Accumulation of toxic elements in an invasive crayfish species (*Procambarus clarkii*) and its health risk assessment to humans

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Highlights:

1) Accumulation of elements in *P. clarkii* tissues was higher compared to *M. nipponense* species.

2) Zinc accumulation was higher in walking legs of *P. clarkii* species.

3) Mercury concentration was higher in muscle tissues.

4) Studied species can be used as a bioindicator for assessing contamination in YRD.

5) Based on EDI and HI calculation, studied elements will not pose any serious health effects.

Abstract

The accumulation and potential health risks of eight trace elements (Cu, Cd, Cr, Mn, Zn, Pb, Hg and As) were analyzed in the commercially important crayfish and oriental river prawn species from the Zhenjiang City, Jiangsu Province, China. The accumulation sequence of elements in different tissues were as gill > walking leg > muscle. Among the analyzed elements, majority of the elements were accumulated in the gill tissue, while Hg was concentrated in abdominal muscle and Zn in walking leg tissues. The elements Zn (95.9 $\mu g\ g^{-1}$) and Cd (0.02 $\mu g\ g^{-1}$) were the most and the least accumulated elements in the abdominal muscle tissue. The calculated estimated daily intake (EDI) and hazard indices (HI) of the analyzed elements in the tissue organs of *Procambarus clarkii* and *Macrobrachium nipponense* species were lower than the Chinese Food Health Criterion and international guideline values and results in no acute toxicities and thus, safe for human consumption. This study also suggests that *Procambarus clarkii* species can be used as an effective bio-indicator organism for examining the toxic heavy metals in the freshwater ecosystems.

Keyword: *Procambarus clarkii*, Mercury, Arsenic, Risk Assessment and Bioaccumulation

1. Introduction

Globally, due to rapid increase in the industrialization and urbanization activities, aquatic environments are contaminated with toxic elements and results in adverse health effects to the aquatic biota (Islam and Tanaka 2004). These toxic heavy metals originate from both natural (volcanic eruptions, oceanic hydrothermal vents and geological processes) as well as anthropogenic sources (industries, mining, combustion of coal, deforestation, pesticide usages, municipal and sewage discharges) and enter into the coastal environment through rivers and stream channels. The level of toxic and heavy elements are mainly associated with sediments compared to water column, due to the presence of different carriers i.e. organic matter clay minerals and metal oxides in the sediment layers (Zhang et al. 2014; Nagarajan et al. 2019). The heavy metals associated with the sediments can be transferred to water bodies by mobility due to changes in the environmental conditions (i.e. variations in pH, redox potential, and acidification of water column) which can become readily bioavailable to the aquatic organism and be amplified from lower tropical level to higher order in the food chain (Chakraborty et al. 2015; Tiquio et al. 2017; Zhong et al. 2018; Anandkumar et al. 2019). Some elements (Cu and Zn) are required in trace amounts for the growth and metabolic activities of the aquatic organism. However, they show toxic effects at elevated concentrations. Other elements (Hg, As, Cd, and Pb) are non-essential and exhibit toxic properties, cause cellular damage and decrease the growth rate of aquatic organisms and even cause death in trace levels (Hosseini et al. 2015). Aquatic organisms accumulate trace elements through absorption from the water column in minor amounts and greater quantities through trophic transfer from prey, whereas humans can be exposed via food-chain and results in the cause of acute and chronic health effects (George et al., 2010; Anandkumar et al., 2018).

Aquatic products are rich in protein, omega-3-fatty acid, vitamins, calcium and more nutrients commonly consumed by humans all over the world (Kalantzi et al. 2016; Anandkumar et al. 2018). In order to maintain a healthy diet, the American Heart Association recommends two servings of fish per week (Neff et al. 2014). According to the aquaculture statistical data from the U.N.'s Food and Agricultural Organization (FAO, Rome, Italy), China is one of the major suppliers for aquaculture products across the globe, with a total amount of 62,575 thousand tons, accounting for 37.42% of the global amount (167,228 thousand tons) in 2014

(CFGN 2016; FAO 2016). “The production cost of freshwater aquaculture was 581,318 Million Yuan (approximately 89,606 Million Dollars), which accounted for 48.4% of the total fishery production cost (approximately 185,016 Million Dollars) in 2016” (Zhang et al. 2014; Zhang et al. 2018). China’s the biggest and giant freshwater prawn culturing and production region is at near Gaoyou City, Jiangsu Province. Thus, many concerns have been raised on the metal contamination and its accumulation level in the aquatic species consumed as food should be monitored. Hence, monitoring the safety of freshwater aquaculture products from China is very important as it is supplied all over the world and for national population.

The red swamp crayfish, *Procambarus clarkii* (Girard 1852), is an invasive freshwater species that originated from north-eastern Mexico and in the south-central United States and currently shows a cosmopolitan distribution all over the world (Barbaresi and Gherardi 2000). In 1929, it invaded into China from Japan and now established stable population in all types of freshwater habitats such as rivers, lakes, ponds, streams and paddy fields (Yi et al. 2018). Even though it is an invasive species, it has become one of the favorite aquatic food products in China due to its unique taste and flavored meat. It is one of the most economically important farmed aquatic species in China, as it is cheaper than other protein sources. The annual consumption rate of *P. clarkii* reaches more than 600,000 metric tons during the summer season (April to June) particularly in the cities located along the middle and lower reaches of the Yangtze River system (Yi et al. 2018). The M. *nipponense* is an indigenous species and distributed throughout China and is farmed on a large scale. This species has high aquaculture potential and it can withstand in low temperatures and bred exclusively in freshwater and commonly available in the lower reaches of the Yangtze River. Crayfish species have been used as a good bio-indicator species to determine the toxic metal contamination in the environment by many researchers. It is widely used because of its following characteristics (i) can able to tolerate environmental stress better than other organisms; (ii) have long life spans (2 to 5 years) and low fecundity with high juvenile survival; (iii) ability to survive in physical contact with contaminated water and soil; (iv) occupy higher tropical level in the food chain; (v) huge in size to get samples from different tissue organs, which make them a suitable study specimen for environmental monitoring studies (Alcorlo et al. 2006; Suárez-Serrano et al. 2010; Gedik et al. 2017).

According to McClain et al. (2007) crayfish have been classified as herbivores, detritivores and omnivores, which magnify the metal levels by feeding the contaminated organisms from the lower trophic levels of the food chain. It feeds on detritus, algae, plant matter, aquatic invertebrates, amphibians and fish (Gherardi 2006). Based on the literature survey, it is observed that the sediment and water in the middle and lower reaches of the Yangtze River area is contaminated with the heavy metals (Pan and Wang 2012; Yu et al. 2018; Wang et al. 2019). Water used for the aquaculture practices normally comes from the surrounding river diversion, well water and natural precipitation (Omar et al. 2013). In addition, the intensity of consuming the farmed and cultured aquatic products is rapidly increasing in these areas. However, the toxic element contents in crayfish and other aquatic products collected from the Zhenjiang city is still unknown and the information's available is sparse. Therefore, determination and investigation of toxic elements in the crayfish and its potential health risks due to consumption is extremely important for human health. Thus, the aim of the present work is to investigate the concentrations of toxic elements in different tissue organs of the *P. clarkii* species collected from the Zhenjiang City and to evaluate the potential health risk to humans by comparing with national and international food safety guidelines and other similar studies.

2. Materials and Methods

2.1. Study Area

Zhenjiang city lies on the southern banks of the fertile Yangtze River delta in the eastern China of Jiangsu Province. It is the place where the famous Yangtze River and Beijing–Hangzhou Grand Canal intersect and the city is known for its surrounding hills. The Yangtze River (length 6300 km), is the third largest river in the World and the longest river in China and covers 1.80-million-km^2, and it has 996 billion cubic meters of total water resources (Yi et al. 2011). It is divided into several reaches and is classified as upper, middle and lower reaches. The Zhenjiang city is located in the lower reaches of the Yangtze River. It plays an important role in China's sustainable economic and social development. According to the Yangtze River yearbook, the provinces in the Yangtze River Delta releases the quantity of Pb and Cr in sewage was 84.3% and 68.8% of China in 2012, respectively (YRYCC 2013). The majority of solid waste and a tremendous amount of wastewater were discharged into the Yangtze River which particularly affects the ecology of the river at middle and lower reaches and results in long term impacts on aquatic habitats and humans (Song et al. 2011; Liu et al. 2019). The Zhenjiang city has been developed with various commercial and residential areas, riverside parks, industries, transportation networks and tourism sectors. It also has an important port in the navigation along the Yangtze River. The local annual mean temperature is 15.5°C and has four obvious seasons (spring, summer, autumn and winter). The hottest and coldest months in the region are July and January respectively. The annual average rainfall is 1088.2 mm, most of which occurs in the summer and autumn season.

2.2. Sample collection and processing

The red swamp crayfish, *Procambarus clarkii*, and the oriental river prawn *Macrobrachium nipponense* were collected for the present study. From each species, a minimum of 30 specimens were collected from a local fish market near Jiangsu University, a famous place for the selling of live fishery products from the catchment area of the Yangtze River basin (Fig. 1). Collected aquatic organisms were packed in polyethylene bags, labeled, placed in an ice box and transferred to the laboratory for analysis. Their average length and weight, scientific and common names, habitat, and the feeding habits were recorded before they were freezed for further analysis and the details are reported in **Table 1**. Later, the freezed samples were thawed at room temperature, and washed with distilled water. Crayfish samples were dissected into abdominal muscle tissues, walking legs and gills. Due to its small size (maximum 8 cm) the whole body tissue of the oriental river prawn is used for analysis. The exoskeletons were removed before drying. All these tissues were dried at 80˚C up to complete dryness. The water content of the tissue samples were calculated based on the difference between the initial wet weight and final dry weights. The dried tissue samples were powdered using an agate mortar and the grinded samples were stored in plastic containers and were placed in the desiccator until further analysis. The metals' concentrations of the analyzed aquatic samples are expressed in µg/g (dry weight).

2.3. Digestion of Aquatic Organisms

The dried powdered tissue were thoroughly homogenized and then subjected to digestion using concentrated nitric acid and hydrogen peroxide (1:1) according to FAO methods (Daziel and Baker 1983) as described in Anandkumar et al. (2019). Finally the digested samples were analyzed in the Flame Atomic Absorption Spectrometer (FAAS) (Analytik Jena Contr AA300) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Thermo Scientific Xseries 2).

Cu, Zn and Mn of all digested samples were determined by FAAS, and Cr, Cd, Pb, Hg and As were determined by ICP-MS. All chemical reagents used during the analysis were of analytical grade and deionized water was used throughout the study. To avoid possible contamination, all glasswares were soaked in diluted nitric acid for 24 h and rinsed with distilled water before use. The instrument was calibrated with chemical standard solutions prepared from the stock solution of metals (Sinopharm Chemical Reagent Co. Ltd, Shanghai). As a part of quality assurance, a quality control sample was run at a frequency of every five samples. Blanks and calibration standard solutions were also analyzed. The precision of the analytical performance was validated by measuring the certified standard reference material (TORT-2, National Research Council, Canada). The overall mean recovery rates for trace elements ranged between 87% and 105%, and this was found to be satisfactory with the results of certified values.

2.4. Bioaccumulation Factor (BAF)

The BAF values of the selected trace elements in the edible muscle tissue were calculated using the following equation (Gobas et al. 2009; George et al. 2011).

$$BAF = \frac{Cm}{Cw}$$

Where, C_m is the mean concentration of element in muscle tissues (mg/kg dry wt) and C_w is the mean concentration of the same element in the water (μg/l), which was adopted from Yi et al. (2011). (Anandkumar 2016)

2.5. Calculation of Estimated Daily Intake (EDI)

The EDT values were calculated by considering the geometric mean concentration of the metals in mg kg^{-1} (wet weight) and per day consumption. The average daily aquatic products consumption for the Chinese population is 32.73 g per capita per day (EFH 2013), as reported by the National Bureau of Statistics of China (CSY 2016). People who live in coastal area of China would eat 105 g fish and crayfish per day (Jiang et al. 2005), and people living in the Yangtze River basin would consume the same dose just like the coastal area of China (Yi et al. 2017). The estimated daily intake of metals was calculated according to (Song et al. 2009; Giri and Singh 2015; Anandkumar et al. 2019).

$$\mathrm{EDI} = \frac{C_{metal} \times \mathrm{Cons}}{B_w}$$

Where EDI characterizes the estimated daily intake of metal through the consumption of aquatic organism for an adult (µg $\mathrm{Kg^{-1}}$/day); C_{metal} is the concentration of metal in biological samples (µg $\mathrm{Kg^{-1}}$) wet weight; Cons signifies the day-to-day consumption rate (g/day), wet weight; B_w is the body weight (Kg) of an adult. The average body weight was (70 kg) for Chinese adults (Liu et al. 2018).

3. Results

The average concentrations of trace/heavy elements (Cu, Cd, Cr, Zn, Mn, Pb, Hg and As) in the most commonly consumed and commercially available red swamp crayfish and the oriental river prawn species were analyzed in their different tissue organs. Among the eight elements studied Cu, Zn and Mn are essential and required in tiny amounts for the organisms; while Cr, Pb, Cd, Hg and As are non-essential and toxic even at low concentration. The order of elemental accumulation in different organ tissues of analyzed species is presented in **Table 2**. The average concentration of elements in *P. clarkii*, and *M. nipponense* species are presented in the **Table 3**, with the average concentrations reported for the similar species from the other literatures.

In the present study, the observed Cu concentration in *P. clarkii*, and *M. nipponense* species was in the range of 17.3 µg $\mathrm{g^{-1}}$ to 93.8 µg $\mathrm{g^{-1}}$ with an average of 50.2 ± 54.1 µg $\mathrm{g^{-1}}$. The highest Cu concentration was observed in the gills followed by walking legs and abdominal muscles of *P. clarkii* species. The highest Zn accumulation was observed in the walking legs followed by the abdominal muscles and gills. Significant difference in the accumulation level was observed in the different organs of the same species. Zn concentration was recorded higher compared to the other elements analyzed in both species. The Zn concentration in all organs varied between 53.9 and 299 µg g^{-1} with an average of 133 ± 173 µg g^{-1}. Chromium concentration was higher in gill tissues of *P. clarkii* sp followed by abdominal muscles and walking legs. The Cr concentration in the present study was in the range of 0.64 to 3.3 µg g^{-1} with an average of 1.4 ± 1.8 µg g^{-1}.

Among the two species analyzed, the highest concentration of Mn was observed in the gill tissues of *P. clarkii* sp followed by walking leg and abdominal muscle tissues. The lowest concentration was observed in the muscle tissues of oriental river prawn *M. nipponense* species. The Mn concentration in the analyzed aquatic species was in the range of 10.6 to 99.8 µg g^{-1} with an average of 43.4 ± 63.1 µg g^{-1}. The observed Pb concentration in *P. clarkii*, and *M. nipponense* species was in the range of 0.0041 to 2.3 µg g^{-1}. The highest Pb accumulation was observed in the gill tissues followed by abdominal muscles and walking leg. Among the elements analyzed, Pb was the least accumulated metal in the walking leg tissues of *P. clarkii* species. The average Pb concentration in the present study was 0.7 ± 1.6 µg g^{-1}. The Cd concentration in the tissue organs of studied aquatic species varied between 0.0063 to 0.0785 µg g^{-1}. Maximum concentration of Cd was observed in the muscle tissues of the oriental river prawn (*M. nipponense*) species. Higher concentration of Cd in the *P. clarkii* species was observed in the gill tissue followed by abdominal muscles and walking legs. Among the elements analyzed, Cd was the least accumulated metal in the muscle tissues of *P. clarkii* species.

Maximum Hg concentration was recorded in the *P. clarkii* species followed by *M. nipponense* species. Interestingly, higher Hg accumulation and distribution was observed in the abdominal muscles followed by the walking leg and gills. The Hg concentration in all organs was in the range of 0.0076 to 0.222 μg g^{-1} with an average of 0.086 ± 0.15 μg g^{-1}. Mercury was the least accumulated metal in the gill tissues of *P. clarkii* species compared to the other analyzed elements. The concentrations of As in crayfish and oriental river prawn species ranged from 1.13 to 3.8 μg g^{-1} with an average of 2.4 ± 1.9 μg g^{-1}. Maximum As accumulation and distribution levels in the crayfish tissues was observed in gill followed by abdominal muscles and walking leg.

The present study results were comparable with the reported values from other parts of the world (**Table 3**). There were some exceptions with some of the studies reporting lower or higher values than the present study for the same species because of the geological nature and metal bioavailability of the study area. The results were relatively close for some elements (Cd and Hg) or comparatively higher than the obtained data for similar crayfish and oriental river prawn species.

4. Discussion

4.1. Accumulation of elements in different tissue organs

In the present study, the accumulation of elements (Cu, Zn, Cr, Pb, Mn, Hg and As) in the tissue organs of crayfish (*P. clarkii*) species was higher than the oriental river prawn (*M. nipponense*) species except of Cd. Among the analyzed elements, Cu, Mn, Cr, Cd, Pb and As show higher concentration in the gill tissue; Hg and Zn show a higher accumulations in abdominal muscle and walking leg tissues. Results of the present study showed that elements' accumulation and distribution levels in crayfish tissues were in the decreasing order of gills >

walking leg > abdominal muscles. Many researchers have reported the same accumulation trend for other heavy metals, stating that higher accumulations was observed in gills and hepatopancreas and muscle tissues with the lowest accumulations (Alcorlo et al. 2006; Suárez-Serrano et al. 2010; Kuklina et al. 2014; Goretti et al. 2016; Gedik et al. 2017; Annabi et al. 2018; Subotić et al. 2019). Each metal exhibitions a different accumulation pattern depending on their role in crayfish metabolism.

Abdominal muscle tissue is of the most concern and an important measure from a health viewpoint, because it is the most edible part of the aquatic organism and commonly consumed by the humans. An aquatic organism accumulates metals via two routes (i) dietary exposure via the food-chain and (ii) through the gill surface by waterborne exposure (Ptashynski et al. 2002). The bioaccumulation of toxic elements greatly increases in organisms when their threshold values are exceeded in that environment. Zinc and Cu always occur in higher concentrations in most of the aquatic organisms, and are essential micronutrients required for their normal growth, physiological functions and metabolism (Handy 1996). On the other hand, the non-essential elements like Cd, Pb, Hg and As are not involved in any type of metabolic activities in the aquatic organisms, their concentrations tend to increase with the increasing concentrations of the elements in the aquatic environments. Zinc is used as an active center for metalloenzymes and activators of other enzyme systems (carbonic anhydrase), while Cu is an integral part of the respiratory pigment haemocyanin, accounting for the high Cu levels observed in the hepatopancreas. Due to the fact that these heavy metals are essential, they are subject to strong regulation, being detoxified by metallothioneins (Canli et al. 1997), eliminated by excretion through faeces or urine, and via haemolymph through excretory organs or gills (Arumugam and Ravindranath 1987).

Generally, crustaceans accumulate some metals in direct proportion to the increase in the bioavailability (direct contact) from surrounding water and via feeding (Anandkumar et al. 2017). However, *P. clarkii*, in the present study, had showed a high level of elemental (Cu, Mn, Cr, Cd, Pb and As) accumulation in its gill tissues regardless their abundance in the ambient water and/or sediment with increasing concentration in aquatic environment and exposure time. Gills of the crayfish are in contact to the external medium and are responsible for metal transfer to organism. This could explain the high metal concentration, recorded herein, in this organ. Meyer et al. (1991) has reported that the gills were the principal site for Cd and Pb accumulation of the crayfish and the muscles exhibited very low values. El-Assal and Abdel-Meguid (2017) observed the highest concentration of essential and non-essential elements in the gills of crustaceans and suggested that gills readily absorb and transport the metals to the other organs via haemolymph. Moreover, the accumulation of elements (Cu, Mn, Cr, Cd, Pb and As) in *P. clarkii* was higher in gills of crayfish than in walking legs and abdominal muscles which can be attributed to the process of filtration of water against the gills and taking up the metals through the body surface. Svobodová et al. (2017) observed that accumulation of toxic metals in the gill tissues of crayfish from two different localities and found the higher concentrations of metals in crayfish collected from the polluted locality compared to the reference site, a less polluted locality. Gills, which are in direct contact with the environment, have a high permeability. Therefore, they are used as a bio-indicator for indicating metal accumulation in organisms as well as to monitor the surrounding water of a particular environment (Alcorlo et al., 2006; Anandkumar et al., 2018).

The accumulations of toxic elements (As, Pb, Cd and Hg) in crayfish muscle, such as hepatopancreas, gill and exoskeleton tissues have been studied by many researchers (Devesa et al. 2002; Alcorlo et al. 2006; Kuklina et al. 2014; Gedik et al. 2017). Inorganic As is considered problematic from a health point of view because of its carcinogenic features. The accumulation of As in sediments, agricultural soils, groundwater, contamination of water bodies by run-off and its bioavailability to aquatic organisms are topics of great concern (Flora 2015). Arsenical pesticides are tremendously toxic which implies that As get accumulated in the soils and remain in the soil for decades, transferred to higher topical level in food chain and cause harmful health effects to humans (Rodríguez-Martín et al. 2006). The accumulation of Hg in *P. clarkii* tissues organs of the present study is found to be higher in muscle tissues. Similar observation of higher accumulation of Hg in the abdominal muscle was reported by Kuklina et al. (2014) in crayfish samples collected from the three drinking water reservoirs in Czech Republic. Suárez-Serrano et al. (2010) observed higher concentration of Hg and Pb in the muscle tissues of crayfish collected near the contaminated sites of the lower Ebro River and Delta. These results indicate a great potential of *P. clarkii* to bioaccumulate toxic elements, especially Hg, in the proximity of contaminated sediments and hence it has the capacity to transfer the toxic elements to higher trophic levels, meanwhile crayfish is consequently consumed by fish, birds and humans, thus facilitating biomagnification of the heavy metals trough the full trophic web (Simon and Boudou 2001; Coelho et al. 2013; Aquino et al. 2017). Hence, crayfish occupy a central position in the food-web they are considered as a vector for transferring the toxic metals to the top level predators (including human populations) through tropic transfer.

The hepatopancreas plays an important role in metabolism of heavy metals, uptake and contributes to their detoxification and expulsion. Furthermore, essential metals such as Cu and Zn may also be subject to regulation either by limiting metal uptake at total body concentration level or by involving specific accumulation strategies of the organism, with active excretion from the metal excess and/or stored in an inert form (Rainbow 2002). Conversely, As, Cd, Hg and Pb (non- essential metals) tend to be detoxified by metallothioneins or phosphoric granules and accumulated permanently in the tissues organs, becoming detrimental elements to aquatic organisms (Anderson et al. 1997; Suárez-Serrano et al. 2010).

4.2. Sources of metals in the Yangtze River

Adsorption and desorption process controls the mobility and bioavailability of metals in soil (Gedik et al. 2016). Organic matter, Fe and Al oxides, soil texture, pH and Oxidation-Redox Potential (ORP) are the most significant factors prevailing elements mobility and release into the overlying water column (Forstner 1985; Chakraborty et al. 2015; Anandkumar et al. 2019). A metal, unlike other pollutants, are non-biodegradable and can accumulate in soils and sediments over longer periods and enters into aquatic organisms via breathing and feeding and affect the top level predators including human (Gedik and Boran 2013). The middle and lower reaches of the Yangtze River are characterized by Paleozoic marine source rocks and Quaternary unconsolidated sediments. The main mineral resources associated with the regional geology are Cu, Zn and Pb (Wang et al. 2018). Several studies have been conducted to examine the levels of heavy metals in the surface sediments of the upper, middle and lower reaches of the Yangtze River (Yi et al. 2011; Yi and Zhang 2012; Wang et al. 2018). According to Yi et al. (2017), Fe and Cr are derived from the natural lithogenic source and erosion is the main process for these elements to flow from the upper reaches of the Yangtze River to the lower reaches. Many authors have suggested that the elevated levels of As, Cu, Hg, Pb, Cd and Zn concentration in the surface sediments collected from the Yangtze River originates from the industrial pollution, general over-use of agrochemicals and chemical fertilizers and the discharge of municipal sewage (Song et al. 2011; Lin et al. 2002; Liu et al. 2019). In addition, the flushing operation caused by flow may not only cause the resuspension of sediment, but also affect the spatial distribution of heavy metals in the sediment from upper to lower reaches of the river.

4.3. Bioaccumulation of Elements

An effect of metal concentration in any tropical level of an aquatic organism depends on bioavailability (uptake from the water body) and bioaccumulation (uptake from the food) factors specific to respective metal and taxa (George et al., 2011; Anandkumar 2017). In the water column, uptake of elements by crustaceans depends upon their bioavailability (Carvalho et al. 1999). The range of bioaccumulation factor for the studied species was 6160-33500 for Cu; 1740-9650 for Zn; 2-1170 for Pb; 15.6-196 for Cd; 489-2520 for Cr; 18.9-555 for Hg and 1080-3880 for As respectively (**Table 4**). The bioaccumulation factors of Cu, Pb, Cr and As elements were higher in gill tissues, whereas Cd, Hg and Zn were more in abdominal muscles and walking leg tissues respectively. Generally, the essential elements (Cu and Zn) exhibit higher bioaccumulation than that of the toxic non-essential elements (Pb, Cd and Hg) in both studied species. Similar interpretations of the higher efficiency of essential elements and a lowest occurrence of non-essential metals in the muscles were noticed in the shrimp tissues from the southwest coast of India (George et al. 2011); crustacean species from the Miri coast, Malaysia (Anandkumar et al., 2019) and Zhanjiang, China (Wu and Yang 2011). The bioaccumulation of elements in the aquatic organisms may vary significantly among different species and depends on exposure frequency, absorption and elimination processes (Hashmi et al. 2002). Apart from this, the two influencing characteristics, i.e. exogenous and endogenous factors, which control the bioaccumulation of elements in the aquatic organisms. Exogenous factors are reflected to environmental parameters such as metal bioavailability, temperature and alkalinity of ambient aquatic environments, whereas, endogenous factors include life cycle, habitat, size, gender, ecology, physiological efficiency and feeding nature. Hence, due to these various factors the studied species showed different levels of element accumulation.

4.4. Estimation of daily intake of metals

An assessment was made between the observed elements in the crayfish and oriental river prawn samples with the reference values, in order to reveal the safe level of consumption for consumers. The possible risks of metals transmitted to human beings are feasibly dependent on the quantity of fishery products consumed by an individual (Kamaruzzaman et al. 2010). The EDI values of studied elements are presented in the **Table 5**. The results of the corresponding EDI, obtained from the present study through the consumption of analyzed crayfish and oriental river prawn species are fall below the provisional tolerable daily intake (PDTI) suggested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA 1999b). Therefore, it is confirmed that the consumption of these species does not pose any health issues for consumers.

4.5. Hazard Level

Among the analyzed elements, As, Hg, Pb and Cd are classified as non-essential toxic elements, which cause adverse health hazards to organisms and humans. Various national and international agencies such as the China Food and Drug Administration (CFDA); FAO (1983); the WHO (1989b); the EC (2014) and the FSSAI (2015) have recommended maximum permissible limits for human consumption. The food standards for aquatic organisms set by these organizations are in wet weight based concentrations (**Table 6**). For comparing with food standards, the metal concentrations in the tissues of aquatic organisms in this study **(Table 3)**, needed to be converted into wet weight by dividing them by factors ranging from 4 to 6 (Anandkumar et al., 2019). In this study, overall a factor 4.54 (i.e. 78% moisture) was adopted. The food standards of crayfish were unavailable, so a comparison of present data with fish was done. By using this factor, the derived wet weight based concentrations of As, Cu, Cd, Cr, Hg, Mn, Pb and Zn in the tissues organs of (*P. clarkii* and *M. nipponense*) species from the fresh markets of Zhenjiang City fell below the maximum permissible limits (MPLs) of the Chinese Food Health Criterion CFHC (1994), WHO (1989), FAO (1983), EC (2014) and FSSAI (2015), whereas Mn and As concentrations were slightly above the acceptable level of WHO (1989) and CFHC (1994) in the tissue organs of both species. Further studies are required to determine the source of As either it derives from the residual products used in the agricultural practices or from the lithology exposed in the catchment of the river basin. However, while comparing with the Hong Kong Government regulations (1987) and Food and Agricultural Organization (1983) Mn and As concentrations recorded in the present study are still below the permissible limits. So the examined species in this study were not associated with any chemical hazards in their muscles and safer for human consumption.

5. Conclusion

The reported dataset from the present study clearly documents that the gill tissue is the main storage organs for analyzed elements in the *P. clarkii* species followed by walking leg and abdominal muscle tissues. As the gills, which are in direct contact with the surrounding environment, have a high permeability, and accumulate more metal concentrations. Among the analyzed elements, Zn has accumulated higher, whereas Cd and Hg have accumulated at the least level in the muscle tissues of *P. clarkii* and *M. nipponense* species respectively. According to the BAF calculation, the essential elements (Cu and Zn) accumulated at a higher efficiency than non-essential elements (Pb, Cd and Hg) due to exogenous and endogenous factors and bioavailability of elements in the surrounding aquatic environment. Based on the Estimated Daily Intake (EDI) and Hazard Index calculations (HI), the analyzed elements will not pose any serious adverse health effects to humans. Compared to the national (CFHC) and international (WHO/FAO) fishery products guidelines the accumulation of elements in the muscle tissues of crayfish and oriental river prawn species were below the maximum permissible limits on wet weight basis with few exceptions and considered safe for human consumption by avoiding the gill tissues because these tissues contains elevated concentration of As and Mn. Since, the crayfish is an invasive species and have the capacity to tolerate high level of metal content compared to the native species. The both analyzed species from the study area can be consumed safely by humans and it can be used as a sentinel species for assessing the heavy metals contamination in freshwater ecosystems and culturing areas.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 1. Morphometric measures of the studied crayfish and oriental river prawn species from the Zhenjiang city

Species	English/ Chinese name	Length (cm)	Weight (g)	Habitat	Feeding nature	Climate
Procambarus clarkii	Crayfish/ 小龙虾	8.6	21.5	Benthic	Omnivores (algae, molluscs, worms, other crustaceans, fungi, bacteria and detritus)	Tropical & Sub-tropical
Macrobrachium nipponense	Oriental River prawn/ 在东方河虾	6.03	3.33	Benthic	Omnivores (algae, molluscs, worms, other crustaceans, fungi, bacteria and detritus)	

Table 2. Elements accumulation in different organs of the Crayfish and Oriental River prawn.

Species	Tissue	Order
Procambarus clarkii	Abdominal Muscle	Zn > Cu > Mn > As > Cr > Pb > Hg > Cd
	Walking Leg	Zn > Mn > Cu > As > Cr > Hg > Cd > Pb
	Gill	Mn > Cu > Zn > As > Cr > Pb > Cd > Hg
Macrobrachium nipponense	Muscle	Zn > Cu > Mn > As > Cr > Pb > Cd > Hg

Table 3. Comparison of elemental concentrations ($\mu g\ g^{-1}$ dry weight) in the muscle tissues of crayfish from the Zhenjiang, City with the other regions

Location	**Sample**	**Cu**	**Zn**	**Mn**	**Cr**	**Cd**	**Pb**	**Hg**	**As**
Present study	*P. clarkii Abdominal Muscle*	17.3	95.9	16	0.714	0.0245	0.241	0.222	1.13
Present Study	*P. clarkii Waking leg*	18	299	47.1	0.64	0.0063	0.004	0.102	1.05
Present Study	*P. clarkii Gill*	93.8	53.9	99.8	3.3	0.0281	2.3	0.0076	3.8
Present Study	*M. nipponense Muscle*	71.8	81.7	10.6	1.2	0.0785	0.323	0.0136	2.17
Czech Republic[1]	*P. clarkii Abdominal Muscle*	32.9	76.9	_	4.2	0.05	<0.5	1.18	_
Louisiana, USA[2]	*P. clarkii Abdominal Muscle*	31	61.6	-	-	0.06	4.5	-	0.2-3.7
Louisiana, USA[2]	*P. clarkii Gill*	204	113	-	-	0.23	4.2	-	0.6-8.7
Italy[3]	*P. clarkii Abdominal Muscle*	187	156	-	-	2.4	0.9	-	-
Egypt[4] (wet weight)	*P. clarkii Abdominal Muscle*	5.5	14.2	2.5	-	0.23	0.9	-	-
Egypt[4] (wet weight)	*P. clarkii Gill*	41.9	7.9	24.1	-	0.273	1.02	-	-
Ebro River, Spain[5]	*P. clarkii Abdominal Muscle*	12.1-82.3	49.2-127	1.7-2.6	-	0.03-0.41	0.22-3.1	0.76-1.64	12.1-82.3
California, USA[6]	*P. clarkii Abdominal Muscle*	44.6	76.9	-	0.43	0.0	0.2	-	0.7
Spain[7]	*P. clarkii Abdominal Muscle*	28.2	65.7	-	1.6	-	1.1	-	0.8
Egypt[8]	*P. clarkii Abdominal Muscle*	32.7	125	-	5.1	-	15.9	-	-
South-Western Sicily, Italy[9]	*P. clarkii Abdominal Muscle*	17.3	74.9	-	0.81	0.0	0.2	-	1.8
USA[10]	*P. clarkii Abdominal Muscle*	3.1	5.9	-	0.5	-	-	-	-
China[11]	*M. nipponense Muscle*	10.7	11.7	-	0.4	0.1	0.72	0.024	0.028

[1]Kuklina et al. (2014), [2]Gedik et al. (2017), [3]Goretti et al. (2016), [4] El-Assal and Abdel-Meguid (2017), [5] Suárez-Serrano et al. (2010), [6] Hothem el al. (2007), [7] Mortimer and Cox (1999)), [8]Abd-Allah and Abd-Allah (2013), [9] Bellante et al. (2015), [10] Madden et al. (1991), [11] Yi et al. (2011).

Table 4. Bioaccumulation factors for metals in the crayfish and oriental river prawn.

Species	**Cu**	**Zn**	**Pb**	**Cd**	**Cr**	**Hg**	**As**
P. clarkii Abdominal Muscle	6160	3090	121	61.3	549	555	1160
P. clarkii Waking leg	6440	9650	2	15.6	489	255	1080
P. clarkii Gill	33500	1740	1170	70.3	2520	18.9	3880
M. nipponense Muscle	25600	2640	162	196	887	33.9	2230

Table 5. Estimated daily intakes (EDI µg kg body wt^{-1} day^{-1} wet weight) of metals by consuming crayfish and oriental river prawn

Species	Cu	Zn	Mn	Cr	Cd	Pb	Hg	As
P. clarkii Abdominal Muscle	5.7	31.7	5.3	0.2	0.0	0.1	0.1	0.4
P. clarkii Waking leg	5.9	98.7	15.5	0.2	0.0	0.0	0.0	0.3
P. clarkii Gill	30.9	17.8	32.9	1.1	0.0	0.8	0.0	1.2
M. nipponense Muscle	23.7	27.0	3.5	0.4	0.0	0.1	0.0	0.7
PTDI	500	1000	140	3	1	3.57	0.23	2.14

PTDI Provisional tolerable daily intake suggested by Joint FAO/WHO committee on Food Additives (JECFA 1999a). The PTDI value of Cr was based on the reference dose (RfD) of Cr (VI) established by US Environmental Protection Agency (2011), For Hg and As adpated from Bhupander and Mukherjee (2011)

Table 6. Maximum Permissible Limit (MPL) of trace metals in fish muscles (µg g^{-1} wet weight) according to National and International Guideline values.

Standard's	Cu	Cr	Pb	Cd	Zn	Mn	Hg	As
WHO (1989a)	30	50	2	1	100	0.5-1	-	
CFHC (1994)	50	2	0.5	0.1	50	-	0.5	0.1
FAO (1983)	30	-	0.5	0.5	40	-	0.5	1.4
HKG (1987)							0.5	2.3
USEPA (2000)	120	8	4	2	120	-	-	
EC (2014)	-	-	0.3	0.5	30	-	-	
FSSAI (2015)	-	-	0.3	0.3	-	-	-	

WHO-World Health Organization; CFHC -Chinese Food Health Criterion; FAO-Food and Agricultural Organization; HKG-Hong Kong Government regulation; USEPA-United States Environmental Protection Agency; EC-European Commission; FSSAI - Safety, Food, Standards Authority of India

Figure 1. Study area showing the sampling points