Research article

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

Abua Ikema,a,b,*, Olukayode James Ayodeji c, James Wetzela,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.
• EW1 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).

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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 PILCR: 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 PILCR > 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).

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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) (USDHH-S/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(loids) 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., 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(loids) 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 hepatotoxicity (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). Cr exerts carcinogenic effects through mutagenesis (Wallace and Djordjevic, 2020).

As microbenthic organisms, crayfish species are in constant contact with bottom sediments (detritus) (Smietana 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; Zha 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 guidelines, 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 ascertainment 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).

**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 sitea | 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. a. 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. a. 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.

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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.

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

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freshwater crayfish species (*F. virilis* and *P. a. acutus*) from Missouri; (2) estimate the dietary intake of metal(loids) 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, -92.426250° 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 (Pfieger, 1999; 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 (Metsuchen, 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 digestor (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: 214.857. 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 R² 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 7.4.4.1, 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), Cd (1.6), Cr (2.5), Cu (2.4), 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):

\[
ARR = (MV/CV) \times 100
\]

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

| Element | Wavelength (nm) | LOD | 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 | 0.98² | 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 |

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) | ICV² | QCS-26² |
|---------|-----------------|------|---------|
|         | 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.

² View mode: All samples were determined under the axial mode.

² Spiked solution (1 mg L⁻¹) analyzed in triplicate.
² Not certified value. Errors are expressed as standard deviation.
elements in crayfish muscle, expressed as mg kg\(^{-1}\) ww was calculated using Eq. (2):
\[
C = \frac{1}{[(AC \times SW) \times VD \times DF]}
\]
(2)
Where C is the element concentration in crayfish muscle (\(\mu\)g g\(^{-1}\)), AC = analytical concentration result (\(\mu\)g 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, 2011), and the EDI/EWI values (this study) were compared with the provided cancer slope factors (CSF; mg kg\(^{-1}\) per day) for iAs, Cr, Ni, and Pb (US EPA, 2019a) according to Eq. (6):
\[
ILCR = \frac{[(EFr \times ED \times IR \times CSF)/(BWt \times AET)] \times 10^{-5}}
\]
(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 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 (CSIRC, 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

IELC 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

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

| Element | Missouri Goldfish Hatchery (P. acus acus, n = 54) | Missouri Goldfish Hatchery (P. acus acus, n = 18) | Ozark Fisheries (P. virilis, n = 40) | Ozark Fisheries (P. virilis, n = 36) | Busby Farm (P. virilis, n = 24) |
|---------|--------------------------------------------------|--------------------------------------------------|-----------------------------------|-----------------------------------|-------------------------------|
| Ag      | < LOD - 0.103                                    | < LOD - 0.097                                    | < LOD - 0.11                      | < LOD - 0.11                      | < LOD - 0.11                  |
| As      | 0.002 ± 0.002                                    | 0.002 ± 0.002                                    | 0.006 ± 0.004                     | 0.006 ± 0.004                     | 0.006 ± 0.004                 |
| Be      | 0.10–0.25                                        | 0.10–0.91                                        | 0.10–3.15                         | 0.30–1.75                         | 0.30–1.75                     |
| Cd      | 0.04 ± 0.01                                      | 0.03 ± 0.01                                      | 0.09 ± 0.01                       | 0.05 ± 0.01                       | 0.01 ± 0.01                   |
| Co      | 0.03 ± 0.01                                      | 0.02 ± 0.01                                      | 0.01 ± 0.01                       | 0.04 ± 0.01                       | 0.02 ± 0.01                   |
| Cr      | 0.11 ± 0.01                                      | 0.04 ± 0.02                                      | 0.11 ± 0.01                       | 0.13 ± 0.01                       | 0.10 ± 0.01                   |
| Cu      | 1.03–21.2                                        | 3.85–7.39                                        | 4.41–35.6                         | 5.69–107                          | 0.30–3.66                     |
| Fe      | 8.97 ± 0.59                                      | 6.22 ± 0.30                                      | 13.4 ± 0.89                       | 27.3 ± 3.05                       | 2.26 ± 0.15                   |
| Mn      | 2.00–23.0                                        | 1.76–3.34                                        | 2.20–19.5                         | 2.66–10.3                         | 0.41–3.83                     |
| Mo      | 9.04 ± 2.44                                      | 2.63 ± 0.13                                      | 4.28 ± 0.53                       | 4.82 ± 0.30                       | 1.39 ± 0.15                   |
| Ni      | 2.00–8.54                                        | 0.29–0.61                                        | 0.20–1.96                         | 0.79–4.94                         |                                |
| Pb      | 1.31 ± 0.22                                      | 0.43 ± 0.03                                      | 0.71 ± 0.07                       | 0.42 ± 0.07                       | 1.82 ± 0.18                   |
| Sn      | 0.40 ± 0.06                                      | 0.87 ± 0.01                                      | 0.87 ± 0.01                       | 0.40 ± 0.04                       | 0.40 ± 0.04                   |
| Zn      | 0.15 ± 0.01                                      | 0.12 ± 0.02                                      | 0.13 ± 0.02                       | 0.15 ± 0.02                       | 0.17 ± 0.04                   |
|        | < LOD - 0.69                                    | < LOD - 0.20                                     | < LOD - 0.83                      | < LOD - 0.99                      | < LOD - 0.99                  |

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

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

| Element | Mean | Std. error | Lower limit | Upper limit | Sigma | Lower limit | Upper limit |
|---------|------|------------|-------------|-------------|-------|-------------|-------------|
| Ag      | 0.003| 0.002      | 0.000       | 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      | 0.002| 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        |
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 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(loids) 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(loids) in muscle ranged from 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(loids) (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). 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 B₁₂. 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 bioaccumulation 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* (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 | 0.01 | 0.00 | 0.00 | 0.00 | 0.30 | 0.40 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 |
| | Max | 19.9 | 155 | 45.3 | 0.11 | 3.15 | 0.21 | 0.11 | 0.32 | 0.83 | 107 | 23.0 | 8.54 | 6.62 | 2.65 | 1.76 | 5.91 | 19.2 |
| This Study; *P. acutus acutus* (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 | 1.40 | 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; *Squilla mantis* (Livorno; Tuscany Coast, Italy) | Mean | 133 | - | 19.1 | 0.21 | 0.05 | 12.1 | 0.21 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | 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 | 0.01 | 0.00 | 0.00 | 0.00 | 0.30 | 0.40 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.17 |
| | Max | 19.9 | 155 | 45.3 | 0.11 | 3.15 | 0.21 | 0.11 | 0.32 | 0.83 | 107 | 23.0 | 8.54 | 6.62 | 2.65 | 1.76 | 5.91 | 19.2 |

**Legislations for metals in crayfish**

| European legislation - Maximum metal levels in Crustaceans | 0.50 | 0.5 |
| European Food Standards Australia New Zealand | 2.0 | 0.5 |
| Codex Alimentarius commission | 0.3 | 30 |
| Ministry of Agriculture, Fisheries and Food (MAFF) | 20 |

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

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

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

§ Crustacean *Squilla mantis* (Bonsignore et al., 2018).

‖ *Crayfish Procambarus clarkii* (Goretti et al., 2016).

¶ *Crayfish Astacus leptodactylus* (Goretti et al., 2016).

**Official Journal of the European Union (2008).**

ι Official Journal of the European Union (2011).

α PSANZ, 2013.

β Inorganic arsenic.

γ WHO/FAO (World Health Organization/Food and Agriculture Organization), 2015.

δ Fish.

ε FAO, 1983.

ζ 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 algaeceides (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, algaeceides 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. clarkia 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·), 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, a 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). 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). 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). 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). 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). 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).
3.2.12. 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 Sn concentration (0.3 mg kg$^{-1}$) was found 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$^{-1}$ (Table 4). The highest average Pb (0.99 mg kg$^{-1}$) 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 mg kg$^{-1}$) found in the shrimp P. semisulcatus muscle (Shalini et al., 2020). Howbeit, the mean Pb in cultured P. clarkia (1.33 mg kg$^{-1}$; Xiong et al., 2020) was higher than our average Pb (Table 7). A low average Pb (<0.04 mg kg$^{-1}$) 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$^{-1}$), and P. a. acutus (0.80 mg kg$^{-1}$) 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), glutatione 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$^{-1}$) was recorded in a sample from Missouri Goldfish Hatchery. In P. a. acutus, the highest Zn concentration (16.0 mg kg$^{-1}$) was found in a sample from Ozark Fisheries. Symptoms of excess Zn

**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.
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 | PTWIa | 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                          |
| Ag      | 5     | 0.01   | 0                                             | 0                                              | 0.01                                          | 0                                           | 0.03                            | 0                                           |
| As      | 15c  | 0.04   | 0.3                                           | 0.45                                           | 4.55                                          | 0.45                                      | 2.6                             | 0.26                                      |
| Be      | 2     | 0.18   | 0.02                                          | 0.17                                           | 0.02                                          | 0.17                                      | 0.02                                          | 0.02                                      |
| Cd      | 25 (2.5)k | 1     | 0.15                                          | 0.11                                           | 0.01                                          | 0.01                                      | 0.01                                          | 0.01                                      |
| Co      | 0.3   | 0.54   | 0.05                                          | 0.22                                           | 0.02                                          | 0.22                                      | 0.02                                          | 0.02                                      |
| Cr      | 300f | 1500   | 0.09                                          | 0.49                                           | 0.05                                          | 0.49                                      | 0.11                                          | 0.11                                      |
| Cu III* | 3500h | 40    | 44                                            | 30.5                                           | 45.7                                          | 0.34                                      | 14.1                                          | 11.1                                     |
| Fe      | 5600i | 20.1  | 2.91                                          | 12.9                                           | 21                                            | 2.1                                       | 23.6                                          | 6.82                                      |
| Mn      | 64    | 0.75   | 0.07                                          | 0.59                                           | 0.06                                          | 0.59                                      | 0.06                                          | 0.06                                      |
| Mo      | 5     | 0.15   | 0.15                                          | 4.83                                           | 0.48                                          | 3.43                                      | 0.34                                          | 0.34                                      |
| Ni      | 35i  | 20     | 1.52                                          | 5.12                                           | 4.34                                          | 0.34                                      | 1.04                                          | 0.06                                      |
| Pb      | 29j  | 3.57j | 0.29                                          | 11.6                                           | 3.48                                          | 0.35                                      | 1.72                                          | 0.89                                      |
| Sn      | 14000k | 600   | 13.4                                          | 51.8                                           | 15                                            | 1.5                                       | 16.9                                          | 3.21                                      |
| Zn      | 7000d | 300   | 60.8                                          | 5.19                                           | 5.58                                          | 5.58                                      | 49                                             | 9.94                                      |

* 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 RfDo 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.
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; \(p = \ldots\))

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**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 \((1.00 \times 10^{-5})\), and \(\sum\)ILCR is the sum of individual cancer risk of exposure to As, Cr, Ni, and Pb via the consumption of crayfish muscle.
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 F. acuta 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 F. acuta. 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 F. acuta, respectively. Strong/moderate correlations indicated similar chemical characteristics or common origins of elements in crayfish. The growth factors, CL, and BW in both species (r²: 1.0; p < 0.05) correlated with maximum strength. Likewise, CL-TL also produced very strong associations in both species (r²: 0.9–1.0; p < 0.05).

In F. virilis, BW moderately or strongly correlated (p < 0.05) with Zn (r²: 0.8), Sn (r²: 0.4), Fe (r²: 0.4), and Cr (r²: 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 (r²: -0.4), and with TL and CL (r²: -0.3). Cu was moderately (r²: 0.4–0.5) correlated with Fe, Zn, and Sn and negatively associated with BW, Ni, and Cr (r²: -0.4–0.5, p < 0.05). Likewise, arsenic (As) significantly (p < 0.05) correlated (r²: 0.3–0.4) with Cu, Ni, Sn, and Zn but negatively associated with BW, CL, and TL (r²: -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. acuta, 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 (RfDo, 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 ΣILCR 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 (F. acuta, n = 18) | Ozark Fisheries (P. acuta acuta, n = 40) | Ozark Fisheries (F. virilis, n = 36) | Busby Farm (F. virilis, n = 24) | Regulatory value |
|---------|-----------------------------------------------|-----------------------------------------------|------------------------------------------|---------------------------------|---------------------------------|-----------------|
| THQ¹    | THQᶜ¹                                        | THQᶜ¹                                        | THQᶜ¹                                    | THQᶜ¹                           | THQᶜ¹                           |                 |
| 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      | (9.7E-05)                                    | (3.5E-05)                                    | (9.3E-05)                                | (1.3E-04)                       | (1.1E-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                                  | 3.8E-07                         | 1.1E-05                         |                 |
| Cr      | (6.1E-06)                                    | (3.4E-06)                                    | (7.9E-06)                                | (8.0E-06)                       | (2.8E-07)                       | (2.8E-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      | (3.6E-05)                                    | (2.8E-05)                                    | (8.3E-05)                                | (2.5E-05)                       | (1.3E-05)                       |                 |
| Pb      | 1.2E-02                                      | 4.6E-02                                      | 1.4E-02                                  | 6.8E-03                         | 7.7E-03                         | 1               |
| Pb      | (3.5E-07)                                    | (5.8E-07)                                    | (4.2E-07)                                | (2.0E-07)                       | (2.3E-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               |
| ΣILCR   | 2.8E-01                                      | 2.0E-01                                      | 2.8E-01                                  | 3.8E-01                         | 2.9E-01                         | 1               |
| ΣILCR   | 1.4E-04                                      | 8.8E-05                                      | 1.8E-04                                  | 1.6E-04                         | 1.2E-04                         |                 |

¹ THQ < 1 implies no harm from consumption of crayfish; THQᶜ = sum of individual THQ values; ILCR (incremental lifetime cancer risk) values for As, Cr, Ni, and Pb are in parenthesis.

² ΣILCR 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, 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 THQAs 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 THQAs value was lower than the estimated non-cancer risk for As obtained for the crustacean *S. mantis* (THQAs: 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 THQ 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 (ZILCR) 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^{-6} 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 (ΣILCR) 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 metalloid(s) in muscle with Cu the highest followed by Fe and Zn. Significant trends in inter-species and gender differences were observed among the metalloid(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/metalloids 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.

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#### 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.

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References

Ali pour, M., Sarafraz, M., Chavoshi, H., Bay, A., Namolaliha, A., Sadani, M., Fakhar, Y., Vahid, Y., Khanghamooy, A.M., 2021. The concentration and probable 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., Dietelso, R., J. F., Schmidt, C.J., Meker, 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.

Anderle, M.B., Preland, J.E., Joibolis, L., Bollinger, J.R., 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. AOAC, Washington, DC. Available online: https://www.astdr.cdc.gov/tox/toxprofiles/index.html. (Accessed 25 February 2021).

Bollinger, J., Bunde, K., Anderson, M.R., Miller, L., Preland, J.E., Joibolis, L., Chen, H., et al., 1997. Bioaccumulation of chromium in red swamp crayfish (Procambarus clarkii). J. Hazard Mater. 54, 1–13.

Bonigsonre, M., Manta, D.S., Mitro, S., Quinci, E.M., Ape, F., Montalto, V., Gristina, M., 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.

ESRI, 2012. USA State Boundaries, 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.

EURACHEM. 2014. Guide: In: Magnussen, B., Onnenmark, U., (Eds.), The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Good Laboratory Practice, Topics, second ed. Products Press, New York, NY. Available online: https://bookshelf.vitalsource.com/#/books/9781351910566. (Accessed 24 November 2019). from VitalSource Bookshelf.

Ersoz, A., Demirel, M., A., Shank, B., Caldeira, K., G., 2015. Monitoring environmental contamination levels. Sci. Total Environ. 559, 339–346.

Green, S.R., Storey, K.B., 2016. Metal accumulation in dragonfly nymphs and crayfish as indicators of constructed wetland effectiveness. Environ. Pollut. 256, 113387.

HESANZ (Food Standards Australia and New Zealand). 2013. Australia New Zealand Food Standards Code, Standard I.4.1. Contaminants and Natural Toxins, Gabd, K., Klongchom, M., Desme, R., Sonnier, J.E., 2017. Distibution of arsenic and other metals in crayfish tissues (Procambarus clarkii) under different production practices. Sci. Total Environ. 574, 322–331.

Gissi, F., Staub, J., Linet, M., Mort, G., A., Adams, S., Schlekat, C.E., 2008. 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., Paffoni, M., Ricciarini, M., Selvaggi, R., Cappelletti, D., 2016. Metal heavy accumulation in sediments and red swamp crayfish (Procambarus clarkii) used as a bioindicator of contamination. Sci. Total Environ. 559, 339–346.

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, 126556.

Hern, R.L., Bergen, D.R., Bauer, M.L., Crayon, J.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.

Huang, Z., Hu, Y., Yang, H., 2013. Potential risk assessment of heavy metals by EFSA (European Food Safety Authority). 2009. Cadmium in food. Sciencel Direct (1), 213–225.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2011. Safety evaluation of certain food Additives and Contaminants. Thirty-Third Report of the Joint FAO/WHO Expert Committee 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. J. 44, 273–317.

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.

Lo Turco, V., Potortì, A.G., Tropea, A., Dugo, G., Di Bella, G., 2013. Human health risk from consumption of aquatic species in arsenic-contaminated shallow urban lakes. Sci. Total Environ. 470, 145318.
Lussier, S.M., Boothman, W.S., Poucher, S., Chaplin, D., Helmstetter, A., 1999. Comparison of dissolved and total metals concentrations from acute tests with six fish species and one invertebrate species. Environ. Toxicol. Chem. 18, 8998-9003.

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.

Mancinielli, G., Papadia, P., Ludoviti, A., Migoni, D., Bardelli, R., Fanizzi, F.P., Vizzini, S., 2018. Beyond the mean: a comparison of trace- and macroelement correlation profiles of two populations of the crustacean Procambarus clarkii. Sci. Total Environ. 62415, 1455–1466.

Mazum, Y., Eversole, A.G., 2004. Observations on the life cycle of Procambarus acutus in South Carolina. Aquat. Ecosyst. Health. 208, 261.

Mazzri, V., Longo, G., Bruno, M.V., Sinattra, F., Copai, 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. 101, 286–297.

McClain, W.R., Romain, R.P., Lutz, C.G., Shirley, M.G., 2007. Louisiana Crawfish Production Manual. Publication # 2637. Available at: https://extension.ornl.gov/sites/default/files/9c/09/Louisiana_Crawfish_Production_Manual.pdf. (Accessed 16 December 2010).

Meerpoel, C., Vidal, A., Andjelkovic, M., De Boevre, M., Tangni, E.K., Huybrechts, B., Depraetere, M., Vangenechten, C., 2012. Metal bioavailability and distribution in Maria Pia Lake sediment in Italy. Water Res. 46, 674–691.

McClain, W.R., Romain, R.P., Lutz, C.G., Shirley, M.G., 2007. Louisiana Crawfish Production Manual. Publication # 2637. Available at: https://extension.ornl.gov/sites/default/files/9c/09/Louisiana_Crawfish_Production_Manual.pdf. (Accessed 16 December 2010).

Meerpoel, C., Vidal, A., Andjelkovic, M., De Boevre, M., Tangni, E.K., Huybrechts, B., Depraetere, M., Vangenechten, C., 2012. Metal bioavailability and distribution in Maria Pia Lake sediment in Italy. Water Res. 46, 674–691.

McClain, W.R., Romain, R.P., Lutz, C.G., Shirley, M.G., 2007. Louisiana Crawfish Production Manual. Publication # 2637. Available at: https://extension.ornl.gov/sites/default/files/9c/09/Louisiana_Crawfish_Production_Manual.pdf. (Accessed 16 December 2010).

McClain, W.R., Romain, R.P., Lutz, C.G., Shirley, M.G., 2007. Louisiana Crawfish Production Manual. Publication # 2637. Available at: https://extension.ornl.gov/sites/default/files/9c/09/Louisiana_Crawfish_Production_Manual.pdf. (Accessed 16 December 2010).
Vuori, K., 1995. Direct and indirect effects of iron on river ecosystems. Ann. Zool. Fenn. 32, 317–329.
Wallace, B.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. Ca 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.