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ORIGINAL PAPER

Bioaccessibility and Health Risk Assessment of Cu, Cd, and Zn in “Colored” Oysters

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Abstract Bioaccessibility describes the fraction of contaminants released from the food matrix into the digestive tracts of humans, which is beneficial for improving the health risk assessment of contaminants. In this study, the bioaccessibilities of cadmium (Cd), copper (Cu), and zinc (Zn) in two severely contaminated green oyster (*Crassostrea angulate*) and blue oyster (*Crassostrea hongkongensis*) populations were investigated. A human health risk assessment of these metals was then performed based on bioaccessibility measurements. Among the three metals, the bioaccessibility was the highest for Cu (42–95 %), and Cd and Zn had comparable bioaccessibility (13–58 %). There was no major difference in the bioaccessibility between green and blue oysters. A significant correlation between the tissue Cu and Zn concentrations was found in these highly contaminated oysters. A health risk assessment showed that all three metals in both oyster species seriously exceeded the levels recommended by the United States Environmental Protection Agency. Thus, oysters from these locations, and the metals contained therein, presented quite high risks for human consumption, which should be a great cause of concern. A significant relationship was only found between metal bioaccessibility and its tissue concentration instead of between metal bioaccessibility and subcellular distribution. In addition, a significant relationship was only observed between metal health risks and its tissue concentration. The influence of metal bioaccessibilities on the health risks was limited. This may suggest that in the case of the colored oysters examined in this study, metal concentration instead of metal subcellular distribution could be the driving factor of the metal bioaccessibility, and metal concentration, instead of metal bioaccessibility, could be the driving factor of the metal health risks.

Total concentrations of contaminants are the most frequent indicators applied in the current human health risk assessment of foods, but they may not always effectively reflect the actual available amount of contaminants ingested with food (Amiard et al. 2008; Brandon et al. 2006; Oomen et al. 2002, 2003; Versantvoort et al. 2005). The bioaccessibility of contaminants appears to be a good substitute for total concentrations. First, bioaccessibility describes the fraction of contaminants released from the food matrix into the human digestive tract. Bioaccessibility provides a cost-effective approximation of the bioavailability of contaminants and is supposed to effectively reflect the health risks of contaminants ingested with food (Cabanero et al. 2004; Laparra et al. 2003, 2008). Second, it is quite simple and quick to measure bioaccessibility. Several in vitro models have been developed for bioaccessibility measurements, which simulated the digestion processes of the human mouth, stomach, and intestine

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(Amiard et al. 2008; Brandon et al. 2006; Intawongse and Dean 2006; Oomen et al. 2002, 2003; Versantvoort et al. 2005). As a consequence, bioaccessibility is helpful in improving the human health risk assessment of contaminants ingested with food.

Some studies have investigated the bioaccessibility of heavy metals in fish, edible seaweed, wheatgrass, vegetables, cereals, and pulses (Almela et al. 2005; Cabanero et al. 2004; He et al. 2010; He and Wang 2011; Hemalatha et al. 2007; Intawongse and Dean 2008; Kulkarni et al. 2007; Laparra et al. 2003; Liu and Zhao 2007). There have been some reports on the bioaccessibility of contaminants in shellfish. For example, previous surveys showed that eating habits and cooking styles influenced the bioaccessibility of metals in some shellfish (Amiard et al. 2008; Houlbreque et al. 2011; Metian et al. 2009). Studies in our laboratories suggested that the subcellular distributions of metals may be related to the bioaccessibility of metals in shellfish (He et al. 2010; He and Wang 2011). In clams, metal speciation was found to affect metal bioaccessibility (Koch et al. 2007). Shellfish pollution has become more and more serious, but it has not yet received enough attention.

Oysters are abundant in nutrition and are widely consumed as an important dietary component in coastal areas. However, oysters also accumulate extraordinarily high concentrations of heavy metals compared with other marine fish and shellfish, which represent a high threat of toxicity to human health. Previous surveys have reported extraordinarily severe pollution of metals in oysters, and colored oysters have been discovered in some estuaries (Han and Hung 1990; Lin and Hsieh 1999; Roosenburg 1969). The multiple occurrences of these colored oysters have attracted the attention of many researchers. Recently, colored oysters with quite serious heavy metal contamination were found in the coastal waters of XiaMen in Southeast China. The subcellular distribution and detoxification mechanism of some metals [silver (Ag), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn)] in these colored oysters were studied (Wang et al. 2011). Then a subsequent transplantation experiment was performed on the same oyster species in the laboratory to investigate whether the biokinetic processes could be reconstructed and the extraordinarily high metal concentration could be built up (Pan and Wang 2012). With the increase in the pollution of the marine environment, these colored oysters are more and more likely to appear in the marketplace and might be accidentally consumed by humans. However, the human health risks of metals from these seriously polluted colored oysters have never been tested in previous studies.

Metals (i.e., Ag, Cd, Cu, and Zn) in some “colorless” oysters from Restronguet Creek in the United Kingdom and French Atlantic coast showed a low bioavailability (Bragigand et al. 2004). However, the bioaccessibility of metals in the seriously polluted colored oysters was still unclear. In this study, we took advantage of the recently discovered green oysters (*Crassostrea angulata*) and blue oysters (*C. hongkongensis*) from a contaminated estuary of XiaMen in Southeast China and examined bioaccessibility and its relationship with the tissue concentration and the subcellular distribution of the metals (Cd, Cu, and Zn) in the soft tissues of these oysters. Moreover, we performed a human health risk assessment of Cd, Cu, and Zn from these green and blue oysters based on the measurements of the bioaccessibility and investigated the relationships of health risks with both the tissue concentration and the bioaccessibility of Cd, Cu, and Zn in these oysters.

Materials and Methods

Oysters Sampling and Reagents

Two species of colored oysters, the blue oyster (*C. hongkongensis*) and the green oyster (*C. angulata*), were collected in this study. Due to the large variation in the sizes, ten blue oyster individuals with a size of 9.9 ± 0.9 cm were collected from a station along the Jiulong River Estuary, XiaMen, China. Green oysters were collected from eight stations along the Jiulong River Estuary, and the sizes of them were more similar. Thus, three green oysters were sampled from each station. The average size of the collected green oysters was 4.2 ± 0.4 cm. These oyster samples were first dissected and the shell removed, and the soft tissues were then obtained and kept at −80 °C for preparation.

α-Amylase, mucin, glucose, glucosamine hydrochloride, pepsin, pancreatin, lipase, bile, KCl, KSCN, NaCl, and $CaCl_2$ for in vitro digestion were purchased from Sigma-Aldrich. Urea, uric acid, glucuronic acid, and bovine serum albumin for in vitro digestion were purchased from Sangon, China. Oyster tissue 1566a (National Institute of Standard and Technology, Gaithersburg, Maryland, USA) was used as the standard reference material for metal analysis. All other reagents were of analytical grade.

Total Concentration and Subcellular Distribution of Metals

The total concentrations and subcellular distribution of Cu, Cd, and Zn in the soft tissues of colored oysters were measured in a previous study in our laboratory (Wang et al. 2011). The soft tissues of the oysters were first dried, homogenized, and then digested by 70 % nitric acid to determine the total concentrations of metals in the soft tissues. Then the total tissue concentrations of Cu, Cd, and

Zn were analyzed by inductively coupled–plasma mass spectrometry (7700X; ICP-MS; Agilent). In addition, fresh oyster soft tissues were subjected to subcellular fractionation using the method described by Wallace et al. (1998, 2003). Briefly, oyster soft tissues were first homogenized in a tissue homogenizer before being subjected to several centrifugations and heat treatments, which were performed as described by Dang and Wang (2010). Five operationally defined subcellular fractions, including metal-rich granules (MRG), organelles, cellular debris, heat-stable protein (HSP), and heat-denaturable protein (HDP), were obtained. These five subcellular fractions were then subjected to digestion by 70 % nitric acid, and the concentrations of Cu, Cd, and Zn in each subcellular fraction were measured to quantify the distributions of the metals in the five fractions.

Metal Bioaccessibility

The bioaccessibility of the Cu, Cd, and Zn in the soft tissues of colored oysters was determined by an in vitro digestion model according to the method modified from Oomen et al. (2002, 2003). The in vitro digestion model mimics the three digestion processes in the mouth, stomach, and small intestine of humans. Four artificial digestive fluids (artificial saliva, gastric juice, duodenal juice, and bile juice) were prepared for this model. The procedures of the in vitro digestion model and the preparation of the artificial digestive fluids were performed as described by Versantvoort et al. (2005). First, 4.5 g of fresh oyster soft tissue was homogenized in a 50-mL Eppendorf tube and subjected to the simulated digestion process in the mouth. Next, 6 mL of artificial saliva was added and incubated for 5 min at 37 °C. Then 12 mL of artificial gastric juice was added and incubated for 2 h at 37 °C to mimic the digestion process in the stomach. Finally, a mixture of 12 mL of artificial duodenal juice, 6 mL of artificial bile juice, and 2 mL of HCO_3^- was added and incubated for another 2 h at 37 °C to simulate the digestion process in the small intestine. All three incubations were performed at 37 °C with a shaker at 55 rpm. The mixture from the three simulated digestion processes was then sent for centrifugation, and the supernatant and pellets were obtained. The supernatant was dried and digested with nitric acid at 80 °C until a clear liquid was obtained, which was submitted for analysis of the metals without further processing. The metal analysis was measured by ICP-MS. The bioaccessibility of the metals was calculated as the ratio of the metal content in the supernatant to that in the soft tissues of the oysters.

Risk Assessment

The health risks of Cu, Cd, and Zn in colored oysters were assessed using a hazard quotient method recommended by the United States Environmental Protection Agency (USEPA 2005). The hazard quotient (HQ) was calculated as the division of the estimated daily intake of metals (EDI) by the reference doses (RfD) of metals established by the USEPA (2005). The EDI of metals was the product of the metal concentration, the metal bioaccessibility, the average daily oyster consumption of metals, and the average body weight (bw) of a person from China (Gu et al. 2006). The average daily oyster consumption of metals was used instead of the average daily seafood consumption because there is no statistical average daily oyster consumption data (FAO 2010). The risks of metals based on the HQ values are interpreted using the following guidelines: $HQ \leq 0.1$: no hazard exists; HQ 0.1–1.0: hazard is low; HQ 1.1–10: hazard is moderate; and $HQ \geq 10$: hazard is high.

Data Analysis

SPSS 16.0 software (Chicago, Illinois, USA) was used for the statistical analysis. Differences between the results were analyzed by a one-way analysis of variance (ANOVA) and Tukey test. The bioaccessibility of each metal in the same oyster species was compared among sampling stations. A significance level of $p < 0.05$ was adopted for all comparisons. The correlations and regression coefficients for the relationship of metal bioaccessibility with its total concentration and subcellular distribution, as well as the relationship of HQ values with the metal bioaccessibility and the tissue concentration, were analyzed using SigmaPlot 10.0.

Results and Discussion

Metal Concentrations in Colored Oysters

Previous measurements by Wang et al. (2011) showed that concentrations of Cu, Cd, and Zn in these colored oysters were exceedingly high and that there was a particularly large variation in the concentration of metals among stations and individuals. In general, most of the Cd, Cu, and Zn concentrations in these colored oysters were higher than the safety levels. The Cd contents was 2.0–12.4 µg/g in green oysters and 1.7–5.1 µg/g in blue oysters, both measurements of which exceeded the safety levels developed by the Standardization Administration of the People's Republic of China (0.1 µg/g) (2001, 2005) and most of which exceeded the safety levels of the USEPA (3–4 µg/g) (Amiard et al. 2008). The Cu concentration was extraordinarily high, reaching 173–2212 and 1063–5314 µg/g in green oysters and blue oysters, respectively, which far exceeded the safety levels of the Standardization Administration of the People's Republic of China (50 µg/g)

(2001). The Zn concentration was 1173–6050 µg/g in green oysters and 2103–7021 µg/g in blue oysters, both of which far exceeded the safety levels of the Food and Agriculture Organization (30–100 µg/g) (Tepe et al. 2008) and the standards of Brazil, Thailand, and Malaysia (50–133 µg/g) (Amiard et al. 2008). This indicated that Zn contamination in these collected oysters was very serious, but it had received little attention, and no safety levels are currently given by Chinese government.

Previous measurements in other colored oysters discovered in Taiwan shown that those oysters accumulated exceeding high Cu concentrations of 5000 µg/g (Lin and Hsieh 1999). These results indicated that the tissue color of these oysters might be associated with the accumulation of high concentrations of Cu. Three metals (Cu, Cd, and Zn) in the soft tissues of colored oysters were measured in our study. The results showed that the contents of Cu, Cd, and Zn in the soft tissues of the colored oysters were all exceedingly high, which illustrated that the tissues colors of these oysters were not only associated with the accumulated Cu but were also related to the accumulated Cd and Zn. Moreover, the Cu and Zn concentrations in blue oysters were found to be higher than those in green oysters, but the Cd concentration was slightly lower. This result showed that blue oysters might have a much higher capacity to accumulate Cu and Zn compared with Cd, whereas green oysters presented the opposite trend.

The relationships among the metals based on their total concentrations in these colored oysters were examined (Fig. 1). No significant correlation of Cd concentrations with Cu and Zn concentrations was observed, which might be attributed to their different bioaccumulation patterns. Different bioaccumulation patterns of essential metals (Cu and Zn) and nonessential metals (Cd and Pb) have been observed in various estuarine and coastal crustaceans, mollusks, and fish (Amiard et al. 1987). However, there was a significant positive correlation between Cu and Zn concentrations in both green and blue oysters in our study ($p < 0.001$). Studies have reported that some metals presented antagonistic or synergistic effects in their accumulation in certain aquatic organisms. For example, ambient calcium inhibited the dietary assimilation of Cd and Zn in a freshwater zooplankton, *Daphnia magna* (Tan and Wang 2008), and Ag and Cu concentrations in the tissues of mussels increased synergistically when they were coexposed to Ag and Cu (Ng and Wang 2007). In our study, it was first shown that Cu and Zn might present a synergistic effect in their accumulation when the oysters were simultaneously exposed to extraordinarily high ambient concentrations of Cu and Zn.

Few relationships were observed between tissue concentration and subcellular distribution of the metals in colored oysters (Supplementary Information Figs. S1, S2, and S3). MRG was a biologically detoxified fraction that could bind with the accumulated metals as a chelating agent and eliminate parts of the metal toxicities (Wallace et al. 1998, 2003). Thus, metal partitioning in MRG was generally increased with increased metal tissue concentration. However, a negative correlation between tissue concentration and subcellular partitioning in MRG was observed for Cu in both green and blue oysters in our study (Fig. S1), which might result from the spillover among the subcellular fractions. The “inappropriate” metals bound with MRG might transfer to other subcellular fractions when the MRG sites were not enough for the binding of metals (Campbell and Hare 2009). Cu partitioning in MRG and the trophically available metal (TAM, defined as a sum of HDP, HSP and organelles) were found to be negatively correlated with the tissue concentration of Cu in green oysters in our study (Figs. S1 and S2), but Cu distributing in cellular debris was positively correlated (Fig. S3). It showed quite a large distribution of Cu in the subcellular fraction of Cellular debris in these colored oysters that were highly contaminated by heavy metals, which might due to the transfer and spillover of the metals from MRG to cellular debris.

Metal Bioaccessibility and Its Relationship with Tissue Concentration in Oysters

As indicated in Fig. 2, the bioaccessibilities of Cu, Cd, and Zn in green and blue oysters were determined. The bioaccessibility of Cu was 66.7–95.0 % and 42.4–94.4 % in green and blue oysters, respectively. However, the bioaccessibility of Cd was 21.0–58.3 % and 25.6–56.8 %, respectively, whereas that of Zn was 13.4–58.0 % and 17.1–51.7 % in green and blue oysters, respectively. There was no significant difference in metal bioaccessibilities among oyster species by sampling station. The bioaccessibilities of Cu, Cd, and Zn in green and blue oysters seemed to be lower than those in oysters without severe contamination. For example, the bioaccessibilities of Cu, Cd, and Zn were 94.5–95.1 %, 78.3–87.8 %, and 74.9–87.5 %, respectively, in cleaner oysters collected from five other places in China, which were all higher percentages than those seen in the blue and green oysters (He and Wang 2013). For two other species of oysters from contaminated waters, the bioaccessibilities of Cd, Cu, and Zn were 62–84 %, 80–97.4 %, and 59–82 %, respectively, which were also higher than those in the blue and green oysters (Amiard et al. 2008).

A correlation analysis between the bioaccessibilities and tissue concentrations of Cu, Cd, and Zn in green and blue oysters was performed (Supplementary Information Table S1; Fig. 2). The bioaccessibilities of the metals were not always correlated with the tissue concentrations in

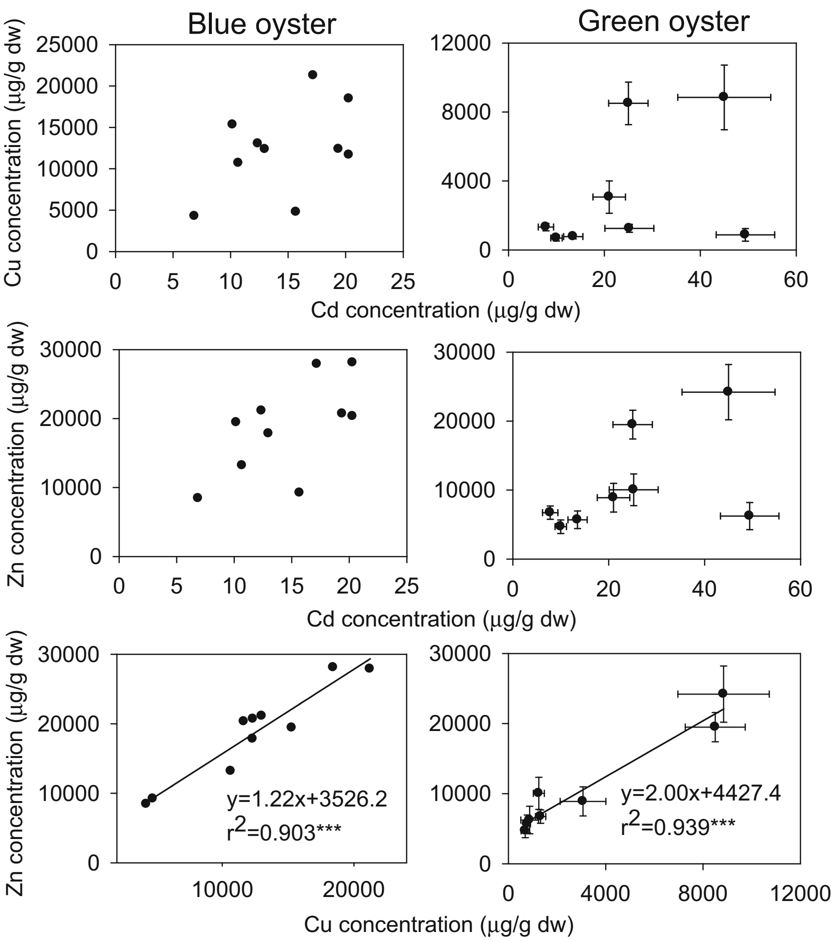


Fig. 1 Cu, Cd and Zn relationship based on their total concentrations in both blue oysters ($n = 10$) and green oysters ($n = 24$). Average total concentrations of oysters from eight stations were used for green oysters. ***$p < 0.001$

some noncontaminated or slightly polluted fish (He et al. 2010; Laird et al. 2009) and shellfish (He and Wang 2013). However, significant negative correlations were observed for the three metals in these exceedingly contaminated colored oysters with a much higher accumulation of heavy metals, except for Cd in the green oysters. The impact of metal tissue concentration on metal bioaccessibility was significant for these exceedingly contaminated colored oysters. In colored oysters, the fraction of metals transferred and stored in insoluble forms increased with the metal tissue concentrations. As shown in Fig. 3, metal partitioning in the insoluble fraction of cellular debris in most of the green and blue oysters was high. The insoluble fractions were not easily degraded by the human digestive systems; thus, metal bioaccessibilities decreased in these colored oysters with increasing metal tissue concentrations (Amiard et al. 2008; Bragigand et al. 2004). Moreover, the higher concentration of metals in oyster tissues required more digestive juices for metal release, but the limited digestion juices in the human lowered protein digestibility and metal release and therefore reduced bioaccessible efficiency (Amiard et al. 2008; He and Wang 2011; Versantvoort et al. 2005). In both green and blue oysters, the tissue concentration of metals alone as an evaluating indicator in the human risk assessment did not provide enough information; metal bioaccessibility must be considered to improve the risk assessment, especially for these severely polluted oysters.

Relationship of Metal Bioaccessibility with Subcellular Distribution

The subcellular distribution of metals in aquatic organisms was usually considered a good predictor of the potential bioaccessibility and bioavailability of the metals. Metals in the subcellular fraction TAM (consisting of organelles, HDP, and HSP) in the prey always indicated that the metals were easily bioavailable, which predicted the potential bioavailability to predators in the trophic transfer (Cheung and Wang 2005; Dang and Wang 2010; Zhang and Wang 2006; Wallace and Luoma 2003). It has been reported that metals in the subcellular fraction of the MRG in prey were

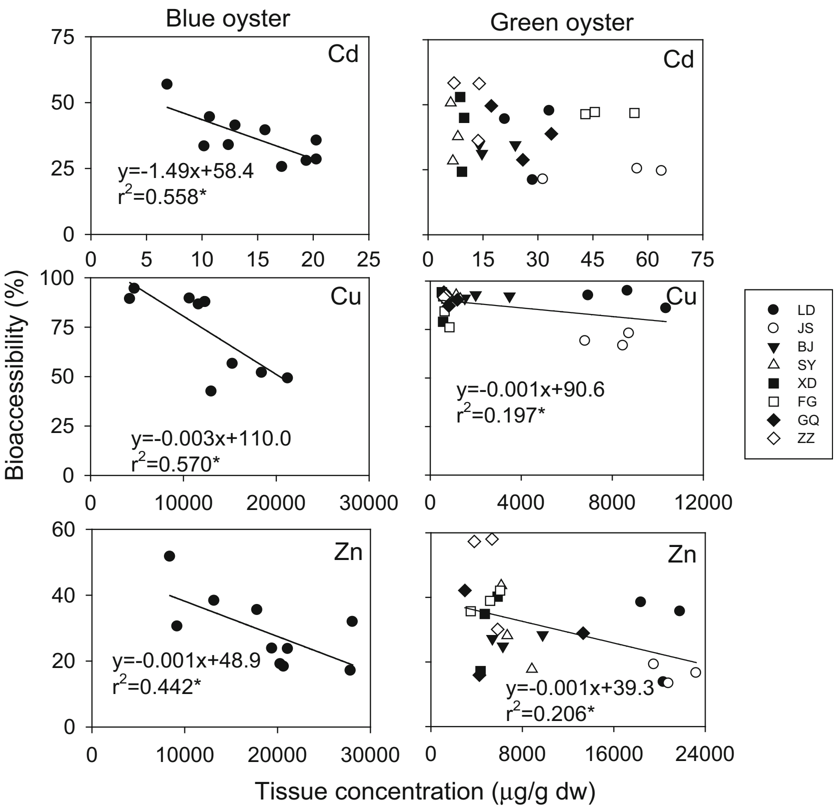


Fig. 2 Relationship between bioaccessibility and tissue concentration of Cu, Cd and Zn in blue oysters ($n = 10$) and green oysters ($n = 24$); LD, JS, BJ, SY, XD, FG, GQ and ZZ were the sampling stations of green oysters; $*p < 0.05$

not easily digested and thus were bioavailable to predators in the trophic transfer of the food chain (Wallace et al. 1998).

Metal bioaccessibility in these severely polluted blue and green oysters was metal-dependent.As indicated in Table 1, the average bioaccessibility of Cu was relatively high (89.0 and 74.7 % in green and blue oysters, respectively), and those of Cd and Zn were comparatively lower. The average bioaccessibility of Cd was 41.8 and 36.7 % in green and blue oysters, respectively, and that of Zn was 33.4 and 29.0 % in green and blue oysters, respectively. The phenomenon that the bioaccessibility of Cu was relatively higher than those of Cd and Zn was observed not only in these severely contaminated colored oysters but also in oysters with only slight metal contamination (He and Wang 2013). However, different results were observed in some uncontaminated fish where the bioaccessibility of Zn was lower than those of Cu and Cd (He et al. 2010) and in some contaminated oysters from France and the United Kingdom where the bioaccessibility of Cu was a little lower than those of Zn and Cd (Bragigand et al. 2004). Such high Cu bioaccessibility in our study might be attributed to the extremely high Cu content and Cu being in a special binding form in oyster tissues. When these oysters were exposed to extraordinarily high concentrations of Cu for a long time, a large proportion of the Cu would first bind with metal-sensitive fractions (MSFs) before being transferred to some subcellular compartments, such as the biologically detoxified metal (BDM), for detoxification. However, the binding sites of these subcellular compartments were limited, so the remainder of the accumulated Cu would transfer to other subcellular fractions or compartments (such as the trophically available metal TAM) in these oysters because of the extraordinarily high Cu concentration in their ambient environment (Campbell and Hare 2009; Wallace et al. 2003; Wallace and Luoma 2003). The TAM showed the trophically available metals, which were composed of the subcellular fractions organelles, HSP and HDP. Most of these fractions that consisted of the TAM were soluble fractions and easily bioaccessible and bioavailable. Thus, a large amount of Cu bonded with TAM was easily digestible, and, accordingly, the bioaccessibility of Cu was relatively high in these green and blue oysters (Campbell and Hare 2009).

The metal subcellular distribution in lightly polluted or nonpolluted shellfish and fish was significantly correlated with metal bioaccessibility and might be a good predictor of metal bioaccessibility. The relationship between metal bioaccessibility and subcellular distribution varied substantially with metal species. The bioaccessibilities of As, Cd, Cu, Se, and Zn in two marine fish species were found

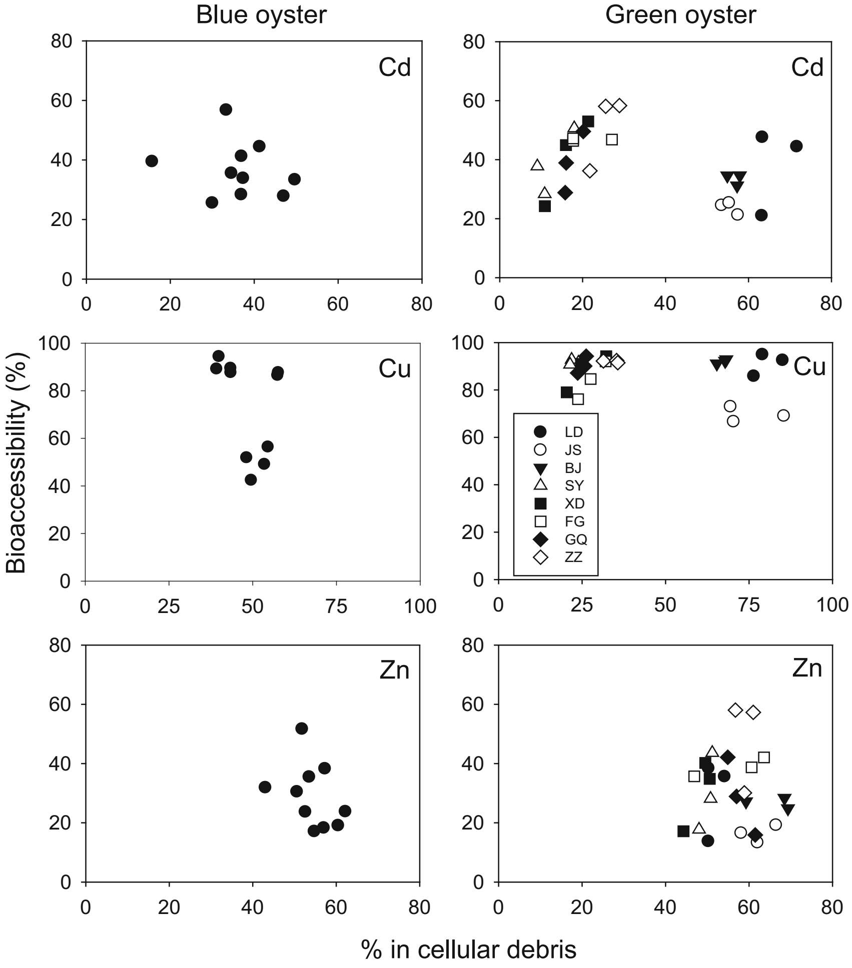


Fig. 3 Relationship between bioaccessibility and subcellular partitioning in Cellular debris of Cu, Cd and Zn in blue oysters ($n = 10$) and green oysters ($n = 24$); LD, JS, BJ, SY, XD, FG, GQ and ZZ were the sampling stations of green oysters

Table 1 Daily intake of metals through oyster consumption by people in China

Oyster species	Average concentration (μg/g ww)[a]	Average bioaccessibility (%)	EDI (μg/kg bw/d)	ADI (μg/kg bw/d)	RfD(μg/kg bw/d)	HQ
C. angulata						
Cd	5.68	41.8	2.901	1	1	2.90
Cu	717.9	89.0	780.8	500	40	19.5
Zn	2438.8	33.4	995.4	300	300	3.32
C. hongkongensis						
Cd	3.85	36.7	1.727	1	1	1.73
Cu	3594.5	74.7	3281.3	500	40	82.0
Zn	5262.5	29.0	1865.0	300	300	6.22

Hazard quotient = EDI/RfD. If the ratio is <1, there is no obvious risk

ADI allowed daily intake from the provisional tolerance weekly intake set by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (2003), *RfD* reference doses of trace elements as established by USEPA (2005)

[a] Average wet weight concentration was converted from the average dry weight concentration (cited from Wang et al. 2011) by a wet/dry weight conversion factor of 4

to be positively correlated with metal distributed in HSP and TAM, whereas they were negatively correlated with metal partitioning in MRG and cellular debris (He et al. 2010). However, the bioaccessibility of methylmercury in 8 marine fish species was proven to be negatively correlated with its subcellular partitioning in MRG and TAM

(He and Wang 2011). The bioaccessibility of Cd in 11 marine shellfish from five stations in China was negatively related to its distribution in cellular debris and positively related to its distribution in HSP and TAM, whereas Cu in these shellfish was negatively correlated with its distribution in cellular debris and positively correlated with its distribution in HDP, HSP and TAM; Zn in these shellfish was only negatively correlated with its distribution in MRG (He and Wang 2013).

Measuring the subcellular distribution of metals may also be an effective method for predicting the bioaccessibility and bioavailability of metals in these severely polluted colored oysters, but this has never been tested in previous studies. Thus, a correlation analysis of metal bioaccessibility and subcellular distribution was performed for the green and blue oysters. However, the same relationship between bioaccessibility and subcellular distribution was not discovered for Cu, Cd, and Zn in colored oysters in this study. There was no significant correlation between the bioaccessibility and any of the subcellular fractions for each of the metals in our study (Table S1; Figs. 3, 4, 5). This result was different from those of shellfish or fish that are not polluted or are only slightly polluted by heavy metals, which might be attributed to their different contamination levels, accumulation patterns, and subcellular distributions.

Increased metal concentrations would lead to higher distribution into the relatively refractory MRG fractions, which may in turn reduce metal bioaccessibility. However, a significant relationship was only found between metal bioaccessibility and its tissue concentration, instead of between metal bioaccessibility and subcellular distribution. This may suggest that in the case of the colored oysters examined in this study, metal concentration instead of metal subcellular distribution could be the driving factor of the metal bioaccessibility.

Risk Assessment

In the current risk assessment of contaminants in foods, only the total tissue concentration of contaminants is widely accepted. However, compared with the total tissue concentration, the bioaccessibility of contaminants could show better performance in the bioavailability of contaminants

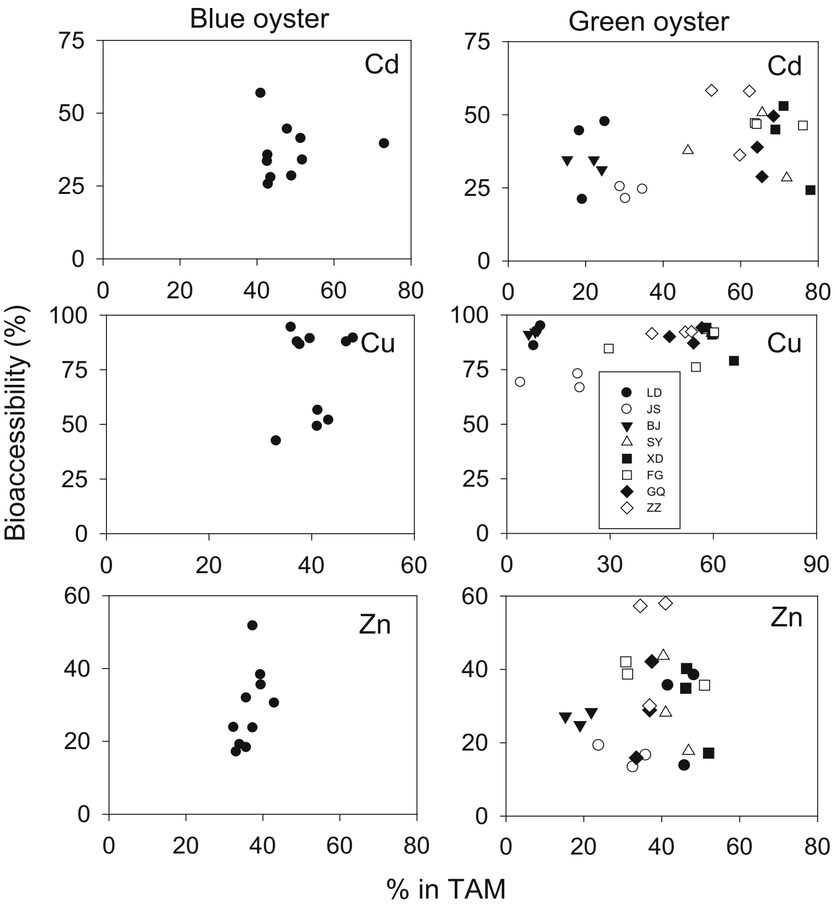


Fig. 4 Relationship between bioaccessibility and subcellular partitioning in TAM of Cu, Cd and Zn in blue oysters ($n = 10$) and green oysters ($n = 24$); LD, JS, BJ, SY, XD, FG, GQ and ZZ were the sampling stations of green oysters; (TAM was defined as a sum of organelles, HDP, and HSP and was reconstructed from % in organelles, HDP and HSP)

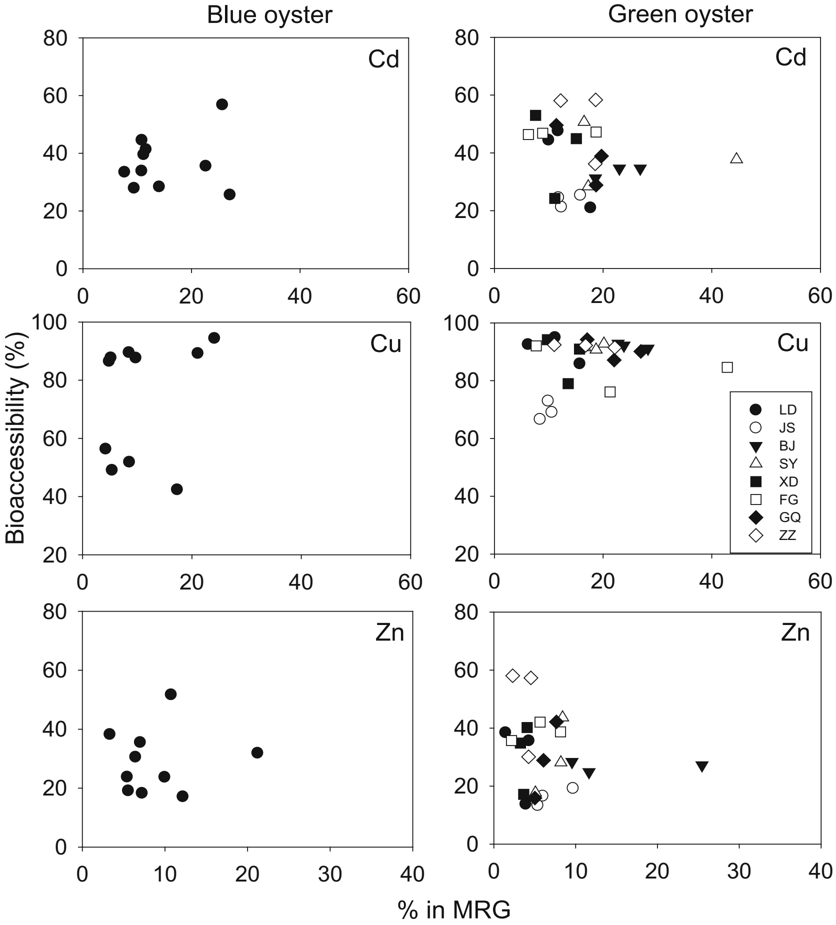


Fig. 5 Relationship between tissue concentration and subcellular partitioning in MRG of Cu, Cd and Zn in blue oysters ($n = 10$) and green oysters ($n = 24$); LD, JS, BJ, SY, XD, FG, GQ and ZZ were the sampling stations of green oysters

ingested with foods and the risk assessments of contaminants. Our results showed that the bioaccessibilities of Cu, Cd and Zn in green and blue oysters were negatively correlated with tissue concentration. Thus, the tissue concentration of contaminants in foods did not always effectively and accurately reflect the actual risks and hazards to humans. Therefore, the tissue concentration measurement alone was not enough; the determination of bioaccessibility also must be incorporated to improve the risk assessment of the contaminants ingested with colored oysters.

In this study, a health risk assessment of Cu, Zn, and Cd in green oysters (*C. angulata*) and blue oysters (*C. hongkongensis*) was performed based on the HQ, which incorporated the tissue concentration, bioaccessibility, EDI, and RfD (USEPA 2005) of the three metals. The HQ values of Cd, Cu, Zn in green oysters and blue oysters have exceeded 1 (Table 1), which shows that the risks and hazards of green and blue oysters were moderate according to guidelines and that the bioaccessible concentration had exceeded the safety levels developed by the USEPA (2005). The HQ value of Cu was extraordinarily high, with a value of 19.5 in green oysters and 82.0 in blue oysters, which showed that the hazards and risks were quite high and that the bioaccessible concentration of Cu exceed the safety levels developed by the USEPA by 19.5 times and 82.0 times, respectively (USEPA 2005). The risk assessment mentioned above was based on the average concentration and average bioaccessibility of the metals. For metals whose concentration and bioaccessibility are higher than the average, the concentration and bioaccessibility in some oyster individuals might present even more hazards and risks. The results of our study showed that these colored oysters present exceedingly serious health hazards to humans, especially for Cu, which should receive more attention and concern from the public.

The relationship of the human health risks (the HQ values) with the tissue concentration and the bioaccessibilities of Cd, Cu, Zn in green and blue oysters was examined. Significant positive correlations were observed between HQ values and tissue concentrations for Cd and Cu in blue oysters and Cd, Cu, and Zn in green oysters (Fig. 6), which indicated that tissue concentration was an important factor affecting the health risks of these metals in these severely contaminated colored oysters. From the

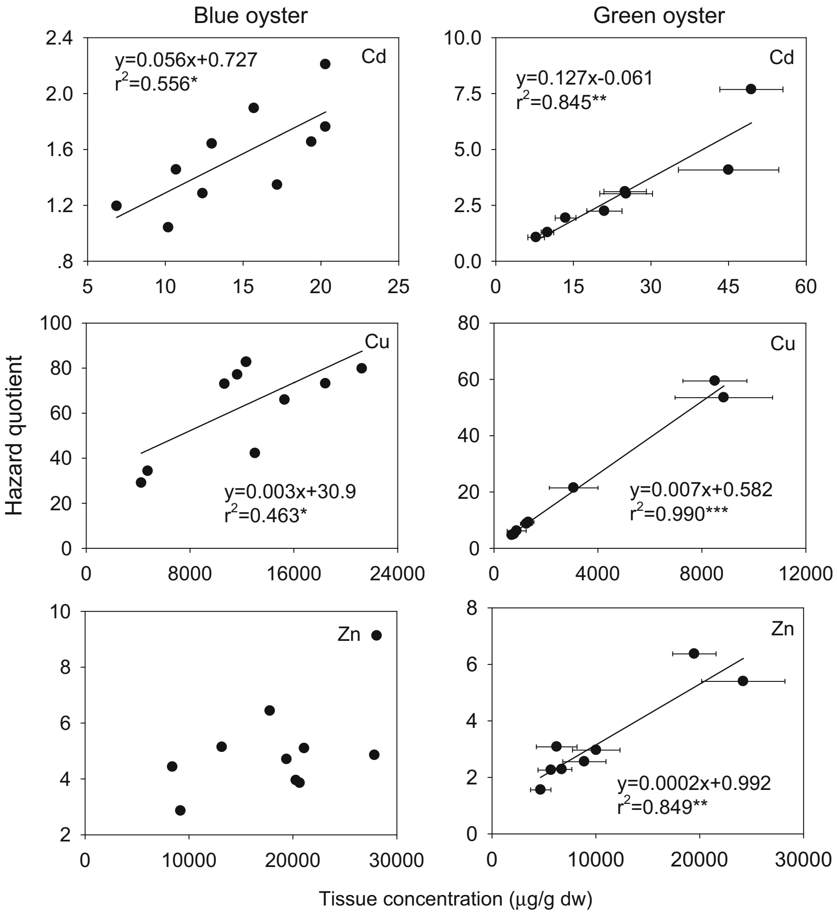


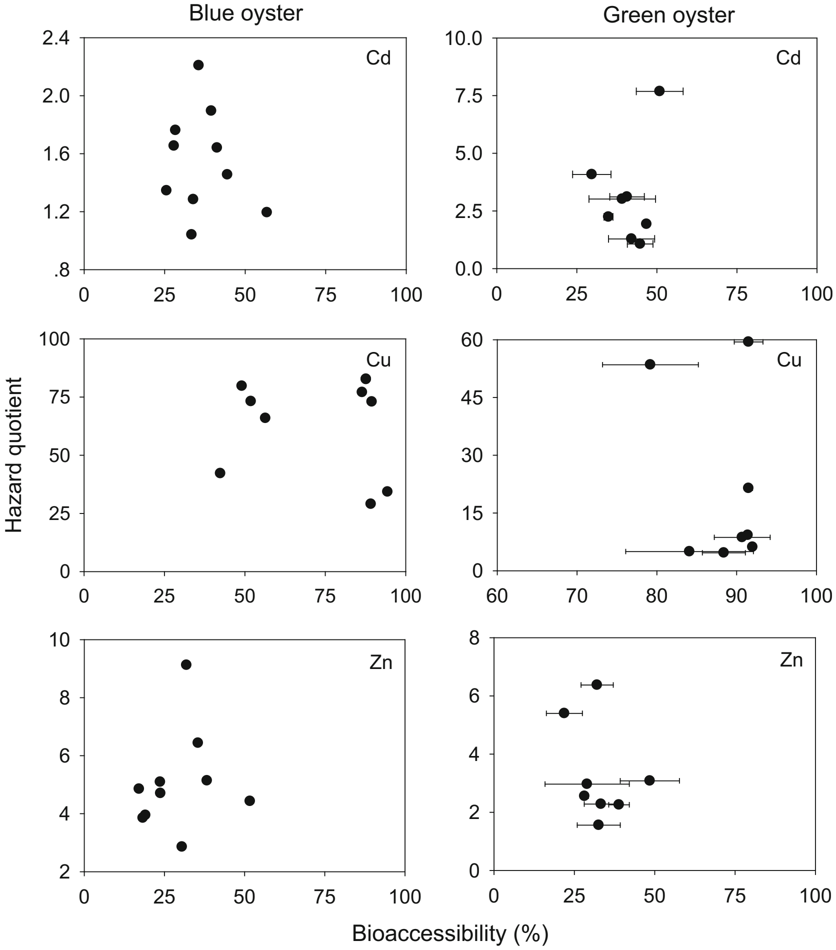
Fig. 6 Relationships of HQ values with tissue concentration of Cu, Cd and Zn in blue oysters ($n = 10$) and green oysters ($n = 24$). Average tissue concentrations and hazard quotients of oysters from eight stations were used for green oysters. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

regression coefficients in Fig. 6, we can see that the correlation between the HQ values and the tissue concentration of Cd, Cu, and Zn in green oysters ($p < 0.01$, $p < 0.001$, $p < 0.01$) was better than those in blue oysters ($p < 0.05$). This result showed that the correlation between the HQ values and the tissue metal concentrations was related to the pollution levels for these severely contaminated colored oysters. The heavier the pollution levels, the poorer the correlations. However, there was no significant correlation between HQ values and the metal bioaccessibilities in green and blue oysters in our study (Fig. 7). The influence of metal bioaccessibilities on the human health risks was limited for these severely contaminated colored oysters.

Conclusions

The bioaccessibilities and human health risks of Cd, Cu, and Zn in soft tissues of green (*C. angulata*) and blue oysters (*C. hongkongensis*) were studied. The total tissue concentrations of the three metals indicated that the green oysters and blue oysters were severely contaminated by Cd, Cu, and Zn, which far exceeded Chinese or international safety levels. The bioaccessibilities of the three metals in these oysters ranged from 13.4 to 95.0 %, which seemed to be lower than that in oysters without severe contamination. The highest bioaccessibility was observed for Cu, which suggests that Cu is the most highly bioavailable metal to humans among the three elements in these oysters. The bioaccessibility of Cu, Cd, and Zn in green and blue oysters decreased with increased tissue concentration. A significant relationship was only found between metal bioaccessibility and metal tissue concentration instead of between metal bioaccessibility and subcellular distribution. This may suggest that in the case of the colored oysters examined in this study, metal concentration instead of metal subcellular distribution could be the driving factor of metal bioaccessibility. The results of the health risk assessment, which combined metal bioaccessibilities, indicated that Cd, Cu, and Zn levels in these severely contaminated green and blue oysters have exceeded the safety levels developed by the USEPA, especially for Cu, which has exceeded the safety levels by more than 19.5 times and 82.0 times in green and blue oysters, respectively. The results showed that these oysters present serious health hazards to humans,

Fig. 7 Relationships of HQ values with bioaccessibility of Cu, Cd and Zn in blue oysters ($n = 10$) and green oysters ($n = 24$). Average tissue concentrations and hazard quotients of oysters from eight stations were used for green oysters



especially for Cu, and this fact should receive more attention and concern from the public. The influence of metal bioaccessibilities on human health risks was limited for these severely contaminated colored oysters. In contrast, tissue concentration was the driving factor affecting the health risks of these metals in these severely contaminated colored oysters, which was positively correlated with the human health risks.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflicts of interest. The submitted work has not received any financial support from any third party, and there is no financial relationship with any entities. All of the financial organizations associated with this work have been disclosed. There is no patent, whether planned, pending or issued, broadly relevant to the submitted work.

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