Ecotoxicology and Environmental Safety 198 (2020) 110670

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Determination and characterization of metal nanoparticles in clams and oysters

Qinfei Zhou[a,b], Lihong Liu[b], Nian Liu[b], Bin He[b], Ligang Hu[b,*], Lina Wang[a,**]

[a] College of Environment and Safety Engineering, Qingdao University of Science and Technology, Qingdao, Shandong, 266042, China
[b] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

ARTICLE INFO

Keywords:
Metal nanoparticles
sp-ICP-MS
Shellfish tissue
Alkali digestion

ABSTRACT

With the extensive application of nanotechnology, metal nanoparticles (MNPs) have been widely used, thus are universally detected in the environment. This has caused increasingly concerns due to their toxicity and the potential health risks they pose to humans. In this work, the concentrations and particle size distributions of MNPs and concentrations of associated metal ionic species in shellfish seafood (clams and oysters) were investigated using single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) and inductively coupled plasma mass spectrometry (ICP-MS). The MNPs in the clam and oyster tissues were extracted via an alkaline digestion method with a recovery rate of 95.9% (for gold nanoparticles (AuNPs)). Then total concentrations of 41 metal elements were measured in the two types of seafood, of which 20 were selected for sp-ICP-MS analysis. The results showed that 5 types of MNPs were detectable in clams (Y, La, Ce, Pr, Gd) and 5 types of MNPs were detectable in oysters (Y, La, Ce, Pr, Nd). Size distributions of MNPs in clams and oysters were in the range of 35–55 nm and 30–65 nm, respectively. Nanoparticle concentrations in clams and oysters ranged from 0.6 to 37.7 ng/g and 4.2–19.7 ng/g, and accounted for 3.4%–50% and 5.5%–46% of the total metal content, respectively. Based on this analysis, the health risks of metals in the two kinds of seafood were evaluated by comparing the Provisional Tolerable Weekly Intake (PTWI) with limits recommended by the World Health Organization (WHO)/Food and Agriculture Organization (FAO). These results provide important information about the presence of metal nanoparticles in seafood and, to the best of our knowledge, this is the first time that the nanoparticles of rare earth elements have been detected and reported in bivalve mollusc tissues.

1. Introduction

Currently nanoparticles (NPs) are widely used in everyday life. For example, nanoparticles are often used in skin care products, nano sensors, electronic products, fertilizers and so on (Bowman et al., 2018; Chen et al., 2018). Amongst these nanomaterials, metal nanoparticles (MNPs) are the most commonly used (Tamm et al., 2016). Metal nanoparticles can constantly migrate and transform in the environment (Tangaa et al., 2016), accumulate in biological tissue (Gajdosechova et al., 2016), and have the potential to adversely impact both the environment and human health (Liu et al., 2019), thus have recently become a point of increasing public concern. The toxicity of metal NPs is strongly related to physical and chemical properties of the particles themselves, including size, surface area, surface charge, chemical composition, and crystal structure (Sharifi et al., 2012). For example, Schrand et al. reported that smaller gold nanoparticles (10–50(Schmid and Riediker, 2008) nm) could be more toxic than larger gold nanoparticles (100–200 nm) in four different cells, as the smaller AuNPs could upregulate the pro-inflammatory expression genes il-1 (il-1), il-6 (il-6) and TNF (Schrand et al., 2010). Studies have also shown that the release of metal nanoparticles on metal ions can promote the production of free radical oxidizing substances (ROS) and increase the oxidative stress of cells, which can increase of the overall metal toxicity (Chen et al., 2017). Thus, exposure to MNPs can potentially pose great risks to food safety and human health (Huang et al., 2010; Hardy et al., 2018). Clams and oysters are the most common and frequently eaten seafood, thus it is important to determine the physical and chemical characteristics of MNPs (particles and ions concentration, size distribution, chemical composition) and to assess the environmental health risk that these nanoparticles pose in clam and oyster seafood.

* Corresponding author.
** Corresponding author.
E-mail addresses: lghu@rcees.ac.cn (L. Hu), lnwang2006@163.com (L. Wang).

https://doi.org/10.1016/j.ecoenv.2020.110670
Received 24 March 2020; Accepted 20 April 2020
Available online 25 April 2020

There are many commonly used methods that can be applied to detect and characterize MNPs, including electronic microscopy (EM), dynamic light scattering (DLS), energy dispersive X-ray spectroscopy (EDS), and ultraviolet visible spectroscopy (uv–vis) (Kim et al., 2012; Cornelis et al., 2013). Although EM technology can be effectively used to characterize the shapes and sizes of MNPs, many methods are limited by high detection limits, which fail to capture the extremely low concentration of MNPs in the environment, and the complexity of the matrix in which MNPs are commonly found (Yang et al., 2016). Recently, single particle mode inductively coupled plasma mass spectrometry (sp-ICP-MS) has been developed as a powerful tool for the analysis of nanoparticles in environmental and biological samples (Merrifield et al., 2017). This technique enables fast and sensitive detection of nanoparticles in complex matrix samples, allowing for quantification of particle size, size distribution, mass concentration, particle number concentration and the detection of the ionic species of metal nanoparticles (Veverkova et al., 2014; Li et al., 2019). Single particle ICP-MS has been successfully used to detect gold, silver, TiO2 and other nanoparticles in natural waters (Frechette-Viens et al., 2019), plants (Hu et al., 2018), chicken meat (Ramos et al., 2017), zebrafish (Sung et al., 2018) and other environmental samples (Peters et al., 2014).

Currently there are three major types of pre-treatment methods used prior to sp-ICP-MS analysis: acid digestion, alkali digestion and enzyme digestion (Deng et al., 2017; Loeschner et al., 2018; Vidmar et al., 2018). Alkaline and enzymatic digestion have been shown to give high recovery rates for nanoparticles from tissues when focusing on quantification endpoints of particle size and total mass. For example, Gray et al. and sung et al. successfully extracted AgNPs and AuNPs from ground beef, fleas, worms by using tetramethylammonium hydroxide (TMAH) (Gray et al., 2013); Yongbo Dan et al. successfully analyzed the absorption characteristics of AuNPs in tomato plants by enzymatic digestion (Dan et al., 2015); and Loeschner et al. and Janja Vidmar et al. successfully extracted the AuNPS from the spleen of rats and human tissue by alkaline and enzymatic digestion (Loeschner et al., 2014). We compared the alkaline and enzymatic digestion methods using shellfish and found that the alkali digestion method resulted in better digestion efficiency.

In this study, we chose two kinds of commonly consumed seafood (clams and oysters), cultured in shallow bays, and characterized the concentrations of metal nanoparticles in those samples. First we compared the metal particle backgrounds of different alkaline solutions. Then we analyzed nanoparticle recovery in alkaline solutions and in biological tissue environments. Subsequently, we characterized the total amount of metal elements in the two kinds of seafood. MNPs were then extracted from clam and oyster tissues by alkaline digestion and detected using sp-ICP-MS, allowing for the characterization of MNP concentration and particle size distribution. Finally, the health risks of metals were assessed in the two kinds of seafood.

2. Materials and methods

2.1. Chemicals and samples

Gold nanoparticle standard solution (particle sizes of 50 nm, PEG-stabilized) was purchased from Nanocomposix (San Diego, CA, USA) and confirmed by TEM (Supporting Information). Multi-element calibration standard 2 A (Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V, Zn, 100 mg/L in 5% HNO_3), and multi-element standard solution (Au, Fd, Pt, Ir, Ru, 100 mg/L, in 1 mol/L HCl; Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Ga, Li, Mg, Mn, Ni, Pb, Sb, Sn, Sr, Ti, Tl, V, Zn, 100 mg/L, in 2.5 mol/L HNO_3; La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Y, 100 mg/L in 1.0 mol/L HNO_3) were purchased from National Standards Network (Beijing, China). Single element standard solutions Hg (1000 mg/L in 2% HNO_3) and W (1000 mg/L in 2% HNO_3) were purchased from National Standards Network (Beijing, China).

A 25% (v/v) aqueous solution of tetramethylammonium hydroxide (TMAH, electronic grade) was obtained from American Alfa Aesar, Japanese TCI, Chinese Aladdin, Chinese Macklin, Chinese Ronlabs. Protease K was a serine protease (Mbiotech, Korea), and proteinase K-buffered was a mixture of calcium acetate and sodium dodecyl sulfate (purity > 98%, sigma-aldrich, USA). Acid digestion was performed using 65% nitric acid (Merck, Germany) and the promoter hydrogen peroxide (Merck, Germany). Triton X-100 was obtained from Sigma-Aldrich (St. Louis, USA). All reagents used were analytical grade or better. Ultrapure water (18.2 MΩ cm) was produced by a Milli-Q system from Millipore (Milford, Billerica, USA).

The clams and oyster seafood samples were collected from Red Island (Qingdao, ShangDong Province). Prior to digestion treatment, tissue surfaces were rinsed with pure water, cut into pieces, and placed in an ultra-low temperature refrigerator (Thermo Scientific Forma, USA).

2.2. Sp-ICP-MS method

The MNPs were analyzed by sp-ICP-MS (Agilent 8800, California, USA) and the data processing was conducted using the Mass Hunter 4.4 Workstation Software (G7201C, Version C.01.04, Agilent Technologies) (Wilbur et al., 2016). The dwell time used in the sp-ICP-MS application module was chosen based on previous studies. It has been determined that a long residence time may cause several small particles to be converted into one large particle for detection. However, a short dwell time can also be problematic, as it can cause one particle to be detected as multiple particles (Lee et al., 2014). Previous studies have recommended a residence time between 0.1 and 20 ms per point (Olesik and Gray, 2012). Therefore, in this study, the dwell time was set to 3 ms. The collection time was set to 60 s in the single particle analysis model. According to the method described by Pace et al., standard size (50 nm) AuNPs were used as references to determine transport efficiency (Pace et al., 2012). To avoid contamination between samples, each sample was rinsed with nitric acid (2%, v/v) and Triton X-100 (0.1%, v/v) prior to measurement. General instrumental operating parameters are presented in Table 1. After each sample was analyzed, the software automatically processed the original data, characterizing particle concentration, mass concentration, ion concentration, and particle size distribution. As this analytical method cannot identify chemical substances, the sizes of all detected metal particles were calculated based on the equivalent mass contained in a theoretical pure metal ball, and expressed by the equivalent particle size. Therefore, these measurements did not necessarily represent the actual particle size. If the shapes, densities and chemical compositions of metallic NPs are to be analyzed in detail, it is also necessary more techniques to characterize the nanoparticles.

Table 1
Instrumental parameters for sp-ICP-MS analysis.

Instrument parameter	Value
RF generator power	1550 (W)
Argon flow	15 ($Lmin^{-1}$)
Carrier gas	1.14 ($Lmin^{-1}$)
Make up gas	0 ($L\ min^{-1}$)
Nebulizer	Micromist
Nebulization Gas Flow	0.95 ($mLmin^{-1}$)
Triple cone	Ni
Peristaltic pump rate	0.1 (rps)
Sample flow rate	0.346 ($mLmin^{-1}$)
Spray chamber temperature	2 (°C)
Data acquisition mode	SP Analysis
Integration time	3 (ms)
Acquisition time	60 (s)

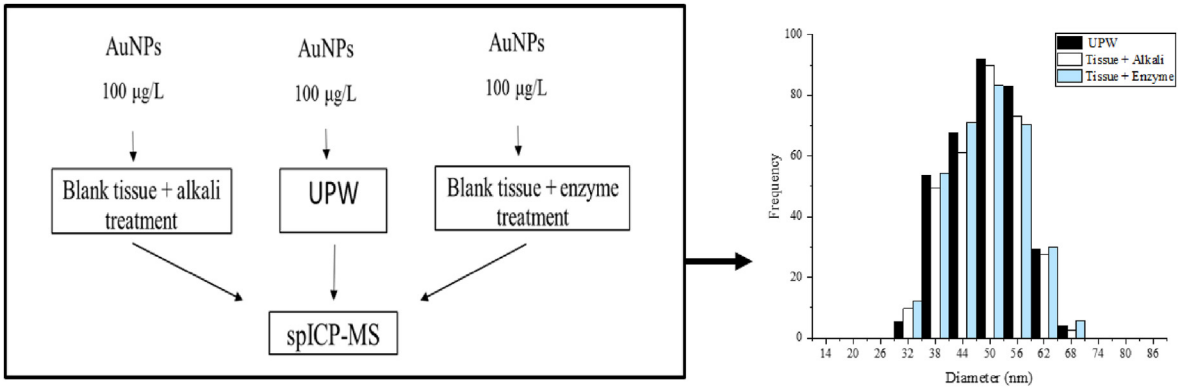

Fig. 1. The standard material AuNPs verifies the recovery rate of alkali treatment method and enzyme treatment.

2.3. Sample pretreatment for sp-ICP-MS analysis

Traditional acid-based tissue digestion methods cannot be applied for MNPs as they can easily dissolve the NPs and cannot often expose the nanoparticle targets in complex tissues. However alkali and enzymes have been successfully applied to NPs in some biological tissues. Thus, using an alkaline digestion technique, a mass of 0.1 g of wet seafood tissue was weighed and mixed 2 mL of TMAH (20% v/v). A vortex oscillator (Thermo Scientific, USA) was used to automatically mix the tube mixture, and helped prevent tissues from sticking to tube walls. The homogenized mixture was sonicated for 60 min at 37 ˚C using an ultrasonic bath instrument (KQ-300gvdv tri-frequency, Kun San ShuMei, China) to accelerate tissue breakdown and prevent particle aggregation. Then, to continue digestion, samples were further mixed using a shaker machine (Sk-o180-e, Scilogex, USA) at 80 r/min at room temperature (RT) for 24 h. The resultant colloid solution was filtered using Advantec paper (0.45 μm membranes, cellulose acetate). Digested samples were diluted to at least 1%TMAH concentration before they were injected into the sp-ICP-MS, and 0.1% Triton X-100 was used to dilute each injection to allow for the detection of single particles.

2.4. Determination of total metal contents by ICP-MS

The total concentrations of various metals were determined by subjecting the clam and oyster samples to acid digestion and quantifying with inductively coupled plasma mass spectrometry (ICP-MS, Agilent 8800, California, USA). Briefly, two seafood tissue samples, approximately 0.1 g each, were collected in 15 mL centrifuge digestion tubes, and 1 mL nitric acid (65% HNO_3) and 50 μL hydrogen peroxide (30% H_2O_2) were added. All samples were digested in a constant temperature water bath pot (Herry Tech HH-M6, Shanghai, China) at 90 ˚C for 1 h. All samples were filtrated through 0.45 μm membrane filters and were then diluted to 40 mL with ultrapure water. In total, 41 different metals were detected in each tissue.

3. Results and discussion

3.1. Selection of digestion solution

To find a suitable pretreatment method for the extraction of MNP from shellfish samples and eliminate background interference, we selected and optimized the particle extract solution. First, we tried two digestion methods and found that alkaline digestion was faster and more effective for seafood tissue (please refer to S1). Then, we compared different alkali solutions, and found there were differences in the background concentrations of metal particles in TMAH reagents of the same or different grades from different companies. We selected TMAH from five different companies (Japanese TCI, American Alfa Aesar, Chinese Aladdin, Chinese Macklin, and Chinese Ronlabs) to compare the backgrounds of several metals (Zn, La, Ce, Ag) during a pre-experiment. Amongst the five TMAH, we found that Alfa Aesar had the lowest background of Ce (Figure S2), Zn, La and Ag. Background information on the metal particles (Y, La, Ce, Pr, Gd, Nd) in the Alfa Aesar TMAH is provided in Supporting Information (Figure S4). In the shellfish tissues, the background of the 20 metal particles detected by Alfa Aesar alkali digestion solution was normal. Therefore, it was determined that the purities of solutions used is very important for the accuracy of single particle analysis.

3.2. Recovery of single particle

To verify the accuracy of the single particle ICP-MS method, we measured the recovery rate of AuNPs. AuNPs (100 μg/L) were added to pure water, the alkaline blank tissue solution and the enzymatic blank tissue solution. After normal alkaline digestion (Section 2.2) and enzyme digestion, solutions were diluted to the same concentration and sp-ICP-MS analysis was performed to compare AuNP recoveries. The recoveries were 95.9% and 103.6% in alkaline treatment solution and enzyme treatment solution, respectively. The pretreatment process for the three solutions and the particle size distributions of detected AuNPs are shown in Fig. 1. There was no significant change in particle size distribution, and the recovery was in the normal range, indicating that this method could be accurately used for AuNP detection.

3.3. Total content of metal elements in clams and oyster sample

The total concentrations of 41 different of elements, including common metals (Al, Ti, V, Mn, Fe, Co, Ni, Cu, Zn, Mo), toxic elements (Cr, Ga, Ge, As, Se, Sr, Cd, Sn, Sb, Ba, Hg), rare earth elements (Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) and others (Ag, W, Pt, Au, Bi) were measured in the clam and oyster samples using ICP-MS. As shown in Fig. 2, ten types of common metal elements (ranging from 347.2 to 53709.3 ng/g and 84.0–101681.7 ng/g), nine types of toxic elements (ranging from 21.2 to 12802.3 ng/g and 5.0–5385.1 ng/g), fifteen types of rare earth elements (ranging from 1.1 to 284.4 ng/g and 1.7–127.3 ng/g) and three types of other elements (ranging from 17.3 to 181.0 ng/g and 3.7–293.2 ng/g) were detected in clam and oyster samples, respectively. Sm was only detected in clams (11.8 ng/g). Another seven elements (Sn, Sb, Tb, Lu, Pt, Hg and Bi) were below the detection limits in both types of seafood samples.

According to regulations set in place for foods (GB 2762–2017) (GB2762-2017,2017) and agricultural products (GB, 18406.4–2001) (GB18406.4–2001,2001) in China, the limits for Cr, Cu, Cd and in shellfish are 1.0, 50 and 2.0 (mg/kg). Limits of Ni, As, Hg in aquatic meats and food are 1.0, 0.5, and 0.05 (mg/kg). The detected concentrations of these 6 kinds of metals in clams were 0.73 (Cr), 2.69 (Cu), 0.37 (Cd), 1.58 (Ni), 7.15 (As), and 0 (Hg) (mg/kg), and concentrations in oysters were 0.83 (Cr), 47.60 (Cu), 2.84 (Cd), 0.18 (Ni),

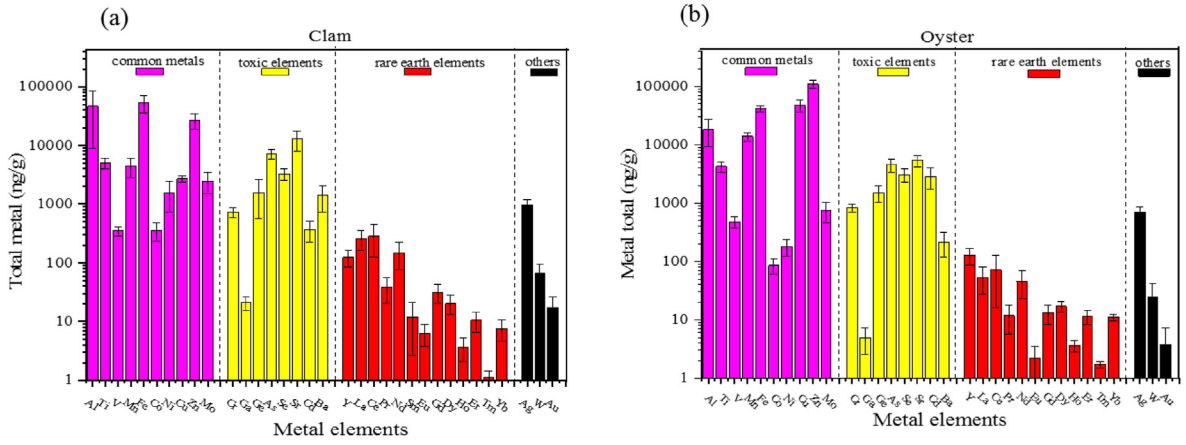

Fig. 2. Total content of metal elements in clams (a) and oysters (b).

4.54 (As), and 0 (Hg) (mg/kg). Therefore, we found that some metals were present at concentrations beyond the standard limits in the tested seafood (clams: Ni, As, oysters: As, Cd).

3.4. Analysis of metal nanoparticles in clams and oysters by sp-ICP-MS

Amongst the elements detected in this study, 20 (Co, Ni, Cu, Zn, Ge, As, Se, Sr, Y, Mo, Ag, Cd, Ba, La, Ce, Pr, Nd, Gd, Dy, Er) were selected for nanoparticle analysis by sp-ICP-MS. Samples were digested with TMAH, diluted to appropriate concentrations for single particle analysis, and then signal response and particle size distribution was detected (a part of data are displayed in Figure S4-S10). Average NMP sizes and size distributions were calculated by Gauss fitting. 5 types of metal nanoparticles (Y, La, Ce, Pr, Gd; concentrations ranging from 31.1 to 284.4 ng/g) were detected in clams and 5 types of metal nanoparticles (Y, La, Ce, Pr, and Nd; concentrations ranging from 11.6 to 127.3 ng/g) were detected in oysters, while nanoparticles of other elements were not detected in these samples.

In the three clam sample tested, nanoparticles were detected in two. A detailed distribution of metal nanoparticles, including particle mass, proportion and concentration size are shown in Table 2. The five detected nanoparticles were all derived from rare earth elements. In sample 3, the Ce nanoparticle (31.7 ng/g) had the highest concentration, accounting for 11% of the total, and the particle size distribution was 51 ± 4 nm (Figure S7). Gd was only detected in one sample (sample 3), with a mass concentration of 2.1 ng/g, accounting for 5.6% of the total, and a particle size distribution of 44 ± 2 nm (Figure S9) (see Table 3).

Opposed to what was observed in clams, Gd nanoparticles were not detected in the three oyster samples. However, Nd nanoparticles were detected, and the other detected nanoparticles (Y, La, Ce, Pr) were the same as those detected in clams. These five detected nanoparticles were also all rare earth elements. In sample 2 Ce particles were found to have the highest concentration (19.7 ng/g), accounted for 13.1% of the total content, and had a particle size distribution of 47 ± 4 nm (Figure S7). Both Y and Pr were only detected in sample 1, with mass concentrations of 4.6 ng/g and 8.4 ng/g, accounting for 5.5% and 15.1% of the total concentration, and demonstrating particle size distributions of 40 ± 4 nm and 36 ± 5 nm (Figure S5 and Figure S8).

Comparison of the two kinds of seafood nanoparticles detected, indicated that the same lanthanide elements particles tended to occur in the tested seafood. This may be because we tested seafood from the same area. However, to the best of our knowledge, no published literature has reported the detection lanthanide nanoparticles in seafood samples. In addition, there is currently a lack of relevant limit standards. Therefore, the detection of the lanthanide nanoparticles in our study suggests that the concentrations of lanthanide and associated nanoparticles in seafood should be of concern (Li et al., 2013).

3.5. Potential health risk of metals in clams and oyster

To estimate the potential health risks of the metals detected in the clam and oyster tissues in this study, a widely used risk assessment model was applied. According to the World Health Organization (WHO)/Food and Agriculture Organization (FAO) (FAO, 2003), the Estimated Weekly Intake (EWI) of seafood was estimated using the following formula (Agusa et al., 2007):

$$\text{EWI (mg /kg.bw)} = c \times \text{FIR} \times 7 / \text{WAB} \quad (1)$$

where c is the metal content in food (mg/kg), FIR is daily food intake rate (g/d), and WAB represents the average human weight (50 kg for an Asian adult).

The Provisional Tolerable Weekly Intake values (PTWI) recommended for shellfish by WHO/FAO were collected for Cr (0.4 mg/kg), Fe (5.6 mg/kg), Cu (3.5 mg/kg), Zn (7.0 mg/kg), As (0.042 mg/kg), Cd (0.007 mg/kg) and Hg (0.005 mg/kg). According to the estimation of weekly heavy metal intake (EWI) (Formula 1) the maximum the daily Food Intake Rate (FIR) for the 6 metal characterized in this study (Fig. 3) were calculated to be 4024 g/day (Cr), 863 g/day (Fe), 9438 g/day (Cu), 2002 g/day (Zn), 43 g/day (As), 154 g/day (Cd) in

Table 2
Mass concentration, particle mass ratio and particle size distribution in clams samples by sp-ICPMS.

Metal particles	Sample 1			Sample 2			Sample 3		
	Mass (ng/g)	Mass ratio (100%)	Size (nm)	Mass (ng/g)	Mass ratio (100%)	Size (nm)	Mass (ng/g)	Mass ratio (100%)	Size (nm)
Y	nd	nd	nd	2.4	3.4	42 ± 5	9.7	6.1	38 ± 5
La	nd	nd	nd	21.6	7.0	40 ± 4	34.6	27.1	41 ± 4
Ce	nd	nd	nd	5.9	8.8	46 ± 4	37.7	11.0	51 ± 4
Pr	nd	nd	nd	0.6	4.0	40 ± 5	4.1	9.2	44 ± 5
Gd	nd	nd	nd	nd	nd	nd	2.1	5.6	44 ± 2

Note: nd means not detected.

Table 3
Mass concentration and Particle mass ratio and particle size concentrated distribution in oyster samples by sp-ICPMS.

Metal particles	Sample 1			Sample 2			Sample 3		
	Mass (ng/g)	Mass ratio (100%)	Size (nm)	Mass (ng/g)	Mass ratio (100%)	Size (nm)	Mass (ng/g)	Mass ratio (100%)	Size (nm)
Y	4.6	5.5	40 ± 4	nd	nd	nd	nd	nd	nd
La	15.0	50.0	40 ± 5	8.5	9.5	38 ± 5	5.4	13.5	35 ± 5
Ce	12.9	46.0	46 ± 4	19.7	13.1	47 ± 4	7.1	19.7	43 ± 4
Pr	8.4	15.1	37 ± 3	nd	nd	nd	nd	nd	nd
Nd	4.2	16.2	56 ± 6	nd	nd	nd	5.5	16.7	59 ± 6

Note: nd means not detected.

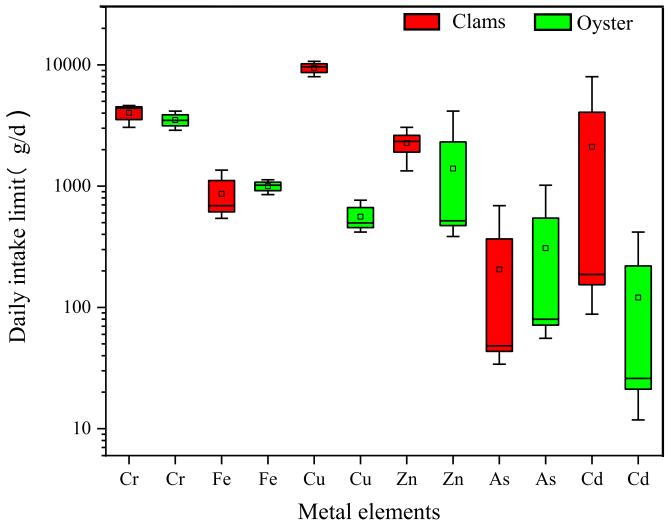

Fig. 3. Maximum daily intake rate in clams and oysters base on PTWI.

clams and 3509 g/day (Cr), 999 g/day (Fe), 560 g/day (Cu), 474 g/day (Zn), 72 g/day (As), 21 g/day (Cd) in oysters. These metal concentrations exceed the recommended standard intakes for As and Cd. Furthermore, when accounting for a long-term shellfish diet, Cu and Zn were also found to be potentially harmful to human health. Overall, concentrations of several detected metals (Cu, As, and Cd in clams; As and Cd in oysters) were much higher than was reported 10 years ago in Qingdao shellfish (clams and oyster) (Cu: 2.4 mg/kg and 55.7 mg/kg, As: 1.44 mg/kg and 0.16 mg/kg, Cd: 0.24 mg/kg and 2.23 mg/kg, Hg: 0.013 mg/kg and 0.054 mg/kg) ten years ago (Table S1) (Du et al., 2009). Increased levels of toxic metals are clearly harmful to human health.

However, China has different views on the risk of rare earth metals in food. The GB 2762-2017 standard had removed of rare earths in foods, and it needs to be revised and improved. Because the toxicity of rare earth elements is getting more and more attention in the environment (Zhang et al., 2013). This study shows that clams and oysters can accumulate rare earth elements, which can be used as a basis for assessing the health risks of rare earth metals in the future.

4. Conclusion

In conclusion, metal nanoparticles present in clams and oysters were detected and characterized using sp-ICP-MS. Six rare earth elements (Y, La, Ce, Gd, Pr, Nd) were detected in the clam and oyster samples using sp-ICP-MS and the nanoparticle sizes were generally in the range of 30–65 nm. However, as there is no established system for detecting rare earth metal nanoparticles in biological tissues, there may be some uncertainty in our method results. Thus, further studies are required. In addition, health risk assessment carried out in this study indicated that concentrations of Ni, As, and Cd in clams and As and Cd in oysters could potentially pose a risk to human health. This is especially important as there are currently no standard limits for metal nanoparticles, especially rare earth elements, in seafood. Therefore, this issue should be further studied, and more focus should be paid to the presence of metal nanoparticles in consumable products in the future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thanks to National Natural Science Foundation of China (91743203, 21605155, 21777179, and 21577153), Chinese Academy of Sciences Strategic Science and Technology Leader Special Category B (No.XDB14010400), Qingdao Municipal Science and Technology Commission (19-6-273-cg) and Thousand Talents Program for Young Talent, Sanming Project of Medicine in Shenzhen (No.SZSM201811070).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.110670.

References

Agusa, T., Kunito, T., Sudaryanto, A., Monirith, I., Kan-Atireklap, S., Iwata, H., Ismail, A., Sanguansin, J., Muchtar, M., Tana, T.S., Tanabe, S., 2007. Exposure assessment for trace elements from consumption of marine fish in Southeast Asia. Environ. Pollut. 145, 766–777.
Bowman, D.M., May, N.D., Maynard, A.D., 2018. Nanomaterials in cosmetics: regulatory aspects. Anal. Cos. Prod. 289–302.
Chen, G.C., Vijver, M.G., Xiao, Y.L., Peijnenburg, W.J.G.M., 2017. A review of recent advances towards the development of (quantitative) structure-activity relationships for metallic nanomaterials. Materials 10, 1013.
Chen, T., Zhang, M., Bhandari, B., Yang, Z.X., 2018. Micronization and nanosizing of particles for an enhanced quality of food: a review. Crit. Rev. Food Sci. Nutr. 58, 993–1001.
Cornelis, G., Pang, L.P., Doolette, C., Kirby, J.K., McLaughlin, M.J., 2013. Transport of silver nanoparticles in saturated columns of natural soils. Sci. Total Environ. 463, 120–130.
Dan, Y.B., Zhang, W.L., Xue, R.M., Ma, X.M., 2015. Characterization of gold nanoparticle uptake by tomato plants using enzymatic extraction followed by single-particle inductively coupled plasma-mass spectrometry analysis. Environ. Sci. Technol. 49, 3007–3014.
Deng, Y.Q., Petersen, E.J., Challis, K.E., Rabb, S.A., Holbrook, R.D., Ranville, J.F., Nelson, B.C., Xing, B.S., 2017. Multiple method analysis of TiO_2 nanoparticle uptake in rice (oryza sativa L.) plants. Environ. Sci. Technol. 51, 10615–10623.
Du, R.X., Fan, Z.X., Wei, A.L., al, e., 2009. Analysis of heavy metal content in shellfish of shandong coastal economy. Shandong Agric. Sci. 8, 58–63.
FAO, 2003. Summary and Conclusions of the 61st Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). FAO/WHO, Rome.
Frechette-Viens, L., Hadioui, M., Wilkinson, K.J., 2019. Quantification of ZnO nanoparticles and other Zn containing colloids in natural waters using a high sensitivity single particle ICP-MS. Talanta 200, 156–162.
Gajdosechova, Z., Lawan, M.M., Krupp, E.M., 2016. In vivo formation of natural HgSe nanoparticles in the liver and brain of pilot whales. Sci. Rep. 6.
GB2762-2017, 2017. Safety quality of agricultural products: safety requirements for

pollution-free aquatic products. Ministry of Health of the People's Republic of China. China Standard Press.
GB18406.4-2001, 2001. Safety quality of agricultural products: safety requirements for pollution-free aquatic products. Ministry of Health of the People's Republic of China. China Standard Press.
Gray, E.P., Coleman, J.G., Bednar, A.J., Kennedy, A.J., Ranville, J.F., Higgins, C.P., 2013. Extraction and analysis of silver and gold nanoparticles from biological tissues using single particle inductively coupled plasma mass spectrometry. Environ. Sci. Technol. 47, 14315–14323.
Hardy, A., Benford, D., Halldorsson, T., Jeger, M.J., 2018. Guidance on risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain: Part 1, human and animal health. EFSA J. 16.
Hu, J., Wang, J.M., Liu, S.X., Zhang, Z.C., Zhang, H.F., Cai, X.X., Pan, J.M., Liu, J.J., 2018. Effect of TiO_2 nanoparticle aggregation on marine microalgae Isochrysis galbana. J. Environ. Sci. 66, 208–215.
Huang, Y.W., Wu, C.H., Aronstam, R.S., 2010. Toxicity of transition metal oxide nanoparticles: recent insights from in vitro studies. Materials 3, 4842–4859.
Kim, B., Murayama, M., Colman, B.P., Hochella Jr., M.F., 2012. Characterization and environmental implications of nano- and larger TiO_2 particles in sewage sludge, and soils amended with sewage sludge. J. Environ. Monit. 14, 1129–1137.
Lee, S., Bi, X.Y., Reed, R.B., Ranville, J.F., Herckes, P., Westerhoff, P., 2014. Nanoparticle size detection limits by single particle ICP-MS for 40 elements. Environ. Sci. Technol. 48, 10291–10300.
Li, L., Wang, Q., Yang, Y., Luo, L., Ding, R., Yang, Z.G., Li, H.P., 2019. Extraction method development for quantitative detection of silver nanoparticles in environmental soils and sediments by single particle inductively coupled plasma mass spectrometry. Anal. Chem. 91, 9442–9450.
Li, X.F., Chen, Z.B., Chen, Z.Q., Zhang, Y.H., 2013. A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China. Chemosphere 93, 1240–1246.
Liu, X.L., Ouyang, W., Shu, Y., al, e., 2019. Incorporating bioaccessibility into health risk assessment of heavy metals in particulate matter originated from different sources of atmospheric pollution. Environ. Pollut. 254, 113113.
Loeschner, K., Brabrand, M.S.J., Sloth, J.J., Larsen, E.H., 2014. Use of alkaline or enzymatic sample pretreatment prior to characterization of gold nanoparticles in animal tissue by single-particle ICPMS. Anal. Bioanal. Chem. 406, 3845–3851.
Loeschner, K., Correia, M., Chaves, C.L., Rokkjaer, I., Sloth, J.J., 2018. Detection and characterisation of aluminium-containing nanoparticles in Chinese noodles by single particle ICP-MS. Food Addit. Contam. Part a-Chemistry Analysis Control Exposure & Risk Assessment 35, 86–93.
Merrifield, R.C., Stephan, C., Lead, J.R., 2017. Single-particle inductively coupled plasma mass spectroscopy analysis of size and number concentration in mixtures of monometallic and bimetallic (core-shell) nanoparticles. Talanta 162, 130–134.
Olesik, J.W., Gray, P.J., 2012. Considerations for measurement of individual nanoparticles or microparticles by ICP-MS: determination of the number of particles and the analyte mass in each particle. J. Anal. Atom. Spectrom. 27, 1143–1155.
Pace, H.E., Rogers, N.J., Jarolimek, C., Coleman, V.A., Gray, E.P., Higgins, C.P., Ranville, J.F., 2012. Single particle inductively coupled plasma-mass spectrometry: a performance evaluation and method comparison in the determination of nanoparticle size. Environ. Sci. Technol. 46, 12272–12280.
Peters, R.J.B., van Bemmel, G., Herrera-Rivera, Z., Helsper, H.P.F.G., Marvin, H.J.P., Weigel, S., Tromp, P.C., Oomen, A.G., Rietveld, A.G., Bouwmeester, H., 2014. Characterization of titanium dioxide nanoparticles in food products: analytical methods to define nanoparticles. J. Agric. Food Chem. 62, 6285–6293.
Ramos, K., Ramos, L., Gomez-Gomez, M.M., 2017. Simultaneous characterisation of silver nanoparticles and determination of dissolved silver in chicken meat subjected to in vitro human gastrointestinal digestion using single particle inductively coupled plasma mass spectrometry. Food Chem. 221, 822–828.
Schmid, K., Riediker, M., 2008. Use of nanoparticles in Swiss industry: a targeted survey. Environ. Sci. Technol. 42, 2253–2260.
Schrand, A.M., Rahman, M.F., Hussain, S.M., Schlager, J.J., Smith, D.A., Ali, S.F., 2010. Metal-based nanoparticles and their toxicity assessment. Wiley Interdiscipl. Rev. Nanomed. Nanobiotechnol. 2, 544–568.
Sharifi, S., Behzadi, S., Laurent, S., Forrest, M.L., Stroeve, P., Mahmoudi, M., 2012. Toxicity of nanomaterials. Chem. Soc. Rev. 41, 2323–2343.
Sung, H.K., Jo, E., Kim, E., Yoo, S.K., Lee, J.W., Kim, P.J., Kim, Y., Eom, I.C., 2018. Analysis of gold and silver nanoparticles internalized by zebrafish (Danio rerio) using single particle-inductively coupled plasma-mass spectrometry. Chemosphere 209, 815–822.
Tamm, K., Sikk, L., Burk, J., Rallo, R., Pokhrel, S., Madler, L., Scott-Fordsmand, J.J., Burk, P., Tamm, T., 2016. Parametrization of nanoparticles: development of full-particle nanodescriptors. Nanoscale 8, 16243–16250.
Tangaa, S.R., Selck, H., Winther-Nielsen, M., Khan, F.R., 2016. Trophic transfer of metal-based nanoparticles in aquatic environments: a review and recommendations for future research focus. Environ. Sci. Nano. 3, 966–981.
Veverkova, L., Hradilova, S., Milde, D., Panacek, A., Skopalova, J., Kvitek, L., Petrzlova, K., Zboril, R., 2014. Accurate determination of silver nanoparticles in animal tissues by inductively coupled plasma mass spectrometry. Spectrochim. Acta B Atom Spectrosc. 102, 7–11.
Vidmar, J., Buerki-Thurnherr, T., Loeschner, K., 2018. Comparison of the suitability of alkaline or enzymatic sample pre-treatment for characterization of silver nanoparticles in human tissue by single particle ICP-MS. J. Anal. Atom. Spectrom. 33, 752–761.
Wilbur, S., Michiko, Y., Sebastien, S., 2016. Characterization of Nanoparticles in Aqueous Samples by ICP-MS. Agilent publication.
Yang, Y., Luo, L., Li, H.P., Wang, Q., Yang, Z.G., Long, C.L., 2016. Separation and determination of silver nanoparticle in environmental water and the UV-induced photochemical transformations study of AgNPs by cloud point extraction combined ICP-MS. Talanta 161, 342–349.
Zhang, L., Chen, B.B., He, M., Hu, B., 2013. Polymer monolithic capillary microextraction combined on-line with inductively coupled plasma MS for the determination of trace rare earth elements in biological samples. J. Separ. Sci. 36, 2158–2167.