =

0.0618247), CL (test statistic = 1.18677; p = 0.27598), and BW (test statistic = 0.106809; p = 0.743806) values were homogenous among the sexes.

Significant inter-species differences ($p < 0.05$) in the concentrations of Be, Ni, and Pb and the growth factor (CL, TL, and BW) values were observed in the species. The levels of Be, Ni, and Pb and the growth factor values were significantly ($p < 0.05$) higher in *P. a. acutus* than in *F. virilis*. Nevertheless, the levels of As, Cd, Co, Fe, and Mo were significantly ($p < 0.05$) higher in *F. virilis* than in *P. a. acutus*. These differences might be attributed to growth inhibition, uptake/release rates of metals/metalloids, detoxification, and environmental factors.

3.4. Spearman's rank correlation

Some of our variables were heteroscedastic and non-normally distributed and herein we used Spearman's rank correlation to compare the interrelationships among the variables. Figure 4a and b present the Spearman's rank correlation ($p < 0.05$) coefficients of the variables measured in *F. virilis* and *P. a. acutus*, respectively. Strong/moderate correlations indicated similar chemical characteristics or common origins of elements in crayfish. The growth factors, CL, and BW in both species (r^2: 1.0; $p < 0.05$) correlated with maximum strength. Likewise, CL-TL also produced very strong associations in both species (r^2: 0.9–1.0; $p < 0.05$).

In *F. virilis*, BW moderately or strongly correlated ($p < 0.05$) with Zn (r^2: 0.8), Sn (r^2: 0.4), Fe (r^2: 0.4), and Cr (r^2: 0.4). Similarly, the association of CL with other variables largely followed the same trend (Figure 4a). Further analysis showed that Mn was significantly ($p < 0.05$) but negatively associated with BW (r^2: -0.4), and with TL and CL (r^2: -0.3). Cu was moderately (r^2: 0.4 - 0.5) correlated with Fe, Zn, and Cr but produced a negative association with Mn (r^2: -0.4). Besides, Pb levels in muscle significantly associated (r^2: 0.2; $p < 0.05$) with TL, CL, and BW but insignificantly ($p > 0.05$) correlated with the elements (Figure 4a).

In *P. a. acutus*, CL was significantly ($p < 0.05$) but negatively correlated (r^2: - 0.3) with As, Cu, Ni, and Sn (Figure 4b). Similarly, BW and TL were negatively associated (r^2: - 0.3; $p < 0.05$) with As, Ni, and Sn and with As and Ni, respectively. This indicates that these elements were not utilized during growth. Cr showed moderate interrelationships with Cu, Fe, and Zn (r^2: 0.4–0.5, $p < 0.05$). Likewise, arsenic (As) significantly ($p < 0.05$) correlated (r^2: 0.3–0.4) with Cu, Ni, Sn, and Zn but negatively associated with BW, CL, and TL (r^2: -0.3). According to Tunca et al. (2013), arsenic can be converted to nontoxic organic forms and thus, can be further detoxified in any part of the animal body.

In the case of *P. a. acutus*, Pb showed negative relationships ($p < 0.05$) with Cr (- 0.3), Cu (- 0.4), and Ni (- 0.3) but showed no significant associations ($p > 0.05$) with the growth factors and the elements (Ag, As, Be, Cd, Co, Fe, Mn, Mo, Sn, and Zn) (Figure 4b). The poor correlations between the growth factors and some elements could be attributed to growth inhibition, lack of utilization of the elements, and detoxification. Crayfishes accumulate metals and metalloids from water, sediment, and diet. Cultured crayfish feed at all levels of the food chain (McClain et al., 2007) including feed wastes from aquaculture.

3.5. Daily/weekly intake of metal(loid)s and health risks via consumption of crayfish muscle

Table 8 summarizes the daily intake (μg day^{-1}) and weekly intake (μg kg^{-1} body weight per week) of metal(loid)s through the consumption of crayfish muscle. For all elements, the EDIs (this study) were below the safe doses (RfD_o, JECFA, and other limits; Table 8). This indicates that the consumption of muscle by the adult population posed no health risks. Moreover, the EWI values (this study) were also below the established PTWI and other regulatory values. In comparison to published data, our

Table 9. Target hazard quotients (THQs) for individual metals/metalloid and total target hazard quotients (TTHQs) for crayfish species and in parenthesis are the lifetime cancer risk (ILCR) of exposure to As, Cr, Ni, and Pb via the consumption of crayfish muscle. The cancer risk was evaluated using the benchmark (1.00E-05), and ∑ILCR is the sum of the individual cancer risk of exposure to As, Cr, Ni, and Pb.

Element	Missouri Goldfish Hatchery (*F. virilis*, n = 54)	Missouri Goldfish Hatchery (*P. acutus acutus*, n = 18)	Ozark Fisheries (*P. acutus acutus*, n = 40)	Ozark Fisheries (*F. virilis*, n = 36)	Busby Farm (*F. virilis*, n = 24)	Regulatory value
	THQ[a]	THQ[a]	THQ[a]	THQ[a]	THQ[a]	
Ag	2.7E-05	0.0E+00	3.4E-05	8.0E-05	0.0E+00	1
As	2.2E-01	1.2E-01	2.1E-01	2.9E-01	2.5E-01	1
As	(9.74E-05)	(5.57E-05)	(9.35E-05)	(1.30E-04)	(1.14E-04)	
Be	1.3E-03	1.2E-03	3.0E-03	1.9E-03	2.9E-04	1
Cd	2.1E-03	1.5E-03	1.0E-03	3.0E-03	1.5E-03	1
Co	2.5E-02	1.0E-02	2.7E-02	2.9E-02	2.3E-02	1
Cr	8.2E-06	4.6E-06	1.1E-05	1.1E-05	3.8E-07	1
Cr	(6.19E-06)	(3.47E-06)	(7.92E-06)	(8.00E-06)	(2.87E-07)	
Cu III	1.6E-02	1.1E-02	2.3E-02	4.8E-02	4.0E-03	1
Fe	5.9E-04	2.6E-04	4.3E-04	4.8E-04	1.4E-04	1
Mn	6.5E-04	2.2E-04	3.6E-04	2.1E-04	9.1E-04	1
Mo	2.1E-03	1.7E-03	1.8E-03	2.2E-03	2.4E-03	1
Ni	1.1E-03	3.4E-03	2.4E-03	7.4E-04	4.1E-04	1
Ni	(3.69E-05)	(2.89E-05)	(8.32E-05)	(2.52E-05)	(1.38E-05)	
Pb	1.2E-02	4.6E-02	1.4E-02	6.8E-03	7.7E-03	1
Pb	(3.52E-07)	(5.86E-07)	(4.23E-07)	(2.09E-07)	(2.37E-07)	
Sn	3.2E-04	1.2E-03	3.6E-04	4.0E-04	7.6E-05	1
Zn	2.9E-03	5.7E-05	2.7E-03	2.3E-03	4.7E-04	1
TTHQ	2.8E-01	2.0E-01	2.8E-01	3.8E-01	2.9E-01	1
∑ILCR[b]	1.41E-04	8.87E-05	1.85E-04	1.63E-04	1.28E-04	

[a] THQ <1 implies no harm from consumption of crayfish; TTHQ = sum of individual THQ values; ILCR (incremental lifetime cancer risk) values for As, Cr, Ni, and Pb are in parenthesis.
[b] ∑ILCR is the sum of the individual cancer risks for As, Cr, Ni, and Pb.

EDI values were close to the corresponding EDIs of Cu, Zn, Mn, Cr, Cd, Pb, and As for *P. clarkii* abdominal muscle (Anandkumar et al., 2020), and those of Cd, Cr, Cu, Ni, Pb and Zn in crustaceans (Liu et al., 2019).

3.6. Non-carcinogenic risk from crayfish muscle consumption

The potential human health risks associated with the consumption of crayfish muscle were evaluated using found elemental concentrations and the RfD_o values. Figure 5a presents the health risk indices (THQ and TTHQ) for metals/metalloid through muscle consumption in the adult population. Among the species, the THQs (range: 0.00–0.29; all samples) values of the elements were within the acceptable limit (i.e., THQ <1). This implied no adverse effects to adult consumers from non-cancer risk and potentially the benefits of consumption of muscle outweighed the risks. In comparison, the THQ_{As} values (range: 0.12–0.29) for As were at least two-fold higher than those of other metals (Figure 5a and Table 9). Similarly, Peng et al. (2016) observed the high contribution of As (THQ/TTHQ = 0.70; P90) relative to other elements in *P. clarkii*. Our THQ_{As} value was lower than the estimated non-cancer risk for As obtained for the crustacean *S. mantis* (THQ_{As}: 0 .77; Bonsignore et al., 2018).

Our estimation of non-cancer risk also revealed that the TTHQs (range: 0.17–0.38) for metals/metalloid through muscle consumption did not exceed the acceptable safe limit (TTHQ <1). Consequently, the crayfish muscle presented an insignificant chance of non-cancer risk to adult consumers. Though the TTHQ of the elements was below 1, As contributions across the sites were in the range of 72%–86%. The TTHQ (in parenthesis; Table 9) across the species and collection sites were as follows: *F. virilis*: Missouri Goldfish Hatchery (0.28); *F. virilis*: Ozark Fisheries (0.38); *P. a. acutus*: Ozark Fisheries (0.28); *P. a. acutus*: Missouri Goldfish Hatchery (0.17); and *F. virilis:* Busby farm (0.29). THQ values (this study), being below 1, followed the same trend as those of Cu, Pb, Zn, Cd, As, and Cr exposure from cultured *P. clarkia* (Xiong et al., 2020). Further, insignificant non-cancer health risk due to As exposure in the adult population was observed in crayfish (*Pacifastacus leniusculus; P. clarkii*) from three lakes in the Puget Sound lowland region (WA, USA; Hull et al., 2021).

3.7. Carcinogenic risk from crayfish muscle consumption

The ILCR values of exposure to As, Cr, Ni, and Pb through the consumption of crayfish muscle and the cumulative cancer risk (ΣILCR) are presented in Figure 5b and Table 9. Irrespective of the species and collection sites, the cancer risk from metal exposure followed the order: As > Ni > Cr > Pb. Based on the 10^{-5} threshold, the cancer risk exceedances of As in all samples analyzed ranged from 98% to 100%. Regarding the Ozark Fisheries site, 25% and 23% As exceedances were observed in *F. virilis* and *P. a. acutus*, respectively. The exceedance of Cr was 19%, 0%, and 0% in *F. virilis* (Missouri Goldfish Hatchery), *P. a. acutus* (Missouri Goldfish Hatchery), and *F. virilis* (Busby farm), respectively. In the case of Ni, the percentage of all samples that surpassed the cancer risk benchmark ranged from 19% to 98% across the collection sites and species. Regarding Ni, 83% of *F. virilis* (Missouri Goldfish Hatchery), and 98% of *P. a. acutus* samples (Ozark Fisheries) were above the recommended cancer risk benchmark. Nonetheless, the cancer risk was insignificant for Pb in all samples (0% of samples were in exceedance). Across the collection sites, the average ILCR for As, and Ni exceeded the benchmark (Figure 5b and Table 9), which indicated potential cancer risk to consumers. Hull et al. (2021) reported cancer risk from exposure to As in crayfish (*Pacifastacus leniusculus; P. clarkii*) from Lake Killarney (WA, USA).

Concerning cancer risk, there was more safety concern since the cumulative risk (∑ILCR) values for the four elements were above the risk threshold (Figure 5b and Table 9). This indicated a potential hazard from muscle consumption by the adult population. In comparison with published data, the ILCR benchmarks applied were exceeded in the case of Cd in *S. mantis* (Bonsignore et al., 2018) and As in *P. clarkii* (Peng et al., 2016) while Pb and As in both cultured and wild crayfish indicated no risk of carcinogenesis (Xiong et al., 2020).

4. Conclusion and recommendation

The present study provided baseline data on two North American native crayfish species from aquaculture, gave an insight into the quality of seafood, and assessed the potential human health risks. Overall, the results showed that the crayfish species accumulated metal(loid)s in muscle with Cu the highest followed by Fe and Zn. Significant trends in inter-species and gender differences were observed among the metal(loid)s and the growth factors. Also, the levels of metals in muscle differed across the collection sites. Significant correlations were observed among the metals, which indicated similar chemical attributes and/or origins. Regarding human health, the average concentrations of As, Cd, and Zn in this study were below the legal limits while Pb, As, and Cu levels, in some samples, exceeded the MALs. Generally, the EDI of metals/metalloid were below the PTWI values, which implied no public safety concern.

Considering the non-carcinogenic risk, the results of the assessment, being less than 1.0 (safe limit), indicated no adverse effects. Nevertheless, the incremental cancer risk assessment results revealed As and Ni carcinogenesis. Thus, moderate consumption of crayfish by the public is advised to reduce dietary exposures. The continuing regulation of the aquaculture industry, improvement of the production processes, and the use of quality water/feeds can enhance the quality of seafood available to consumers. Globally, we recommend more studies that evaluate metal toxicity in cultured crayfish to safeguard public health.

Declarations

Author contribution statement

Abua Ikem, Olukayode James Ayodeji, James Wetzel: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the United States Department of Agriculture - National Institute of Food and Agriculture (USDA-NIFA) [Grant award #: 2015-38821-24386].

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors are grateful to the Missouri Goldfish Hatchery, Ozark Fisheries, and Lincoln University staff at Busby farm for aiding sample collection. Much gratitude to Jimmie Garth, Chanelle Quinn, Tyler Edwards, Mikayla Morris, and other students not mentioned for their assistance during sample collection and laboratory work. The authors express appreciation to Austen Dudenhoeffer and Dr. Samson Tesfaye (Center of Excellence in Geospatial Information Science, Lincoln

University, MO) for assisting the map preparation. The authors acknowledge the United States Geological Survey (USGS; "National Hydrology Dataset" (NHD), 2017) and ESRI ("States": Shapefile for the United States of America) for the datasets applied during the preparation of our sampling map. We thank the anonymous reviewers for their valuable comments and suggestions.

References

Alipour, M., Sarafraz, M., Chavoshi, H., Bay, A., Nematollahi, A., Sadani, M., Fakhri, Y., Vasseghian, Y., Khaneghah, A.M., 2021. The concentration and probabilistic risk assessment of potentially toxic elements in fillets of silver pomfret (*Pampus argenteus*): a global systematic review and meta-analysis. J. Environ. Sci. 100, 167–180.

Allert, A.L., Distefano, R.J., Fairchild, J.F., Schmitt, C.J., Mckee, M.J., et al., 2013. Effects of historical lead-zinc mining on riffle-dwelling benthic fish and crayfish in the Big River of southeastern Missouri, USA. Ecotoxicology 22, 506–521.

Amiard, J.-C., Amiard-Triquet, C., Barka, S., Pellerin, J., Rainbow, P.S., 2006. Metallothioneins in aquatic invertebrates: their role in metal detoxification and their use as biomarkers. Aquat. Toxicol. 76, 160–202.

Anandkumar, A., Li, J., Prabakaran, K., Zhang, X.J., Leng, Z., Nagarajan, R., Du, D., 2020. Accumulation of toxic elements in an invasive crayfish species (*Procambarus clarkii*) and its health risk assessment to humans. J. Food Compos. Anal. 88, 103449.

Anderson, M.B., Preslan, J.E., Jolibois, L., Bollinger, J.E., George, W.J., 1997. Bioaccumulation of lead nitrate in Red Swamp crayfish (*Procambarus clarkii*). J. Hazard Mater. 54, 15–29.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, twentieth ed. APHA, AWWA and WEF, Washington DC.

ATSDR (Agency for Toxic Substances and Disease Registry), 2019. CERCLA Priority List of Hazardous Substances. Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, 1600 Clifton Road NE, Atlanta, GA 30333, USA. Available online: https://www.atsdr.cdc.gov/spl/#2019spl. (Accessed 14 April 2020).

ATSDR (Agency for Toxic Substances and Disease Registry), 2021. Toxicological Profiles, Agency for Toxic Substances and Disease Registry, Division of Toxicology and Environmental Medicine, 1600 Clifton Road NE, Atlanta, GA 30333, USA. Available online: https://www.atsdr.cdc.gov/toxprofiledocs/index.html. (Accessed 25 February 2021).

Bollinger, J.E., Bundy, K., Anderson, M.B., Millet, L., Preslan, J.E., Jolibois, L., Chen, H., et al., 1997. Bioaccumulation of chromium in red swamp crayfish (*Procambarus clarkii*). J. Hazard Mater. 54, 1–13.

Bonsignore, M., Manta, D.S., Mirto, S., Quinci, E.M., Ape, F., Montalto, V., Gristina, M., Traina, A., Sprovieri, M., 2018. Bioaccumulation of heavy metals in fish, crustaceans, molluscs and echinoderms from the Tuscany coast. Ecotoxicol. Environ. Saf. 162, 554–562.

Cauduro, J., Ryan, A., 2017. Ultra-fast ICP-OES Determination of Trace Elements in Water, as Per US EPA 200.7. Application Note. Agilent Technologies Melbourne, Australia.

Crandall, K.A., Buhay, J.E., 2008. Global diversity of crayfish (*Astacidae, Cambaridae*, and *Parastacidae*—Decapoda) in freshwater. Hydrobiologia 595, 295–301.

Desaulty, A., Petelet-Giraud, E., 2020. Zinc isotope composition as a tool for tracing sources and fate of metal contaminants in rivers. Sci. Total Environ. 728, 138599.

Dippong, T., Hoaghia, M., Mihali, C., Cical, E., Calugaru, M., 2020. Human health risk assessment of some bottled waters from Romania. Environ. Int. 267, 115409.

Dippong, T., Mihali, C., Hoaghia, M., Cical, E., Cosma, A., 2019. Chemical modeling of groundwater quality in the aquifer of Seini town – someș Plain, Northwestern Romania. Ecotoxicol. Environ. Saf. 168, 88–101.

Dippong, T., Mihali, C., Goga, F., Cical, E., 2017. Seasonal evolution and depth variability of heavy metal concentrations in the water of Firiza-Strîmtori Lake, NW of Romania. Studia UBB Chemia LXII (1), 213–228.

DiStefano, R.J., Litvan, M.E., Meyer, A.W., Taylor, C.A., 2008. Identifying Crayfish: a Guide for Bait Vendors and Aquaculturists. Missouri Department of Conservation, Jefferson City. Available online: https://huntfish.mdc.mo.gov/sites/default/files/downloads/crayfish_id_brochure_6-08_0.pdf. (Accessed 24 January 2021).

EFSA (European Food Safety Authority), 2009. Cadmium in food. Scientific opinion of the panel on contaminants in the food chain. (Question No EFSA-Q-2007-138). EFSA J. 980, 1–139. Available online: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3595. (Accessed 24 April 2020).

EFSA (European Food Safety Authority), 2014. Scientific Opinion on the risks to public health related to the presence of chromium in food and drinking water. EFSA J. 12 (3), 3595. Available online: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3595. (Accessed 24 April 2020).

EFSA (European Food Safety Authority), 2015. Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water. EFSA Panel on Contaminants in the Food Chain (CONTAM). EFSA J. 13 (2), 4002. Available online: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2015.4002. (Accessed 24 April 2020).

EGV (Expert Group on Vitamins and Minerals), 2003. Safe Upper Levels for Vitamins and Minerals. Food Standards Agency, 125 Kingsway, London WC2B 6NH, UK. Available online: https://cot.food.gov.uk/sites/default/files/vitmin2003.pdf. (Accessed 11 April 2020).

ESRI, 2012. USA State Boundaries, *ESRI "States" Shapefile for the United States of America*, USA. https://www.esri.com.

EURACHEM, 2014. Guide. In: Magnusson, B., Örnemark, U. (Eds.), The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, second ed. ISBN 978-91-87461-59-0.

Eversole, A.G., Turker, H., Kempton, C.J., 1999. Lipid content of *Procambarus acutus acutus* (Girard). Freshw. Crayfish 12, 194–204.

FAO (Food and Agriculture Organization), 1983. Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products. FAO Fishery Circular No. 464. Food and Agriculture Organization of the United Nations, Rome, pp. 5–10.

FAO/WHO (Food and Agriculture Organization/World Health Organization), 2005. Dietary exposure assessment of chemicals in food. In: Report of an FAO/WHO Consultation, Annapolis, Maryland, USA, 2–6 May 2005. Available online: https://apps.who.int/iris/bitstream/handle/10665/44027/9789241597470_eng.pdf?sequence=1&isAllowed=y. (Accessed 25 March 2020).

FAO (Food and Agriculture Organization), 2020. The State of World Fisheries and Aquaculture 2020 – Sustainability in Action. FAO, Rome. http://www.fao.org/3/ca9229en/CA9229EN.pdf. (Accessed 25 February 2021).

Fletcher, D.E., Lindell, A.H., Stankus, P.T., Fletcher, N.D., Lindell, B.E., McArthur, J.V., 2020. Metal accumulation in dragonfly nymphs and crayfish as indicators of constructed wetland effectiveness. Environ. Pollut. 256, 113387.

FSANZ (Food Standards Australia and New Zealand), 2013. Australia New Zealand Food Standards Code, Standard 1.4.1, Contaminants and Natural Toxicants.

Gedik, K., Kongchum, M., DeLaune, R.D., Sonnier, J.J., 2017. Distribution of arsenic and other metals in crayfish tissues (*Procambarus clarkii*) under different production practices. Sci. Total Environ. 574, 322–331.

Gissi, F., Stauber, J.L., Binet, M.T., Golding, L.A., Adams, M.S., Schlekat, C.E., Garman, E.R., Jolley, D.F., 2016. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. Environ. Pollut. 218, 1308–1323.

Goretti, E., Pallottini, M., Ricciarini, M.I., Selvaggi, R., Cappelletti, D., 2016. Heavy metals bioaccumulation in selected tissues of red swamp crayfish: an easy tool for monitoring environmental contamination levels. Sci. Total Environ. 559, 339–346.

Green, S.R., Storey, K.B., 2016. Regulation of crayfish, *Orconectes virilis*, tail muscle lactate dehydrogenase (LDH) in response to anoxic conditions is associated with alterations in phosphorylation patterns. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 202, 67–74.

Gunderson, M.P., Boyd, H.M., Kelly, C.I., Lete, I.R., McLaughlin, Q.M., 2021. Modulation of endogenous antioxidants by zinc and copper in signal crayfish (*Pacifastacus leniusculus*). Chemosphere 275, 129982.

Heidari, M., Darijani, T., Alipour, V., 2021. Heavy metal pollution of road dust in a city and its highly polluted suburb; quantitative source apportionment and source-specific ecological and health risk assessment. Chemosphere 273, 129656.

Hothem, R.L., Bergen, D.R., Bauer, M.L., Crayon, J.J., Meckstroth, A.M., 2007. Mercury and trace elements in crayfish from Northern California. Bull. Environ. Contam. Toxicol. 79, 628–632.

Hull, E.A., Baraja, M., Burkart, K.A., Fung, S.R., Jackson, B.P., Barrett, P.M., Neumann, R.B., Olden, J.D., Gawel, J.E., 2021. Human health risk from consumption of aquatic species in arsenic-contaminated shallow urban lakes. Sci. Total Environ. 770, 145318.

Huner, J., 2019. Freshwater Crayfish Aquaculture in North America, Europe, and Australia: *Families Astacidae, Cambaridae, and Parastacidae*, first ed. Products Press, New York, NY. Available online: https://bookshelf.vitalsource.com/#/books/9781351991506/. (Accessed 24 November 2019). from VitalSource Bookshelf.

Ikem, A., Shank, B., Caldwell, J., Garth, J., Ahuja, S., 2015. Estimating the daily intake of essential and nonessential elements from lamb m. *longissimus thoracis et lumborum* consumed by the population in Missouri (United States). J. Food Compos. Anal. 40, 126–135.

ISO (International Organization for Standardization), 2017. General Requirements for the Competence of Calibration and Testing Laboratories. ISO/IEC 17025. ISO, Geneva, Switzerland.

ISO (International Organization for Standardization), 2019. Accuracy (Trueness and Precision) of Measurement Methods and Results – Part 2: Basic Method for the Determination of Repeatability and Reproducibility of a Standard Measurement Method. ISO 5725-2 (E). ISO, Geneva, Switzerland.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1989. Evaluation of Certain Food Additives and Contaminants. Thirty-Third Report of the Joint FAO/WHO Expert Committees on Food Additives. World Health Organization Technical Report Series 776.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Safety evaluation of certain food additives and contaminants. WHO Food Addit. Ser. 44, 273–312.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2011. Safety evaluation of certain food additives and contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Addit. Ser. 960, 1–237.

Lahman, S.E., Trent, K.R., Moore, P.A., 2015. Sublethal copper toxicity impairs chemical orientation in the crayfish, *Orconectes rusticus*. Ecotoxicol. Environ. Saf. 113, 369–377.

Larson, E.R., Olden, J.D., 2017. Field Sampling Techniques for Crayfish. In: Biology and Ecology of Crayfish. Taylor & Francis Group, pp. 287–324.

Li, J., Huang, Z., Hu, Y., Yang, H., 2013. Potential risk assessment of heavy metals by consuming shellfish collected from Xiamen, China. Environ. Sci. Pollut. Res. 20, 2937–2947.

Liu, Q., Xu, X., Zeng, J., Shi, X., Liao, Y., Du, P., Tang, Y., Huang, W., Chen, Q., Shou, L., 2019. Heavy metal concentrations in commercial marine organisms from Xiangshan Bay, China, and the potential health risks. Mar. Pollut. Bull. 141, 215–226.

Lo Turco, V., Potortì, A.G., Tropea, A., Dugo, G., Di Bella, G., 2020. Element analysis of dried figs (*Ficus carica L.*) from the Mediterranean areas. J. Food Compos. Anal. 90, 103503.

Lussier, S.M., Boothman, W.S., Poucher, S., Champlin, D., Helmstetten, A., 1999. Comparison of dissolved and total metals concentrations from acute tests with saltwater organisms. Environ. Toxicol. Chem. 18, 889e898.

MAFF, 1998. Ministry of Agriculture, Fisheries and Food. Monitoring and Surveillance of Non-radioactive Contaminants in the Aquatic Environment and Activities Regulating the Disposal of Wastes at Sea, 1995 and 1996. Aquatic Environment Monitoring Report No. 51. Center for Environment, Fisheries and Aquaculture Science, Lowestoft, UK.

Mancinelli, G., Papadia, P., Ludovisi, A., Migoni, D., Bardelli, R., Fanizzi, F.P., Vizzini, S., 2018. Beyond the mean: a comparison of trace- and macroelement correlation profiles of two lacustrine populations of the crayfish *Procambarus clarkia*. Sci. Total Environ. 62415, 1455–1466.

Mazlum, Y., Eversole, A.G., 2004. Observations on the life cycle of *Procambarus acutus* in South Carolina culture ponds. Aquacult 238, 249–261.

Mazzei, V., Longo, G., Brundo, M.V., Sinatra, F., Copat, C., Oliveri Conti, G., Ferrante, M., 2014. Bioaccumulation of cadmium and lead and its effects on hepatopancreas morphology in three terrestrial isopod crustacean species. Ecotoxicol. Environ. Saf. 110, 269–279.

McClain, W.R., Romain, R.P., Lutz, C.G., Shirley, M.G., 2007. Louisiana Crawfish Production Manual. Publication # 2637. Available at: https://articles.extension.org/sites/default/files/w/9/9c/Louisiana_Crawfish_Production_Manual.pdf. (Accessed 16 December 2018).

Meerpoel, C., Vidal, A., Andjelkovic, M., De Boevre, M., Tangni, E.K., Huybrechts, B., Devreese, M., Croubels, S., De Saeger, S., 2021. Dietary exposure assessment and risk characterization of citrinin and ochratoxin A in Belgium. Food Chem. Toxicol. 147, 111914.

Milenkovic, B., Stajic, J.M., Stojic, N., Pucarevic, M., Strbac, S., 2019. Evaluation of heavy metals and radionuclides in fish and seafood products. Chemosphere 229, 324–331.

Nain, P., Kumar, A., 2020. Ecological and human health risk assessment of metals leached from end-of-life solar photovoltaics. Environ. Pollut. 267, 115393.

Neris, J.B., Olivares, D.M.M., Santana, C.S., Emenike, P.C., Velasco, F.G., Andrade, S.F.R., Paranhos, C.M., 2021. HERisk: an improved spatio-temporal human health risks assessment software. Sci. Total Environ. 772, 145044.

Official Journal of the European Union, 2008. Commission Regulation (EC) No 629/2008 of 2 July 2008 Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs); L 173/176.

Official Journal of the European Union, 2011. Commission Regulation (EU) No 420/2011 of 29 April 2011 Amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs; L 111/3.

Peng, Q., Nunes, L.M., Greenfield, B.K., Dang, F., Zhong, H., 2016. Are Chinese consumers at risk due to exposure to metals in crayfish? A bioaccessibility-adjusted probabilistic risk assessment. Environ. Int. 88, 261–268.

Pflieger, W.L., 1996. The Crayfishes of Missouri, first ed. Missouri Department of Conservation, Jefferson City, Missouri.

Plessl, C., Gilbert, B.M., Sigmund, M.F., Theiner, S., Avenant-Oldewage, A., Keppler, B.K., Jirsa, F., 2019. Mercury, silver, selenium and other trace elements in three cyprinid fish species from the Vaal Dam, South Africa, including implications for fish consumers. Sci. Total Environ. 659, 1158–1167.

Rainbow, P.S., 1995. Physiology, physicochemistry and metal uptake– A crustacean perspective. Mar. Pollut. Bull. 31, 55–59.

Rainbow, P.S., Amiard-Triquet, C., Amiard, J.C., Smith, B.D., Langston, W.J., 2000. Observations on the interaction of zinc and cadmium uptake rates in crustaceans (amphipods and crabs) from coastal sites in UK and France differentially enriched with trace metals. Aquat. Toxicol. 50, 189–204.

Rainbow, P.S., Amiard, J.-C., Amiard-Triquet, C., Cheung, M.-S., Zhang, L., Zhong, H., Wang, W.-X., 2007. Trophic transfer of trace metals: subcellular compartmentalization in bivalve prey, assimilation by a gastropod predator and in vitro digestion simulations. Mar. Ecol. Prog. Ser. 348, 125–138.

Rainbow, P.S., Luoma, S.N., 2011. Metal toxicity, uptake and bioaccumulation in aquatic invertebrates - modelling zinc in crustaceans. Aquat. Toxicol. 105, 455–465.

Rainbow, P.S., Smith, B.D., 2013. Accumulation and detoxification of copper and zinc by the decapod crustacean *Palaemonetes varians* from diets of field-contaminated polychaetes *Nereis diversicolor*. J. Exp. Mar. Biol. Ecol. 449, 312–320.

Renu, K., Chakraborty, R., Myakala, H., Koti, R., Famurewa, A.C., Madhyastha, H., Vellingiri, B., George, A., Gopalakrishnan, A.V., 2021. Molecular mechanism of heavy metals (lead, chromium, arsenic, mercury, nickel and cadmium) - induced hepatotoxicity - a review. Chemosphere 271, 129735.

Rowe, C.L., Hopkins, W.A., Zehnder, C., Congdon, J.D., 2001. Metabolic costs incurred by crayfish (*Procambarus acutus*) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups. Comp. Biochem. Physiol., C 129, 275–283.

Royston, J.P., 1983. Some techniques for assessing multivariate normality based on the Shapiro-Wilk W". Appl. Stat. 32, 121–133.

Schmidt, L., Novo, D.L.R., Druzian, G.T., Landero, J.A., Caruso, J., Mesko, M.F., Flores, E.M.M., 2021. Influence of culinary treatment on the concentration and on the bioavailability of cadmium, chromium, copper, and lead in seafood. J. Trace Elem. Med. Biol. 65, 126717.

Shalini, R., Jeyasekaran, G., Shakila, R.J., Arisekar, U., HemaMalini, A., 2020. Concentrations of trace elements in the organs of commercially exploited crustaceans and cephalopods caught in the waters of Thoothukudi, South India. Mar. Pol. 154, 111045.

Sharafi, K., Yunesian, M., Nodehi, R.N., Mahvi, A.H., Pirsaheb, M., 2019a. A systematic literature review for some toxic metals in widely consumed rice types (domestic and imported) in Iran: human health risk assessment, uncertainty and sensitivity analysis. Ecotoxicol. Environ. Saf. 176, 64–75.

Sharafi, K., Yunesian, M., Nodehi, R.N., Mahvi, A.H., Pirsaheb, M., Nazmara, S., 2019b. The reduction of toxic metals of various rice types by different preparation and cooking processes - human health risk assessment in Tehran households, Iran. Food Chem. 280, 294–302.

Sioen, I., De Henauw, S., Van Camp, J., Volatier, J., Leblanc, J., 2009. Comparison of the nutritional–toxicological conflict related to seafood consumption in different regions worldwide. Regul. Toxicol. Pharmacol. 55, 219–228.

Skalicky, F., 2018. Critter of the Week: Virile Crayfish. Missouri Department of Conservation. Available at: https://www.columbiatribune.com/news/20180429/critter-of-week-virile-crayfish. (Accessed 22 March 2020). from Columbia Tribune URL.

Śmietana, N., Panicz, R., Sobczak, M., Nędzarek, A., Smietana, O., 2020. Variability of elements and nutritional value of spiny-cheek crayfish (*Faxonius limosus*, Rafinesque, 1817) Variability of elements and nutritional value of *F. limosus*. J. Food Compos. Anal. 94, 103656.

Soedarini, B., Klaver, L., Ressink, I., Widianarko, B., van Straalen, N.M., van Gestel, C.A.M., 2012. Copper kinetics and internal distribution in the marbled crayfish (*Procambarus sp.)*. Chemosphere 87 (4), 333–338.

Suami, R.B., Al Salah, D.M.M., Kabala, C.D., Otamonga, J.-P., Mulaji, C.J., Mpiana, P.T., Poté, J.W., 2019. Assessment of metal concentrations in oysters and shrimp from Atlantic coast of the democratic Republic of the Congo. Heliyon 5, e03049.

Suárez-Serrano, A., Alcaraz, C., Ibáñez, C., Trobajo, R., Barata, C., 2010. *Procambarus clarkii* as a bioindicator of heavy metal pollution sources in the lower Ebro River and Delta. Ecotoxicol. Environ. Saf. 73, 280–286.

Tavoloni, T., Stecconi, T., Galarini, R., Bacchiocchi, S., Dörr, A.J.M., Elia, A.C., Giannotti, M., Siracusa, M., Stramenga, A., Piersanti, A., 2021. BFRs (PBDEs and HBCDs) in freshwater species from Lake Trasimeno (Italy): the singular case of HBCDs in red swamp crayfish Tamara. Sci. Total Environ. 758, 143585.

Taylor, R.M., Watson, G.D., Alikhan, M.A., 1995. Comparative sub-lethal and lethal acute toxicity of copper to the freshwater crayfish, *Cambarus robustus* (Cambaridae, Decapoda, Crustacea) from an acidic metal-contaminated lake and a circumneutral uncontaminated stream. Water Res. 29, 401–408.

Traina, A., Bono, G., Bonsignore, M., Falco, F., Giuga, M., Quinci, E.M., Vitale, S., Sprovieri, M., 2019. Heavy metals concentrations in some commercially key species from Sicilian coasts (Mediterranean Sea): potential human health risk estimation. Ecotoxicol. Environ. Saf. 168, 466–478.

Tunca, E., Ucuncu, E., Ozkan, A.D., Ulger, Z.E., Tekinay, T., 2013. Tissue distribution and correlation profiles of heavy metal accumulation in the freshwater crayfish *Astacus leptodactylus*. Arch. Environ. Contam. Toxicol. 64, 676–691.

Ullah, I., Zhao, L., Hai, Y., Fahim, M., Alwayli, D., Wang, X., Li, H., 2021. Metal elements and pesticides as risk factors for Parkinson's disease - a review. Toxicol. Rep. 8, 607–616.

US EPA (United States Environmental Protection Agency), 2001. Methods for the Determination of Metals in Environmental Samples, Supplement 1 (EPA/600/R-94/111), Washington DC, USA.

US EPA (United States Environmental Protection Agency), 1989. Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part A), Interim Final. EPA 540/1-89/002, Washington DC, USA.

US EPA (U.S. Environmental Protection Agency), 1991. Risk Assessment Guidance for Superfund, Volume I: Human Health Evaluation Manual (Part B, Development of Risk-Based Preliminary Remediation Goals). Office of Emergency and Remedial Response. EPA/540/R-92/003.

US EPA (U.S. Environmental Protection Agency), 2009. Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part F, Supplemental Guidance for Inhalation Risk Assessment). Final. EPA-540-R-070-002, OSWER 9285.7-82. Office of Superfund Remediation and Technology Innovation, US EPA, Washington, D.C. Available online: https://www.epa.gov/sites/production/files/2015-9/documents/partf_200901_final.pdf. (Accessed 2 February 2021).

US EPA (United States Environmental Protection Agency), 2012. Human Health Risk Assessment Strategic Research Action Plan 2012 – 2016. EPA 601/R-12/007. United States Environmental Protection Agency, p. 43. Available online: https://www.epa.gov/research/human-health-risk-assessment-strategic-research-action-plan-2012-2016. (Accessed 8 January 2021).

US EPA (United States Environmental Protection Agency), 2019a. Regional Screening Level (RSL) Summary Table (TR=1E-06, HQ=1) November 2019. Available online: https://semspub.epa.gov/work/HQ/199626.pdf. (Accessed 20 April 2020).

US EPA (United States Environmental Protection Agency), 2019b. Guidelines for Human Exposure Assessment (EPA/100/B-19/001). Risk Assessment Forum, US EPA, Washington, D.C.. Available online: https://www.epa.gov/sites/production/files/2020-01/documents/guidelines_for_human_exposure_assessment_final2019.pdf. (Accessed 25 April 2020)

US EPA (United States Environmental Protection Agency), 2020. Integrated Risk Information System. Available online: https://www.epa.gov/iris. (Accessed 22 April 2020).

USDHHS/USDA (United States Department of Health and Human Services/United States Department of Agriculture), 2015. 2015–2020 Dietary Guidelines for Americans, eighth ed., p. 144 Available online: https://health.gov/sites/default/files/2019-09/2015-2020_Dietary_Guidelines.pdf. (Accessed 6 January 2020).

USGS (United States Geological Survey), 2017. National Hydrology Dataset. NHD, USA. http://www.usgs.gov.

Varol, M., Sünbül, M.S., 2017. Organochlorine pesticide, antibiotic and heavy metal residues in mussel, crayfish and fish species from a reservoir on the Euphrates River, Turkey. Environ. Pollut. 230, 311–319.

Varol, M., Sünbül, M.R., 2018. Biomonitoring of trace metals in the Keban Dam Reservoir (Turkey) using mussels (*Unio elongatulus eucirrus*) and crayfish (*Astacus leptodactylus*). Biol. Trace Elem. Res. 185, 216–224.

Vuori, K., 1995. Direct and indirect effects of iron on river ecosystems. Ann. Zool. Fenn. 32, 317–329.

Wallace, D.R., Djordjevic, A.B., 2020. Heavy metal and pesticide exposure: a mixture of potential toxicity and carcinogenicity. Curr. Opin. Toxicol. 19, 72–79.

Wang, H., Matsushita, M.T., 2021. Heavy metals and adult neurogenesis. Curr. Opin. Toxicol. 26, 14–21.

Wei, J., Cen, K., 2020. Assessment of human health risk based on characteristics of potential toxic elements (PTEs) contents in foods sold in Beijing, China. Sci. Total Environ. 703, 134747.

Wei, K., Yang, J., 2016. Copper-induced oxidative damage to the prophenoloxidase-activating system in the freshwater crayfish *Procambarus clarkia*. Fish Shellfish Immunol. 52, 221–229.

WHO (World Health Organization), 1980. International agency for research on cancer (IARC). In: Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Metals and Metallic Compounds, 23. IARC, Lyon.

WHO (World Health Organization), 1993. Guidelines for drinking-water quality. In: Recommendations, , second ed.1. World Health Organization, Geneva, Switzerland.

WHO (World Health Organization), 1996. Trace Elements in Human Nutrition and Health. WHO Technical Report Series. WHO, Geneva.

WHO/FAO (World Health Organization/Food and Agriculture Organization), 2015. Codex Alimentarius Commission. General Standard for Contaminants and Toxins in Food and Feed. CODEX STAN 193e1995.

Xiong, B., Xu, T., Li, R., Johnson, D., Ren, D., Liu, H., Xi, Y., Huang, Y., 2020. Heavy metal accumulation and health risk assessment of crayfish collected from cultivated and uncultivated ponds in the Middle Reach of Yangtze River. Sci. Total Environ. 739, 139963.

Zhang, R., Yu, K., Li, A., Zeng, W., Lin, T., Wang, Y., 2020. Occurrence, phase distribution, and bioaccumulation of organophosphate esters (OPEs) in mariculture farms of the Beibu Gulf, China: a health risk assessment through seafood consumption. Environ. Pollut. 263, 114426.

Zhao, D., Zhang, X., Liu, D., Ru, S., 2019. Cu accumulation, detoxification and tolerance in the red swamp crayfish *Procambarus clarkia*. Ecotoxicol. Environ. Saf. 175, 201–207.

Zhou, M., Wu, Q., Wu, H., Liu, J., Ning, Y., Xie, S., Huang, W., Bi, X., 2021. Enrichment of trace elements in red swamp crayfish: influences of region and production method, and human health risk assessment. Aquacult 535, 736366.