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# Primary Investigation of Heavy Metal Contamination Status in Molluscs Collected from Chinese Coastal Sites

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Heavy metals have brought severe pollution in aquatic system for their wide use in industries. Due to the special deposition properties, heavy metals can persist in environment for a long time. They can be effectively accumulated in plants and animals, transferred to human bodies by food chains and their levels can be increased by biological enrichment. Acute exposure and chronic low-dose exposure to heavy metals can lead severe adverse effects to organs, the central nervous system and the immune system. Therefore, some related regulations to restrict the emission of heavy metals and the maximum permissible levels (MPLs) in food have been set up in many countries/areas or organizations to protect human health.

China was famous for its high-speed economic development during the past twenty years. But the concomitant environmental deterioration emerged along with it, especially the worsening of the marine environment. According to China Marine Environmental Quality Communique in 2001 (Zhan et al., 2002), most estuaries, bays and seas abutting big cities had high contamination of heavy metals Many marine mollusks annihilated and even the living marine mollusks in the coastal sea area abutting the pollution sources are not suitable for consumption. The fisher folk lost their main food source and ran the risk of being poisoned. However, little data on heavy metal pollution in mollusks of Chinese seawater were published, and the corresponding criteria concerning about the marine environmental quality was absent in China. Accordingly, it was of great importance to carry out a research on the contamination status of heavy metals in Chinese mollusks. In this work 64 popular and delicious mollusks were collected from six coastal sites. The main aims of our study were to investigate the heavy metal contaminations in mollusks and provide basic data to help the establishment of the biological criteria concerning about the marine environmental quality.

## MATERIALS AND METHODS

Agilent 7500i inductively coupled plasma-mass spectrometer (ICP-MS)(Agilent

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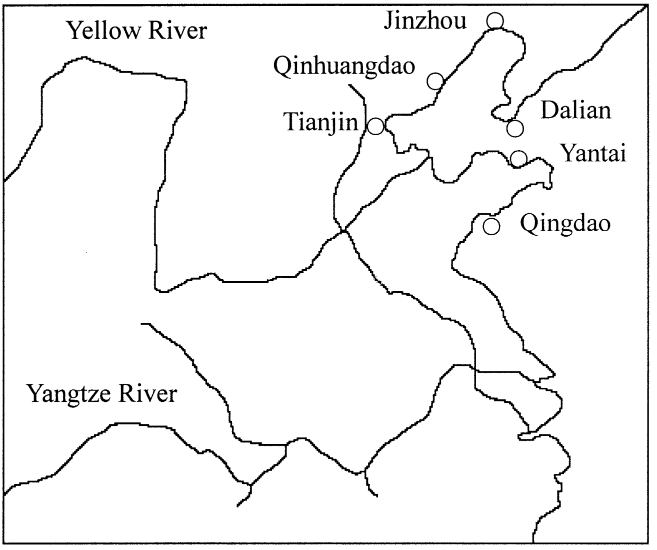


**Figure 1**. Sampling sites for mollusk samples.

Technologies Co. Ltd., USA) was used for the determination of elements. The working parameters are listed below: RF power 1450 w; Sampling depth 9.5 mm; Carrier gas flow rate 0.85 l/min; Makeup gas flow rate 0.3 l/min; Spray Chamber temperature 2℃; Sample uptake rate 0.4 ml/min. The orifice of nickel sampling cone and skimmer cone is 1.0mm and 0.4mm, respectively. Nitric acid and hydrogen peroxide were guarantee reagents (G.R.) and purchased from Merck and Beijing Chemical Reagents Company, respectively. The mixture of 1.0 μg/ml Sc (45), Ge (72) and In (115) in 1% v/v nitric acid was used as calibration internal standard.

Figure 1 shows the sampling sites for mollusks. These sites spread out along the Bohai and Huanghai Sea in the northeast of China where the heavy industries and the fish culture were relatively highly developed and serious environmental pollution occurred in the aquatic system. The mollusks were commercially obtained on the local retail market during the fishing time, which were popular for the local residents and the tourist guests.

The soft tissues of mollusks were excised by stainless steel scalpel blades, and then thoroughly rinsed with Milli-Q water to remove extraneous impurities. After the sufficient homogenate by a blender, the samples were kept at -18℃ until analysis. Approximately 1g (wet weight) of soft tissues were weighed in a PTFE digestion container. Each sample was added with 3ml of concentrated nitric acid and left to predigest overnight at 40℃. After cooling, 2ml of 30 % hydrogen peroxide was added. Thereafter the container was covered and placed in a high-pressure stainless steel bomb then put in an oven. The oven temperature was increased to 160℃ and kept for 4h. After cooling, the solution was diluted with Milli-Q water and transferred into PET bottle to 50g. As, Cd, Cr, Hg, Pb and Sn were determined with ICP-MS. All analyses were repeated thrice by external calibration method. The accuracy of the method was validated by the determination of Prawn (GBW 08572) and Mussel (GBW 08571) certified reference materials.

## RESULTS AND DISCUSSION

In order to prove the validity of the method, we analyzed two certified reference materials and the results are reported in Table 1. The determined values of all elements were in good agreement with the certified values, suggesting the proposed method was feasible in the determination of heavy metals in biota samples. The absolute detection limit of As, Cd, Cr, Hg, Pb and Sn were 0.46, 0.16, 1.41, 0.37, 3.15, 0.29 ng/g, separately.

**Table 1.** Comparison of heavy metal contents (μg/g) in certified reference materials.

| Element | Prawn (GBW 08572) | | Mussel (GBW 08571) | |
|---|---|---|---|---|
| | Certified | Found | Certified | Found |
| Cr (53) | 0.240 ± 0.03 | 0.280 ± 0.05 | 0.570 ± 0.04 | 0.630 ± 0.03 |
| As (75) | 1.420 ± 0.03 | 1.660 ± 0.01 | 6.100 ± 0.6 | 6.850 ± 0.08 |
| Cd (111) | 0.023 ± 0.002 | 0.025 ± 0.002 | 4.500 ± 0.3 | 4.780 ± 0.06 |
| Hg (202) | 0.201 ± 0.002 | 0.188 ± 0.032 | 0.067 ± 0.004 | 0.061 ± 0.003 |
| Pb (208) | 0.298 ± 0.010 | 0.263 ± 0.009 | 1.960 ± 0.05 | 1.666 ± 0.002 |

Heavy metal contents in the collected mollusks were measured by the method described above and the results are shown in Table 2. It was clear that all samples were contaminated with different levels of heavy metals. In order to investigate the detailed pollution status, we further discussed each tested heavy metal's level in mollusk samples and their possible sources. Comparison of pollution in tested mollusks with the maximum permissible levels (MPLs) was carried out to clarify the pollution extent and the possible hazards to human health.

Cd contents in mollusks varied from 0.06 μg/g to 28.08 μg/g, with the mean value of 2.16 μg/g. Compared with MPLs of heavy metals in food set up by WHO, there were 22 mollusks contained Cd levels exceeding 2 μg/g, as could be seen from Table 2. As a whole, *Rapana venosa*, *Amusium*, *Scapharca subcrenata* and

**Table 2.** Heavy metal contents in mollusk samples (μg/g, wet weight).

| Sites | Species | Sample name | As | Cd | Cr | Hg | Pb | Sn |
|---|---|---|---|---|---|---|---|---|
| Yantai | Gastropods | Rapana venosa | 5.17 | 2.02 | 0.08 | 0.01 | 0.13 | 0.03 |
| | | Neptunea arthritica cumingii | 32.71 | 1.61 | 0.40 | 0.06 | 0.18 | 0.05 |
| | | Neverita didyma | 6.65 | 0.21 | 0.28 | 0.03 | 0.13 | 0.03 |
| | Bivalves | Mytilus edulis | 1.48 | 0.88 | 0.18 | 0.04 | 0.29 | 0.03 |
| | | Crassostrea talienwhanensis | 1.31 | 1.63 | 0.16 | 0.03 | 0.18 | 0.05 |
| | | Sinonovacula constricta | 1.55 | 0.77 | 0.23 | 0.02 | 0.18 | 0.05 |
| | | Amusium | 1.15 | 3.15 | 0.17 | 0.02 | 0.22 | 0.02 |
| | | Scapharca subcrenata | 1.10 | 1.39 | 0.15 | 0.02 | 0.25 | 0.09 |
| | | Meretix meretrix | 1.37 | 0.19 | 0.28 | 0.01 | 0.42 | 0.06 |
| | | Mactra veneriformis | 0.57 | 0.14 | 0.32 | 0.01 | 0.21 | 0.06 |
| | | Ruditapes philippinarum | 2.74 | 0.11 | 0.14 | 0.03 | 0.13 | 0.03 |
| Tianjin | Gastropods | Neverita didyma | 6.94 | 0.77 | 0.78 | 0.04 | 0.24 | 0.03 |
| | Bivalves | Amusium(small) | 2.32 | 1.43 | 0.90 | 0.01 | 0.14 | 0.17 |
| | | Amusium(large) | 2.54 | 6.09 | 1.28 | 0.02 | 0.42 | 0.04 |
| | | Mytilus edulis | 1.59 | 1.42 | 0.59 | 0.04 | 0.34 | 0.27 |
| | | Meretix meretrix | 1.46 | 0.38 | 1.04 | 0.01 | 0.19 | 0.07 |
| | | Cyclina sinensis | 1.42 | 0.11 | 1.44 | 0.01 | 0.24 | 0.18 |
| | | Ruditapes philippinarum | 2.04 | 0.24 | 1.18 | 0.01 | 0.21 | 0.06 |
| | | Mactra veneriformis | 2.38 | 0.07 | 0.82 | 0.01 | 0.17 | 0.41 |
| | | Crassostrea talienwhanensis | 1.35 | 1.21 | 1.00 | 0.01 | 0.17 | 0.03 |
| | | Sinonovacula constricta | 3.10 | 0.19 | 1.51 | 0.02 | 0.35 | 0.08 |
| | | Scapharca ubcrenata(middle) | 1.32 | 2.02 | 0.81 | 0.01 | 0.16 | 0.05 |
| | | Scapharca subcrenata(large) | 0.93 | 1.60 | 0.99 | 0.02 | 0.32 | 0.12 |
| | | Scapharca broughtonii | 2.45 | 3.93 | 0.85 | 0.02 | 0.20 | 0.06 |
| | | Atrina pectinata | 3.02 | 3.43 | 1.14 | 0.01 | 0.49 | 0.08 |
| Dalian | Gastropods | Rapana venosa | 11.31 | 22.25 | 0.08 | 0.03 | 0.19 | 0.05 |
| | | Neptunea arthritica cumingii | 53.83 | 2.65 | 0.27 | 0.04 | 0.12 | 0.02 |
| | | Neverita didyma | 12.31 | 0.65 | 0.37 | 0.05 | 0.19 | 0.09 |
| | Bivalves | Mytilus edulis | 2.86 | 1.11 | 0.29 | 0.04 | 0.42 | 0.08 |
| | | Crassostrea talienwhanensis | 2.60 | 1.26 | 0.19 | 0.02 | 0.22 | 0.08 |

**Table 2** Continued.

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Sinonovacula constricta | 1.26 | 0.06 | 0.16 | 0.02 | 0.15 | 0.03 |
| | | Amusium | 1.33 | 3.68 | 0.19 | 0.03 | 0.31 | 0.03 |
| | | Scapharca subcrenata | 1.08 | 2.21 | 0.15 | 0.01 | 0.10 | 0.03 |
| | | Meretix meretrix | 1.22 | 0.18 | 0.29 | 0.02 | 0.24 | 0.07 |
| | | Mactra veneriformis | 1.04 | 0.16 | 0.44 | 0.02 | 0.27 | 0.05 |
| | | Ruditapes philippinarum | 1.72 | 0.36 | 0.29 | 0.03 | 0.10 | 0.04 |
| Qingdao | Gastropods | Rapana venosa | 9.52 | 4.21 | 1.05 | 0.07 | 0.14 | 0.00[a] |
| | | Neptunea arthritica cumingii | 2.66 | 0.20 | 0.93 | 0.04 | 0.07 | 0.02 |
| | | Umbonium thomasi | 7.88 | 5.59 | 2.23 | 0.05 | 0.17 | 0.02 |
| | | Eulima bilineata | 3.32 | 1.08 | 3.48 | 0.08 | 0.20 | 0.01 |
| | | Natica clausa | 5.47 | 0.88 | 1.90 | 0.04 | 0.02 | 0.01 |
| | Bivalves | Chlamys farreri | 1.83 | 1.62 | 1.06 | 0.03 | 1.21 | 0.40 |
| | | Ruditapes philippinarum | 2.48 | 0.35 | 1.52 | 0.02 | 0.19 | 0.01 |
| | | Crassostrea gigas | 2.10 | 0.75 | 1.20 | 0.02 | 0.24 | 0.22 |
| | | Solen grandis | 2.99 | 0.11 | 1.86 | 0.02 | 0.11 | 0.01 |
| Qin huang dao | Gastropods | Neverita didyma | 13.88 | 1.38 | 1.28 | 0.03 | 0.20 | 0.10 |
| | Bivalves | Amusium | 2.32 | 3.13 | 1.21 | 0.01 | 0.32 | 0.03 |
| | | Hiatula diphos | 4.17 | 0.53 | 1.01 | 0.03 | 0.20 | 0.07 |
| | | Meretix meretrix | 1.45 | 0.38 | 1.01 | 0.00[b] | 0.20 | 0.05 |
| | | Ruditapes philippinarum | 2.67 | 0.58 | 1.94 | 0.01 | 0.33 | 0.12 |
| | | Cyclina sinensis | 1.75 | 0.18 | 1.47 | 0.01 | 0.36 | 0.13 |
| | | Crassostrea talienwhanensis | 1.28 | 2.04 | 0.60 | 0.01 | 0.13 | 0.03 |
| | | Sinonovacula constricta | 1.22 | 2.26 | 0.25 | 0.01 | 0.09 | 0.03 |
| | | Solen grandis | 2.95 | 0.32 | 1.30 | 0.02 | 0.29 | 0.23 |
| | | Scapharca subcrenata | 1.68 | 5.17 | 0.26 | 0.02 | 0.09 | 0.03 |
| | | Scapharca broughtonii | 3.18 | 4.43 | 0.86 | 0.02 | 0.33 | 0.03 |
| | | Mya arenaria | 2.63 | 0.09 | 1.01 | 0.01 | 0.61 | 0.19 |
| | | Cryptomya busoensis | 7.00 | 0.21 | 1.08 | 0.01 | 0.34 | 0.07 |
| | | Barnea dilatata | 2.01 | 0.08 | 0.84 | 0.01 | 0.44 | 0.05 |
| Jinzhou | Gastropods | Rapana venosa | 5.24 | 28.08 | 0.03 | 0.09 | 0.13 | 0.04 |
| | | Neverita didyma | 2.44 | 0.74 | 0.05 | 0.04 | 0.05 | 0.06 |
| | Bivalves | Scapharca subcrenata | 1.47 | 1.84 | 0.14 | 0.03 | 0.39 | 0.10 |
| | | Meretix meretrix | 1.15 | 1.67 | 0.28 | 0.08 | 0.46 | 0.05 |
| | | Mactra veneriformis | 1.41 | 0.47 | 0.09 | 0.02 | 0.13 | 0.07 |

a: The actual value was 0.001 μg/g.

b: The actual value was 0.002 μg/g.

*Scapharca broughtonii* manifested more Cd levels in the soft tissues, especially *Rapana venosa* from Jinzhou and Dalian. Several *Umbonium thomasi*, *Atrina pectinata*, *Crassostrea talienwhanensis* and *Sinonovacula constricta* also possessed higher Cd contents. The exceptionally high Cd level in *Rapana venosa* was probably caused by the Cd pollution in the sea. Liaodong Bay as one of the three bays of Bohai Sea suffered the most serious pollution due to the drainage of wastewater and waste residue from the chemical industries (Qiao, 1991). The Cd content in seawater and sediment was once high as 1.41 μg/L and 4.1 μg/g, respectively. As an acute toxic element, Cd can cumulate in human body for a long time and whose biological half-life are sixteen to thirty-eight yeas (Wang et al., 1998). Once entering human body, about 50% of Cd deposited in liver and kidney, which might affect people's health, or even to threaten their lives. Much attention should be paid when digesting these species of mollusks.

The levels of As ranged from 0.58 μg/g to 53.83 μg/g in samples with the mean value of 4.33 μg/g. Among all 64 samples, only *Scapharca subcrenata* collected in Tianjin and *Mactra veneriformis* from Yantai were contaminated with As below 1 μg/g. From Table 2, we found that gastropods loaded higher As contents than bivalves. *Rapana venosa*, *Neptunea arthritica cumingii* and *Neverita didyma* in all sampling sites showed high As pollution statues. According to the previous report, As was generally lower than 0.5 μg/g in human's food but could reach to 1-100 μg/g in halobios due to the process of biotransformation and enrichment (Larsen, 1995). The results obtained here were coincident with the conclusion above. Although the main species of As existed in halobios is arsonobetaine, which is of little toxicity and can be excreted from the bodies directly by urine, the toxic inorganic As still exists in halobios and its concentration increases with the increase of As contamination and could do harm to people's health when As polluted sea biota were drawn on for long period.

The collected mollusks contained Cr with the mean level of 0.77 μg/g (0.03 μg/g – 3.48 μg/g). There were 24 samples covered Cr level more than 1.0 μg/g (it is the limited value regulated by Hongkong government). The Cr contamination in the Bohai Sea was not outstanding and the probable pollution sources might be its main use in alloys and the general chemical industries.

Pb contamination in Bohai Sea was also very famous for the drainage of wastewater from zinc plant in Huludao. According to the investigation in 1988 (Qiao, 1991), the geometric mean of Pb contents in sediments presented to be 29.7 μg/g. Fortunately, Pb content in the selected mollusks was very low and was in the range of 0.02 μg/g -1.21 μg/g, with the mean level of 0.26 μg/g. This value was neglectable comparing with the enacted level established by WHO, 2.0 μg/g. Pb was not easy to be assimilated by organisms and its excretion was effectively influenced by calcium and protein contents in food. Approximately 90% Pb could be discharged by excretion (Wang et al., 1998). However, low levels of Pb could not be ignored, as Pb and its salts were capable of damaging the nerve system, hematosis and kidney in human body.

As one of the most poisonous elements, Hg pollution was mainly caused by the wastewater drainage of oil refining plant, chloroalkaline industry and petrochemical industry in Jinxi city, which is adjacent to the Liaodong Bay. Compared with the MPLs set up by WHO, 0.5ppm, it was exciting that Hg contents detected here were relatively low. The concentrations ranged from 0.002 μg/g to 0.09 μg/g with the mean value of 0.03 μg/g. Similar to As, Hg contents in gastropod species were generally higher than those in bivalves. The possible reasons might be that the bivalves are grass-eating mollusks, while the collected gastropod species are predatory flesh-eating mollusks and the main food of which are bivalves. So from the view of trophic level, the gastropods are superior to bivalves and can bioaccumulate As and Hg effectively. Though our results of Hg contents in mollusks satisfied us, the early survey (Fu et al., 1992) about the Hg accumulation in human hair in Jinzhou Bay indicated that the Hg loading in fisher folk was higher than that in peasant due to their different food species. Excess seafood ingestion made the fisher folk ran more risk of Hg poison.

Sn pollution in aquatic system was mainly introduced in the form of organotin compounds by the use of antifouling paints, PVC stabilizers, pesticides and other usages. Sn pollution in Chinese coastal sites was paid much attention (Jiang et al., 2001; Zhou et al., 2001) and it was quite serious in the big ports. The mean content detected here was 0.08 μg/g. As tin usually existed in virulent alkyl forms such as tributyltin, the pollution status was worth being worried about yet.

The contamination levels of As, Cd, Cr, Hg, Pb and Sn in mollusks were detected in 64 samples colleted from Chinese northeast ocean area in this paper. Universal pollution of these six tested heavy metals existed in these samples, wherein cadmium contamination was obviously most severe. Gastropods possessed higher levels of As and Hg than bivalves. Mollusks were popular seafood for people. It might be dangerous for people's health if these polluted seafoods were uptaken for a long period. Much attention should be paid to the food safety. Strict policies have been established to restrict heavy metals from releasing to aquatic system in China. Heavy metal pollution would be an agonizing problem over a long period of time because of the long-persistence of heavy metals in environment. Necessary steps should be taken to prevent the potential deleterious effects of those polluted seafoods on people.

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