Investigation of toxic elements in *Carassius gibelio* and *Sinanodonta woodiana* and its health risk to humans

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Abstract

Increasing toxic metal content in aquatic products has become a universal burden due to the risk to aquatic organisms and human health risks associated with consumption of these products. In this study, toxic metal distribution and accumulation in the organs of fish and bivalve species of economic and culinary importance from the lower reaches of the Yangtze River are examined, and the corresponding health risks are also investigated. In general, the viscera and gill show higher concentration of metals than other tissues. The order of the accumulation sequence of metals in muscle tissue of fish and bivalve is: Zn > Cu > Mn > Cr > As > Hg > Pb > Cd and Mn > Zn > Cu > As > Cr > Pb > Cd > Hg respectively. Maximum accumulation of Mn (507.50 µg g^{-1}) and Pb (0.51 µg g^{-1}) in the gill tissues indicates the major uptake of these metals from the water column. According to the Hazard Index (HI) calculations (based on USEPA), the analyzed metals will not cause any harmful health effects to individuals for both normal and habitual fish consumers, except for Hg and As in habitual consumers, if these species are consumed at a larger amount. Compared to the Chinese Food Health Criterion and other international standards (WHO/FAO), metal concentrations in the edible muscle tissues of the studied species are lesser than the acceptable levels and found to be fit for human consumption.

Keywords: Yangtze River, Fish, Bivalve, Risk Assessment, Arsenic, Mercury

1. Introduction

One of the side effects of urbanization and industrialization is the contamination of the environment. Although toxic elements are mostly anthropogenic in origin, those originating from lithogenic processes (natural processes) also contribute to environmental contamination (Gibbs 1973; Jain 2004; Yi and Zhang 2012; Yuan et al. 2019). Sediments washed down from the catchment areas of rivers are the chief contributors of heavy metal pollutants to the oceans. There are two major ways by which the total metal concentrations in river sediment increases: the geological parent resources and climatic conditions in the river basins, and the metals added by human actions (Singh et al. 1999; Jain and Sharma 2001; Liu et al. 2019). These toxic elements finally reach the aquatic environment through runoff of river/ streams sediments where they remain as suspended particles and bounds with clay and organic matters (Prabakaran et al. 2019). Heavy metals might migrate from water and sediments into aquatic food chain, getting magnified as they move up the trophic level (Gu et al. 2015). Because of their persistence, bioavailability, recalcitrance, toxicity and biomagnification in the food chain, the contamination of the aquatic environment by metals is of great concern all over the World (Chi et al. 2007; Görür et al. 2012; Anandkumar et al. 2018).

Fish play a significant role in human diet as they are rich in protein, omega-3-fatty acids, vitamins, minerals. These nutritive qualities are important for reducing the high cholesterol levels and maintain a healthy and fit immune system (Copat et al. 2012). Fishes are at the uppermost trophic level of the aquatic food chain. As a result, they acquire a substantial quantity of toxic elements through their gill, skin, oral consumption of food and via gastrointestinal tract (Dallinger et al. 1987). Excessive contaminants in an aquatic environment would disturb the normal activities of fish, resulting in cell damage, degeneration of tissues or even death. The toxic elements accumulated in fish are known to cause serious health effects such as growth hindrance, deficiency in the immune system, psychosocial impairment and other illnesses associated with malfunction in humans (Iyengar and Nair 2000).

Among the aquatic organisms, fish and mussels are considered as ideal organisms for gauging pollution and possible environmental damages (Viarengo et al. 2007). Bivalves play a significant role in the aquatic food chain as they exemplify the primary consumers in the aquatic ecosystem (Hamdani and Soltani-Mazouni 2012). Mussels are mostly bottom dwellers feeding on suspended substances and plankton in the water. They have direct contact with polluted water and sediments, which makes them susceptible towards accrual of toxic elements in their soft tissues (Sarma et al. 2013). Thus, mussels are used as sensitive indicators for chemical pollution in freshwater bodies. The accumulation of toxic metals in aquatic organisms depends on the hydrodynamics of the environment, metal bioavailability, feeding nature, exposure frequency, species size, gender and reproductive cycle of the organisms (Łuszczek-Trojnar et al. 2013).

The culture and capture fishery products are widely consumed all over China and are also exported to various countries (Yi et al. 2017). In 2010, China was the major producer and exporter of fish produces at the global level, accounting for 35% of fish production (FAO 2012). In 2015, China (mainland only) produced 65.2 million tonnes of food fish, with 47.6 million tonnes (73 percent) from aquaculture and 17.6 million tonnes (27 percent) from capture. The Yangtze River has a highly diverse freshwater fish community, with 361 fish species from 29 families and 131 genera, accounting for 36% of all freshwater fish species in China (Fu et al. 2003). However, increased industrialization, urbanization and other anthropogenic activities including dumping of municipal and sewage wastes have escalated pollution levels in the middle and lower reaches of the Yangtze River (Yi et al. 2011). This has led to damages to the aquatic organisms residing in both freshwater and coastal ecosystems of China (Liu et al. 2010; Jia et al. 2018). Therefore, we believe that determining toxic metal accumulation in tissues of aquatic organisms will in turn help us understand the concentration of contaminants in water and their accumulation in the food web. Due to the abundant availability of aquatic products in the middle and lower reaches of the Yangtze River, the intensity of consuming these products is rapidly increasing. However, the toxic metal contents in commonly consuming fish and other aquatic products (bivalves) collected from the Zhenjiang city, lower reaches of the Yangtze River are still unknown and the information available is sparse

The objectives of this study is to investigate i) the distribution of selected metal in the different tissue organs of fish and bivalve species that are commonly consumed by the local population of Zhenjiang city in the lower reaches of the Yangtze River Delta, and ii) to evaluate the health risks associated with the consumption of fish and bivalve species.

2. Materials and Methods

2.1. Study Area

Zhenjiang city is located on the southern banks of the Yangtze River Delta (YRD) in the eastern China of Jiangsu Province, where the famous Yangtze River and Beijing–Hangzhou Grand Canal intersect. The Yangtze River is the 3^{rd} largest in the World and the longest river (length 6300 km), in China, covering an area of ~ 1,800,000 km^2. It has 996 billion cubic meters of total water resources and delivers > 470 Mt of sediment annually into its estuary, thus and building one of the largest deltaic system (Yi et al. 2011). Based on the topographic and hydrologic characteristics, the Yangtze River is classified into three reaches as upper (from the source to Yichang in Hubei Province; 4510 km length), middle (from Yichang to Hukou in

Jiangxi Province; 940 kms) and lower (downstream from Hukou; 850 kms) reaches. The middle and lower reaches of the Yangtze River are characterized by Paleozoic marine sedimentary rocks and Quaternary unconsolidated sediments. The major mineral resources associated with the regional geology are Cu, Zn and Pb (Wang et al. 2018). The middle and lower reaches of the river are a highly urbanized and densely populated with around 400 million inhabitants (Yang et al. 2006; Zeng and Wu 2013). The Yangtze River plays an important role in China's sustainable economic and social development. According to (YRYCC 2013), the Yangtze River Delta has released 84.31% of Pb and 68.84% of Cr to the sewage during the year 2012. The majority of sewage and wastewater discharged into the Yangtze River basin are subsequently carried into the East China Sea (ECS) which particularly affect the ecology of the river and results in long term impacts on aquatic habitats and humans (Song et al. 2011; Liu et al. 2019). The Zhenjiang city is famed for various commercial and residential areas, riverside parks, industries, transportation networks and tourism sectors etc. and has an important port for navigation along the Yangtze River. The local mean annual temperature is recorded as 15.5°C with the maximum (July) and minimum (January) temperatures of 28°C and 2.7°C respectively.

2.2. Sample collection and processing

Two commercially available and most commonly consumed species, the Prussian carp (*Carassius Gibelio*) (Bloch, 1782) and the Chinese pond mussel (the Eastern Asiatic freshwater clam or swan-mussel), (*Sinanodonta woodiana*) (Rea, 1834) were collected from the local markets for the present study. The local fish market near Jiangsu University is famous for the live fishery products caught from the catchment area of the Yangtze River region (Figure. 1). Collected specimens (minimum of 30 no's) were preserved on ice bags and shifted to the laboratory for chemical analysis. The morphometric measures of the collected species were recorded before they were frozen for further analysis and the details are reported in (**Table 1**). Later, the collected samples were defrosted at normal room temperature, followed by washing with DI water. Fish samples were dissected and the muscle, gonad and gill tissues were considered for the chemical analysis. The bivalve samples were dissected and the mantle, foot muscles, gills and gonad tissues were collected for analysis. The dissected tissue materials were dried at 60˚C to complete dryness. The exoskeletons (shells) were detached before drying. The moisture (water) content of the tissue samples was determined as the variance between the initial and dry weight. Generally, a factor 4.54 (i.e. 78% moisture) was adopted for the present study. The dried tissue samples were powdered using an agate mortar and were stored in plastic containers which were kept in the desiccator until further analysis.

2.3. Digestion process of Aquatic Organisms

The dried ground tissue samples (fish and bivalve samples) were homogenized thoroughly from which one gram of sample was subjected to acid digestion with 10ml of conc. HNO_3 (65%) and H_2O_2 (30%) according to FAO methods (Daziel and Baker 1983). After digestion, the mixture was cooled and adjusted to 50ml in the volumetric flask with distilled water. The digested samples were analyzed in the Flame Atomic Absorption Spectrometer (FAAS) (Analytik Jena Contr AA300) for Cu, Zn and Mn concentrations. Cr, Cd, Pb, Hg and As were determined in Inductively Coupled Isotopic Mass Spectrometer (ICP-MS) (Thermo Scientific Xseries 2). In order to avoid possible impurities, all the glassware used for the experiments were drowned in diluted HNO_3 for 24 h and rinsed with distilled water before use and analytical grade chemical reagents were used for the analysis. The equipment was standardized with working standard solutions prepared from the stock solution of metals (Shanghai Sinopharm Chemical Reagent Limited Company, Shanghai, China). A quality control sample was analyzed at an interval of every five samples to ensure the quality of analyses in addition to blanks and calibration standard solutions. The standard reference material (TORT-2, National Research Council, Canada) was used to validate the accuracy and precision of the analytical performance. The mean recovery rates for trace elements ranged inclusively between 97% and 115% based on certified reference material TORT-2 (NRC, Canada), and this range is within the satisfactory level of certified values.

2.4. Estimated Daily Intake (EDI) calculation

The EDI was determined by taking the arithmetical mean of the metals' concentration in mg kg^{-1} (wet weight) and per day consumption. The Chinese population has regular daily aquatic foodstuffs consumption of 32.73 g per capita per day (EFH 2013) as described by the National Bureau of Statistics of China (CSY 2016). The population in the coastal belts of China consumes ~ 105 g fish and shellfishes per day (Jiang et al. 2005), which is comparable to the consumption by the people living in the Yangtze River Basin (Yi et al. 2017). The estimated daily intake of metals was estimated using the formulae given below (Song et al. 2009; Giri and Singh 2015; Ouattara et al. 2020; Anandkumar et al. 2020).

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

Where, C_{metal} is the metal concentration in the aquatic organism (μg Kg^{-1}; wet weight); Cons represents the daily consumption rate (g/day; wet weight); and B_w is the average body weight (Kg) of an individual, which is considered as 70 kg for Chinese adults (Liu et al. 2018).

2.5. Human Health Risk Assessment

The method proposed by the United States Environmental Protection Agency (USEPA 2000) was adopted for the assessment of human health risk through the consumption of studied fish species. The level of exposure resulting from oral human consumption of particular trace metals in the fish edible tissues is expressed by calculating the average daily dose (ADD; average daily intake of a specific chemical over a lifetime). It has been calculated using the following equation, which is adopted from Anandkumar et al. (2018)

$$ADD\ (mg/kg/day) = \frac{Cm * IR * EF * ED}{BW * AT}$$

Where, C_m is the mean concentration of element/metal in fish muscle (mg/kg dry wt.), IR is the rate of ingestion (0.0312 kg/day for normal and 0.1424 kg/day for habitual fish consumers), EF is the exposure frequency (365 days/year), ED is the exposure duration over a lifetime (assumed as 70 years), BW is the body weight (assumed as 70 kg for normal adults), and AT is the average lifetime (70 years × 365 days/year). Risk assessment was assessed by calculating the hazard index (HI), which is a non-cancer index of adverse health effects from the intake of specific trace metal contaminants in food. HI is expressed as the ratio of the ADD to the oral reference dose (RfD) of the trace metal according to the following equation (USEPA 2000).

$$Hazard\ Index\ = \frac{ADD}{Oral\ RfD}$$

Where oral RfD is the oral reference dose of trace metals (mg/kg/days) based on the safe upper level of elements oral intake for an adult human with an average body weight of 70 Kg. The oral RfD for Cu, Pb, Zn, Mn, Cd, Hg, As and Cr is 0.04, 0.00357, 0.3, 0.14, 0.001, 0.0003, 0.0003 and 1.5 mg/kg/day, respectively (USEPA 2015). The Hazard Index (HI) values below 1.0 indicate a non-probable occurrence of adverse health effects to humans. On the contrary, if the calculated HI is greater than 1.0, the occurrences of adverse health effects to humans are expected.

3. Results and Discussion

Among the analyzed elements, Zn and Mn concentrations are recorded higher, while Cd and Hg show the lowest concentration in the muscle tissues of both fish and bivalve species. The order of elemental accumulation in various organ tissues of analyzed fish and bivalve species are presented in **Table 2**. The average concentration of elements in *Carassius gibelio* and *Sinanodonta woodiana* species are presented in **Table 3**, with the range and mean values stated for the similar species from the supplementary literature.

3.1. Distribution of elements

3.1.1. Copper

Copper is an important nutrient for aquatic biota and humans, required for normal growth and production of hemoglobin (Sivaperumal et al. 2007). However, excess intake may result in adverse health effects. In the present study, Cu concentration in the fish tissues varied between 2.25 and 7.01 μg g^{-1} with an average of 3.70 μg g^{-1}. The maximum Cu concentration was observed in the viscera followed by skin, gill and muscles of *Carassius gibelio* species. Copper concentration in the edible muscle tissue was lower than the values reported for the same freshwater fish species from Serbia (36.19 μg g^{-1}; Skoric et al. 2012), Iran (7.4 μg g^{-1}; Ebrahimpour et al. 2011), the Luan River, China (5.07 μg g^{-1}; Wang et al. 2015) and the Hooghly River, India (16.22 to 47.97 μg g^{-1}; De et al. 2010), but higher than the reported values from Huainan, China (1.14 to 1.77 μg g^{-1}; Wang et al. 2015). The Cu concentration in

Sinanodonta woodiana varied between 2.82 and 8.39 µg g^{-1}, with the declining order as gill > gonad > mantle > foot muscles. The average Cu concentration in the bivalve tissues was lesser (4.88 µg g^{-1}), related to the values reported from Poland (57.5 µg g^{-1}; Krolak and Zdanowski 2001), the Liuyang River, China (18.5 µg g^{-1}; Jia et al. 2018), but greater than the values specified for *Anodonta cygnea* muscle tissues from Iran (0.209 µg g^{-1}; Pourang et al. 2001).

3.1.2. Zinc

Generally, aquatic organisms contain a high level of Zn because it is one of the common elements in the Earth's crust and an important element required for normal growth, metabolism and enzymatic reactions (Casarett and Doull 1975). They have the affinity to get accrued in fatty tissues of the aquatic organisms and affect the reproductive functioning in fishes (Bhupander and Mukherjee 2011). Overexposure of Zn results in acute and chronic toxicity such as Parkinson's disease, diarrhea, anemia, damage to the pancreas and arteriosclerosis (Gorell et al. 1997; Anandkumar et al. 2017). The highest Zn accumulation in the present study was detected in the viscera followed by gill, skin and muscles. Zinc concentration was higher compared to other metals and a significant difference was observed in the accumulation levels in various organs of the same species. Zinc absorption in the fish tissues varied between 63.72 and 344.26 µg g^{-1} (average of 181.81 µg g^{-1}) which was lower than the values reported from the Serbia (286.11 µg g^{-1}; Skoric et al. 2012) and Bangladesh (42.83 to 418.00 µg g^{-1}; Rahman et al. 2012), but higher compared to the reported values from the Luan River, China (57.4 µg g^{-1}; Wang et al. 2015) and Iran (19.40 µg g^{-1}; Ebrahimpour et al. 2011). The Zn accumulation in *Sinanodonta woodiana* was in the range of 102.09 to 292.75 µg g^{-1}. The maximum accumulation was recorded in gills followed by gonad > mantle > foot muscles. The average Zn concentration of the bivalve species was lower (183.25 µg g^{-1}), compared to the values reported from the Liuyang River, China (581.30 µg g^{-1}; Jia et al. 2018) and Taihu Lake, China (1252 µg g^{-1}; Liu et al. 2010), but higher than the values reported from Poland (119.2 µg g^{-1}; Krolak and Zdanowski, 2001).

3.1.3. Manganese

Mn is an element of low toxicity and has substantial biological importance in humans, plants and animals. A small amount of Mn is required for numerous chemical processes of cholesterol, carbohydrates and proteins in the body. Manganese is present in enzymes such as oxidoreductases, lyases, hydrolases and isomerases and involved in bone formation (Goldhaber 2003). Higher accumulation of Mn results in a lung embolism, nerve injury, bronchitis and infertilities (ATSDR 2012). When human consumption exceeds the recommended daily intake doses, it may cause health problems of the respiratory tract and nervous system (Davies and Mundalamo 2010). The highest Mn accumulation was observed in the gill tissues followed by viscera, skin and muscles. The Mn absorption in the fish tissues varied between 1.23 and 16.45 µg g^{-1} with an average of 7.50 µg g^{-1}, which was lesser than the reported values from Serbia (33.86 µg g^{-1}; Skoric et al. 2012), Bangladesh (9.43 to 51.17 µg g^{-1}; Rahman et al. 2012) and Nigeria (53.02 µg g^{-1}; Asuquo et al. 2014) but higher than the values reported from the Eastern China (0.54 µg g^{-1}; Zhong et al. 2018). The Mn concentration in *Sinanodonta woodiana* species varied between 227.90 and 507.50 µg g^{-1} with an average of 369.82 µg g^{-1}. Among the analyzed elements, Mn concentration was higher in the tissues of bivalve. The maximum accumulation was recorded in gill tissues pursued by gonad, mantle and foot muscles. The average values of Mn in the present study were several folds lesser than the reported values from Poland (821.5 µg g^{-1}; Krolak and Zdanowski, 2001), the Liuyang River, China (12179.42 µg g^{-1}; Jia et al. 2018), Taihu Lake, China (11886.00 µg g^{-1}; Liu et al. 2010) and Italy (11258.00 µg g^{-1}; Ravera et al. 2003).

3.1.4. Chromium

Chromium is available in two different forms as Cr (III) and Cr (VI) and they enter into the aquatic habitat through natural processes (mineralization of rocks and volcanic dust) and human activities (industrial effluents, mining, etc). Cr (III) is required for normal function in our diet and the other form Cr (VI) is extremely toxic to organisms and humans (Losi et al. 1994; Anandkumar et al. 2017). Chromium compounds are vital for insulin molecules to carry glucose into the cells for glycolysis (the first step in ATP production) (Malik et al. 2009). Prolonged exposure to chromium can cause stomach agitation, ulcers, contraction, cramp and even mortality (Lingard and Norseth, 1979). The highest Cr concentration in the fish tissues was noticed in the muscle tissues followed by viscera, skin and gill tissues. Chromium concentration in the present study was in the ranges of 0.49 to 0.95 µg g^{-1} with an average of 0.67 µg g^{-1}, which was lesser compared to the reported values of freshwater fish species from Bangladesh (0.47 to 2.07 µg g^{-1}; Rahman et al. 2012), the Hooghly River, India (ND to 3.89 µg g^{-1}; De et al. 2012) and the Luan River, China (6.67 µg g^{-1}; Wang et al. 2015), but greater than the reported values from the Taihu Lake, China (0.387 µg g^{-1}; Chi et al. 2007). The Cr absorption in *Sinanodonta woodiana* ranged from 0.54 to 0.73 µg g^{-1} (average of 0.64 µg g^{-1}). The predominant accumulation was observed in the gonadal tissue followed by the gills, mantle and foot muscles. The average Cr concentration in the bivalve species was lesser compared with the identical species from the Liuyang River, China (2.88 µg g^{-1}; Jia et al. 2018) but greater than the value reported from Italy (0.40 µg g^{-1}; Ravera et al. 2003).

3.1.5. Cadmium

Compared to other elements, cadmium receives special attention due to its capability of producing a chronic toxic effect even at a low concentration level. Cadmium arises from the natural and anthropogenic origin with runoff from agricultural fields, particularly from phosphate manures which dissolved in the water column and resides in the environment for longer periods (Nagarajan et al. 2014; Khan et al. 2018). Later, they may be bioaccumulated in the liver and kidney of organisms through the food chain and cause harmful health effects (Barber and Sharma 1998). Cadmium concentration in fish tissues of the present study ranged from 0.009 to 0.056 µg g^{-1} with an average of 0.021 µg g^{-1}. Among the metals analyzed, Cd was the least accrued metal in the muscle tissues of *Carassius gibelio* species. Maximum Cd content in the fish tissues was detected in viscera followed by gill, muscle and skin tissues. The average Cd concentration in the current study was lesser compared with the same species from the Yangtze River, China (0.132 µg g^{-1}; Yi et al., 2011), the Hooghly River, India (0.62 to 1.20 µg g^{-1}; De et al. 2011), Bangladesh (0.09 to 0.87 µg g^{-1}; Rahman et al. 2012) and Nigeria (0.8 µg g^{-1}; Asuquo et al. 2014), but greater compared to the values reported from Huainan, China (0.005 to 0.010 µg g^{-1}; Wang et al. 2015). The Cd concentration in *Sinanodonta woodiana* ranged from 0.034 to 0.135 µg g^{-1} with an average of 0.094 µg g^{-1}. The predominant accumulation was observed in the gonad followed by gill, mantle and foot muscles. The average Cd concentration in the muscles of bivalve was lesser than the values reported from Poland (0.90 µg g^{-1}; Krolak and Zdanowski, 2001), the Liuyang River, China (0.95 µg g^{-1}; Jia et al. 2018), Italy (10.00 µg g^{-1}; Ravera et al. 2003), Austria (0.1 to 1.13 µg g^{-1}; Gundacker 2000) but higher than that of from Korea (0.024 µg g^{-1}; Habte et al., 2015).

3.1.6. Lead

Similar to cadmium, lead is a non-essential element and a serious environmental pollutant which originates mostly from anthropogenic sources and partially from the natural sources. Dust expulsions of coal and gas-fired power stations, emission from vehicles, anti-rust agents, paints and industrial effluents are the main sources of lead pollution (Harrison and Laxen 1981; Anandkumar et al. 2017). They are non-biodegradable and have a long residence time and are toxic to biota and humans even in low concentration. Lead can cause neurotoxicity, nephrotoxicity and numerous further adverse side effects through chronic inhalation (García-Lestón et al. 2010). Among the analyzed elements, Pb was the least accumulated metal in gill tissues of fish species. Lead concentration was predominant in muscle tissues followed by viscera, skin and gill tissue. The Pb accretion in the present study varied between 0.003 and 0.174 µg g^{-1} (average of 0.121 µg g^{-1}) which was lower than the reported values of freshwater fish species from Nigeria (10.01 µg g^{-1}; Asuquo et al. 2014), the Hooghly River, India (12.40 to 19.96 µg g^{-1}; De et al. 2011), Bangladesh (1.76 to 10.27 µg g^{-1}; Rahman et al. 2012) and Huainan, China (0.12 to 0.36 µg g^{-1}; Wang et al. 2015), and similar to that of from the Eastern China (0.12 µg g^{-1}; Zhong et al. 2018). The Pb accumulation in *Sinanodonta woodiana* varied from 0.278 to 0.512 µg g^{-1} (average of 0.390 µg g^{-1}). Predominant accumulation was recorded in the gills pursued by mantle, gonad and foot muscles. The average Pb accumulation in the muscle tissues of bivalve was lesser compared to the reported values from Poland (49.8 µg g^{-1}; Krolak and Zdanowski 2001), the Liuyang River, China (5.82 µg g^{-1}; Jia et al. 2018), Italy (23.00 µg g^{-1}; Ravera et al. 2003) and Austria (0.1 to 21.30 µg g^{-1}; Gundacker 2000) but greater than the average reported values from Korea (0.195 μg g^{-1}; Habte et al. 2015) and Iran (0.255 μg g^{-1}; Pourang et al. 2010).

3.1.7. Mercury

Mercury is a serious environmental contaminant, toxic to aquatic biota and humans even in small quantities. Mercury is present as methylmercury (MeHg) in fishery products because aquatic organisms possess an extraordinary ability to turn inorganic mercury into organic compounds (MeHg) which is easily transferable further through the aquatic food chain (Bhupander and Mukherjee 2011). Overexposure to mercury can cause neurological modifications in adults because the target organ is the brain; they have the ability to simply bypass the blood-brain and placental barriers (Diez 2009). Interestingly, higher Hg accumulation was observed in the muscle tissues followed by viscera, skin and gills. Mercury concentration in the fish tissues varied between 0.016 and 0.454 μg g^{-1} with an average of 0.201 μg g^{-1} which was lesser compared to the reported values from the Serbia (0.79 μg g^{-1}; Skoric et al. 2012), but higher than that of from India (0.14 μg g^{-1}; Malik et al. 2009). The Hg concentration in *Sinanodonta woodiana* ranged from 0.014 to 0.032 μg g^{-1}. Among the analyzed elements, Hg was the least accumulated metal in tissue organs of bivalve species. The predominant accumulation was recorded in gill tissues followed by gonad, foot muscles and mantle. The average Hg absorption in the bivalve species was lower (0.021 μg g^{-1}), than the values reported from Korea (0.052 μg g^{-1}; Habte et al. 2015) for the same species.

3.1.8. Arsenic

Arsenic prevails in both terrestrial and aquatic habitat due to natural and anthropogenic processes (Rahman et al. 2012). Though ubiquitous, it is toxic for humans and animals. The inorganic arsenic forms are more perilous to human than the organic ones (Bhupander and Mukherjee 2011). Chronic exposure to inorganic As may persuade various effects on the skin, cardiovascular and hematopoietic system (Jia et al. 2017). The highest accumulation in the fish tissues was observed in the muscle tissue followed by skin, viscera and gills. The concentration of As in the present study varied between 0.515 and 0.823 μg g^{-1} (average of 0.688 μg g^{-1}), which is lower compared to the value reported for the similar species from Bangladesh (1.97 to 6.24 μg g^{-1}; Rahman et al. 2012), but greater than that of from the Luan River (0.10 μg g^{-1}; Wang et al., 2015) and Eastern China (0.17 μg g^{-1}; Zhong et al. 2018) and almost identical to the values recorded from the Serbia (0.69 μg g^{-1}; Skoric et al. 2012). The As adsorption in *Sinanodonta woodiana* species ranges from 1.95 to 3.93 μg g^{-1} with an average of 2.90 μg g^{-1}. The predominant accumulation was observed in gills followed by gonads, foot muscles and mantle. The observed average value of As in the present study was several folds lesser than the reported values for the same species from the Liuyang River, China (13.97 μg g^{-1}; Jia et al. 2018), the Taihu Lake, China (15.00 μg g^{-1}; Liu et al. 2010), Italy (13.00 μg g^{-1}; Ravera et al. 2003), and Korea (3.545 μg g^{-1}; Habte et al. 2015).

3.2. Accumulation of elements in various tissue organs

In the present study, the analyzed metals (Cu, Zn, Mn, Cr, Cd, Pb, Hg, and As) in tissue organs of *Carassius gibelio* and *Sinanodonta woodiana* species show significant variations in their accumulation pattern. Essential elements (Cu and Zn) are accumulated higher in the tissue organs compared to the non-essential elements (Cd, Pb, Hg, and As). Many researchers have observed a similar accumulation pattern of higher concentration of essential elements in the tissue organs compared to the non-essential elements (Zubcov et al. 2012; Wang, Chu, et al.

2015; Varol et al. 2017; Anandkumar et al. 2018). Among the analyzed metals in the fish species, Cu, Zn, and Cd show higher concentration in the viscera tissue; Cr, Pb, Hg and As shows higher accumulation in muscle tissue and Mn accumulated higher in the gill tissue, whereas in bivalve species accumulation of Cu, Zn, Mn, Pb, Hg and As was higher in gill tissues; Cr and Cd show higher accumulation in gonad tissue. A similar observation of high concentration of metals in viscera was detected for fish raised in polyculture systems in Hong Kong (Liang et al. 1999) and fish from the Baotou Urban section of the Yellow River (Wang et al. 2010). Moreover, higher accumulation of Cd in viscera compared to the other tissue of the fish is due to the presence of metallothionein proteins, which might bind certain elements such as Cd and Cu for detoxification (Ploetz et al. 2007). The high Mn content in the gills indicates that the metal has accumulated from the water. Another reason for the higher concentration of metals in gill tissues is due to the high density of chloride cells competent in picking up cations, such as heavy metal ions (Costa and Fernandez 2002). The accumulation pattern of Mn in the tissue organs are gills > viscera > skin > muscle. Similar observations were also reported in *Carassius auratus, Pelteobagrus fulvidraco, and Squaliobarbus curriculus* collected from the Xiang River, Southern China (Jia et al. 2017). Muscle tissue is commonly considered as the organ with the low accumulation ability for toxic metals. However, this is not always the case, previous studies by Liu et al. (2015) and Jia et al. (2017) have reported the higher concentration of non-essential elements (Cr, As and Cd) in the muscle tissue of *S. fuscescens*, *P. fulvidraco* and *C. carassius* compared to gill and liver tissues. This can be due to the distinct circulation pattern of heavy metals between various fish species (Liu et al. 2015). The obvious cause for higher metal accumulation in muscle tissue needs additional investigation.

Elemental concentrations in different tissues of aquatic organisms (fishes and shellfish) always exhibited great variation (Fernandes et al. 2007). It is well accepted that bioaccumulation of metals by the aquatic organism depends primarily on either surrounding ambient water or from dietary exposure via food chain (levels in prey) (Terra et al. 2008). The liver is the primary organ for accumulation, metabolic processes of trace metals and detoxification with a large number of metallothioneins (MT) proteins, which are regarded as biomarkers and as cysteine bonding metals (Staniskiene et al. 2006; Huang et al. 2013). Gills are the dominant sites for metal uptake from the water column of a specific environment (Rao and Padmaja 2000). The gills filter out the metal ions from the habitual water column through the process of osmoregulation and gas exchange (Ahmed et al. 2016). When fishes and shellfishes are disclosed to high levels of contaminants in water, they naturally show greater concentrations in the gills (Reynders et al. 2008). Whereas, in the situation of metal exposure from food, metal levels in the gills are usually lesser than in other tissues (Pedlar and Klaverkamp 2002). Skin also shows higher metal accumulation when fish are exposed to higher concentrations of dissolved trace elements (Dural et al. 2007). So, the liver, intestine and gills always show higher accumulation of elements in the aquatic organisms related to the muscle tissue (Görür et al. 2012).

Carassius gibelio species have accumulated the highest levels of toxic elements (As, Hg, Cd, and Pb) in the muscle, gill and viscera. This may be due to the omnivorous feeding performance of the fish and it mainly feeds on zooplankton, shrimp and fingerlings. They are also active swimmers that bioaccumulate and bio-magnify the toxic metals in their tissue organs (Tao et al. 2012). Freshwater mussels live in the riverbed, channels, ponds and lakes, they are microscopic plant-eating animals that suck water through a siphon and absorb the suspended particles and dissolved trace metals from the water column and accumulate in their body tissues

(Kurnia et al. 2010). Fish living in the middle-lower layer of the water column are expected to accumulate more heavy metals compared to those fish in the surface layer of the water column (Yi et al. 2017). In the aquatic environment, sediments act as a sink for heavy metals and the level of metals is always greater in the sediments than that of in the water column. According to existing research in the Yangtze River Basin, the concentrations of heavy metals were 100–10,000 times greater in the sediment than in the water column (Yi et al. 2008). Meanwhile, suspended sediments tend to adsorb heavy metals from the water and both are transported downstream, thus increasing the concentration of heavy metals in the downstream of the river (Yi et al. 2011). Aquatic organisms that reside in the bottom layers are predisposed to higher concentrations of heavy metals. The higher uptake of contaminated zoobenthic predators and other feeds infers a higher concentration of metal levels in their tissues (Yi et al., 2011). Metal accumulation in an organism is controlled by the equilibrium between uptake and elimination kinetics (Wang and Rainbow 2008). The different body parts such as muscles, gills, gonad and viscera exhibit varying metal absorption rates, which are greatly influenced by the bioavailability of the elements in the surrounding environment, physiology, exposure frequency and feeding behavior of the organisms (Canli and Atli 2003). In addition, environmental factors such as pH, temperature, nutrients, organic carbon, organic matter and geological processes of an ecosystem influence the bioavailability and bioaccumulation rates of elements in the aquatic organisms (Zhou et al. 2008; Rejomon et al. 2010; Rahman et al. 2012; Anandkumar et al. 2018).

3.3. Assessment of daily intake of metals

An assessment was made among the detected elements in the *Carassius gibelio* and *Sinanodonta woodiana* species with the mentioned values, in order to reveal the safe level of ingestion for humans. The possible threats of metals transferred to human beings are feasibly reliant on the number of fishery products consumed by an individual (Kamaruzzaman et al. 2010). The EDI values of studied elements are presented in **Table 4**. The results of the analogous EDI, achieved from the current study through the consumption of analyzed species fall below the provisional tolerable daily intake (PTDI) Joint FAO/WHO Expert Committee on Food Additives; (JECFA 1999b) except for Mn in the gill tissues of bivalve species. Therefore, the result specifies that the intake of these aquatic species on the basis of mean day-to-day exposure of the local residents is safe and does not pose any health issues.

3.4. Human Health Risk Assessment

Finfishes and shellfishes are the main aquatic products of the Yangtze River. Toxic metal pollution in the river must have an impact on the quality of the aquatic products. The calculated HI values of the analyzed elements in the muscle tissues and other organs of the fish and bivalve species are presented in **Table 5**. The determined HI values for muscle tissues of both species are <1 for all the analyzed elements, and will not cause any deleterious health effects at both ingestion rates on consumers except for Hg in fish muscle tissues and Mn in foot muscle tissues of bivalve species for habitual consumers and As for both ingestion rates. However, the HI values for the other tissue organs indicate that Zn, Mn, Hg and As were greater than 1, which indicates that these elements would be expected to cause non-ignorable health effects in humans when these species are consumed at a more capacity. Consequently, as far as Mn and As were concerned, restricted consumption of bivalve should be regulated or the daily consumption rate should be reduced (less than 105g per day). The bivalve species should be exposed to the depuration process before consumption in order to reduce the contaminant level in their tissue organs.

3.5. Hazard Level

Among the studied elements, As, Hg, Pb and Cd are categorized as non-essential toxic elements that pose adverse health hazards to aquatic biota and humans even at trace levels. Various national and international agencies such as China's Ministry of Health, MOH (2012); FAO (1983); the WHO (1989b); the EC (2014) and the FSSAI (2015) recommend maximum tolerable guidelines for human consumption. The food criteria for aquatic animals established by these organizations are in wet weight based concentrations (**Table 6**). In order to compare with food quality criterions, the observed metal absorptions in the corresponding tissues of aquatic creatures in the present study (**Tables 3**), were transformed into wet weight by isolating the dry weight by factors ranging from 4 to 6 (Anandkumar et al. 2019). By employing this factor, the derived wet weight based concentrations of As, Cu, Cd, Cr, Hg, Mn, Pb and Zn in the tissue organs of *Carassius gibelio* and *Sinanodonta woodiana* species from the fresh markets of Zhenjiang City fell under the maximum permissible limits (MPLs) of the Chinese Food Health Criterion CFHC (1994), WHO (1989), FAO (1983), EC (2014) and FSSAI (2015). However, Zn, Mn and As concentration was somewhat above the tolerable level of WHO (1989) and CFHC (1994) in the other tissue organs of both species but lower in the edible muscle tissue. The high concentration of Mn in the gills showed that the main uptake passage of this element from the water. Identification of the explicit source of As is required, which may be either derived from the residual products used in the agricultural practices or from geological source rocks exposed in the catchment area and/or in river delta regions. However, while comparing with the Hong Kong Government regulations (1987) and the Food and Agricultural Organization (1983) Mn and As concentrations are still below the permissible limits in the consumable muscle tissues, so the investigated species in this study does not pose any biochemical hazards in their muscle tissues and harmless for consumption.

4. Conclusion

The data in this paper suggests that the toxic elements in the consumable muscle tissues of *Carassius gibelio* and *Sinanodonta woodiana* species from the Zhenjiang City, the lower reaches of the Yangtze River Delta are beneath the maximum permissible limits (MPLs) of the Chinese Food Health Criterion and other international guideline values of HKG (1987), WHO (1989) and EC (2014) on wet weight basis with the exception of As and Mn in the other tissue organs. The maximum concentration of elements in fish was observed in the viscera followed by gill, skin and muscle, whereas in the bivalve, maximum concentration was recorded in the gill tissue followed by gonad, mantle and foot muscle. The metal binding proteins such as metallothioneins may play a prominent role in regulating the concentrations of metals in different tissues. A significant difference in the metal concentration was observed among the studied species and tissue organs, which is attributed to differences in the life cycle, habitat, physiology, exposure frequency and feeding behavior of the organisms. Compared to national and international standards, the elemental concentrations in the edible muscle tissue are lower than the maximum permissible limits but excessive in the other tissue organs for Mn and Zn. According to the human health risk index calculations, Hg and As may cause non-ignorable health effects in humans if these species are consumed at a larger amount. Whereas, remaining elements will not pose any adverse health effects to humans according to the ingestion rates proposed by USEPA. Based on the calculation indices and human health point of view, the levels of Mn, Hg and As observed in the tissue organs of studied species should be considered to be an important warning signal. Therefore, with respect to these metals, a reduced consumption rate of bivalve species is advised and the depuration process of bivalve mollusk is recommended before consumption.

Conflict of Interest Statement

The authors state that there are no conflicts of interest.

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Table 1. Morphometric measures of the studied Prussian carp and bivalve species from the Zhenjiang city

Species	English/ Chinese name	Length (cm)	Weight (g)	Habitat	Feeding nature	Climate
Carassius gibelio	Prussian Crap	17.9 ± 0.45	130.21 ± 7.84	Benthic	Omnivores (algae, molluscs, worms, other crustaceans, fungi, bacteria, fishes and detritus)	Tropical & Sub-tropical
Sinanodonta woodiana	Chinese pond mussel	10.23 ± 0.68	86.49 ± 15.77	Benthic	Filter Feeders (planktons and algae)	

Table 2. Elements accumulation in various organs of the Prussian carp and Bivalve

Species	Tissue	Order
Carassius gibelio	Viscera	Zn > Mn > Cu > As > Cr > Pb > Cd > Hg
	Gill	Zn > Mn > Cu > As > Cr > Hg > Cd > Pb
	Skin	Zn > Mn > Cu > As > Cr > Hg > Pb > Cd
	Muscle	Zn > Cu > Mn > Cr > As > Hg > Pb > Cd
Sinanodonta woodiana	Mantle	Mn > Zn > Cu > As > Cr > Pb > Cd > Hg
	Foot Muscle	Mn > Zn > Cu > As > Cr > Pb > Cd > Hg
	Gill	Mn > Zn > Cu > As > Cr > Pb > Cd > Hg
	Gonad	Mn > Zn > Cu > As > Cr > Pb > Cd > Hg

Table 3. Comparison of elemental concentrations (μg g^{-1} dry weight) in the muscle tissues of Prussian carp and Bivalve species from the Zhenjiang, City with the other regions

Location	Sample	Cu	Zn	Mn	Cr	Cd	Pb	Hg	As
Present study	*Carassius gibelio Gill*	2.662	247.409	16.455	0.491	0.010	0.003	0.016	0.515
Present Study	*Carassius gibelio Muscle*	2.255	63.721	1.230	0.950	0.011	0.174	0.454	0.823
Present Study	*Carassius gibelio Skin*	2.869	71.848	3.755	0.579	0.009	0.151	0.284	0.717
Present Study	*Carassius gibelio Viscera*	7.016	344.266	8.570	0.660	0.056	0.157	0.051	0.699
Present study	*Sinanodonta woodianaMantle*	3.683	156.254	359.550	0.616	0.074	0.404	0.014	1.915
Present Study	*Sinanodonta woodiana Foot muscle*	2.826	102.096	227.900	0.542	0.034	0.278	0.016	2.232
Present Study	*Sinanodonta woodiana Gill*	8.399	292.756	507.500	0.689	0.133	0.512	0.032	3.926
Present Study	*Sinanodonta woodiana Gonad*	4.627	183.251	384.350	0.739	0.135	0.368	0.022	3.520
Huainan China[1]	*Carassius auratus Gibelio Muscle*	1.14-1.77	8.63-10.5	-	-	0.005-0.01	0.12-0.36	-	-
Huainan China[1]	*Carassius auratus Gibelio Gill*	1.70-352	12.32-19.23	-	-	0.032-0.073	0.80-1.43	-	-
Huainan China[1]	*Carassius auratus Gibelio Viscera*	4.67-12.81	16.15-24.53	-	-	0.13-0.165	0.52-1.84	-	-
Yangtze River, China[2]	*Carassius auratus auratus Muscle*	1.09	9.40		0.33	0.17	0.89	0.014	0.019
Yangtze River, China[3]	*Carassius auratus Muscle*	0.93	6.445	-	0.19	0.132	0.811	0.0079	-
Eastern China[4]	*Carassius carassius Muscle*	0.89	19.80	0.54	2.98	0.04	0.12	-	0.17
Luan River, China[5]	*Carassius auratus Muscle*	5.07	57.40	-	6.67	0.05	1.12	-	0.10
Taihu Lake, China[6]	*Carassius auratus Muscle*	1.89	180.00	-	0.387	0.013	0.287	-	-
Taihu Lake, China[6]	*Carassius auratus Gonad*	13.61	249.00	-	1.038	0.01	0.201	-	-
Xiang River[7]	*Carassius auratus*	0.19-23.13	4.69-87.28	0.22-7.241	-	0.021-0.093	0.056-0.183	-	0.042-0.082
Iran[8]	*Carassius auratus Gibelio Muscle*	7.40	19.40	-	0.70	0.29	1.30	-	-
Iran[8]	*Carassius auratus Gibelio Gill*	11.90	31.30	-	1.00	0.40	3.10	-	-
India[9]	*L. rohita and C. idella Muscle*	0.59	1.88	-	0.58	0.427	1.32	0.14	-
Nigera[10]	*Freshwater fishes (4 species)*	3.01	63.10	53.02	-	0.8	10.01	-	-
Bangladesh[11]	*Freshwater fishes (7 species)*	8.33-43.18	42.83-418	9.43-51.17	0.47-2.07	0.09-0.87	1.76-10.27	-	1.97-6.24
Hooghly River, India[12]	*Esturaine fishes*	16.22-47.97	12.13-44.74	-	ND-3.89	0.62-1.20	12.40-19.96	-	-
Korea[13]	*Anodonta woodiana*	-	-	-	-	0.024	0.195	0.052	3.545
Poland[14]	*Anodonta sp.*	57.50	119.20	821.50	-	0.90	49.8	-	-
Liuyang River, China[15]	*Anodonta woodiana*	25.99	581.30	12179.42	2.88	0.95	5.82	-	13.97
Taihu Lake, China[16]	*Anodonta woodiana*	18.50	1252	11886	-	23.10	2.8	-	15.00
Danube River, Austria[17]	*Anodonta sp.*	0.90-12.70	71-862	-	-	0.1-1.13	0.1-21.3	-	-
Italy[18]	*Anodonta cygnea*	34.00	642.00	11258	0.40	10.00	23.00	-	13.00
Iran[19]	*Anodonta cygnea*	0.209	-	-	-	0.117	0.255	-	-

[1]Wang et al. (2015), [2]Yi et al. (2011), [3]Yi et al. (2012), [4] Zhong et al. (2018), [5] Wang et al. (2015), [6] Chi el al. (2007), [7] Jia et al. (2017), [8] Ebrahimpour et al. (2011), [9] Malik et al. (2009), [10] Asuquo et al. (2004), [11] Rahman et al. (2012), [12] De et al. (2010), [13] Habte et al. (2015), [14] Krolak and Zdanowski (2001), [15] Jia et al. (2018), [16] Liu et al. (2010), [17] Gundacker (2000), [18] Ravera et al. (2003), [19] Pourang et al. (2010).

Table 4. Estimated daily intakes (EDI μg kg body wt^{-1} day^{-1} wet weight) of metals by consuming Prussian carp and Bivalve species

Species	Cu	Zn	Mn	Cr	Cd	Pb	Hg	As
Carassius gibelio Gill	0.879	81.645	5.430	0.162	0.003	0.001	0.005	0.170
Carassius gibelio Muscle	0.744	21.028	0.406	0.313	0.004	0.057	0.150	0.271
Carassius gibelio Skin	0.947	23.710	1.239	0.191	0.003	0.050	0.094	0.236
Carassius gibelio Viscera	2.315	113.608	2.828	0.218	0.019	0.052	0.017	0.231
Sinanodonta woodianaMantle	1.215	51.564	118.652	0.203	0.025	0.133	0.004	0.632
Sinanodonta woodiana Foot muscle	0.933	33.692	75.207	0.179	0.011	0.092	0.005	0.737
Sinanodonta woodiana Gill	2.772	96.609	167.475	0.227	0.044	0.169	0.010	1.296
Sinanodonta woodiana Gonad	1.527	60.473	126.836	0.244	0.045	0.121	0.007	1.161
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 5. Indicating HI for muscle consumption calculated at mean ingestion and subsistence rates for Prussian carp and Bivalve species

Sample	Cu [a]	Cu [b]	Zn [a]	Zn [b]	Mn [a]	Mn [b]	Cr [a]	Cr [b]	Cd [a]	Cd [b]	Pb [a]	Pb [b]	Hg [a]	Hg [b]	As [a]	As [b]
Carassius gibelio Gill	0.025	0.115	0.095	0.432	0.004	0.018	0.000	0.001	0.005	0.022	0.022	0.099	0.674	**3.075**[*]	**1.222**[*]	**5.577**[*]
Carassius gibelio Muscle	0.030	0.135	0.368	**1.678**[*]	0.052	0.239	0.000	0.001	0.004	0.020	0.000	0.002	0.024	0.110	0.764	**3.489**[*]
Carassius gibelio Skin	0.078	0.357	0.511	**2.334**[*]	0.027	0.125	0.000	0.001	0.025	0.115	0.020	0.090	0.075	0.344	**1.038**[c]	**4.736**[*]
Carassius gibelio Viscera	0.032	0.146	0.107	0.487	0.012	0.055	0.000	0.001	0.004	0.017	0.019	0.086	0.422	**1.926**[*]	**1.065**[*]	**4.859**[*]
Sinanodonta woodianaMantle	0.041	0.187	0.232	**1.060**[*]	**1.145**[*]	**5.224**[*]	0.000	0.001	0.033	0.151	0.050	0.230	0.020	0.092	**2.844**[*]	**12.982**[*]
Sinanodonta woodiana Foot muscle	0.031	0.144	0.152	0.692	0.726	**3.312**[*]	0.000	0.001	0.015	0.070	0.035	0.158	0.024	0.111	**3.316**[*]	**15.135**[*]
Sinanodonta woodiana Gill	0.094	0.427	0.435	**1.985**[*]	**1.616**[*]	**7.374**[*]	0.000	0.001	0.059	0.270	0.064	0.291	0.047	0.214	**5.833**[*]	**26.622**[*]
Sinanodonta woodiana Gonad	0.052	0.235	0.272	**1.243**[*]	**1.224**[*]	**5.585**[*]	0.000	0.001	0.060	0.275	0.046	0.210	0.033	0.149	**5.229**[*]	**23.866**[*]

[a] 0.0312 kg/day (mean ingestion rate) [b] (0.1424 kg/day (subsistence ingestion rate)); [*]HI> 1, adverse health effects are excepted to occur.

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

Standards	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 (after Anandkumar et al. 2020)