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RESEARCH ARTICLE

Concentrations of trace metals in tissues of *Chionoecetes* crabs (*Chionoecetes japonicus* and *Chionoecetes opilio*) caught from the East/Japan Sea waters and potential risk assessment

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Abstract The concentrations of trace metals (As, Cd, Co, Cu, Fe, Hg, Pb, and Zn) were measured in muscle and hepatopancreas of two *Chionoecetes* crabs (*Chionoecetes japonicus* and *C. opilio*) caught from the East/Japan Sea (EJS) in order to assess the potential health risk by the consumption of deep sea crabs. The highest metal concentrations in muscle and hepatopancreas were As and Fe, respectively, while the lowest metal concentration in two tissues was Pb. The mean concentrations of Cd, Co, Cu, Fe, and Pb in *Chionoecetes* crabs were one or two orders of magnitude higher in hepatopancreas than in muscles. The mean concentrations of As, Cu, and Hg in muscle and hepatopancreas were relatively higher in *C. japonicus* than in *C. opilio*. The estimated daily intakes (EDI) of all metals in muscle were below 0.1% of the provisional tolerable daily intake (PTDI) adopted by the Joint FAO/WHO Expert Committee on Food Additives. Similarly, the target hazard quotient (THQ) of all trace metals in muscle was less than 1.0. These results imply that *Chionoecetes* crabs caught from EJS do not have an adverse impact on the Korean health. Based on the mean metal concentrations, PTDI, and THQ, the daily maximum permissible intakes of *C. japonicus* and *C. opilio* were estimated to be approximately 240 and 410 g/day, respectively.

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Introduction

Trace metals in the ocean play an important role in serving as micronutrients. However, some trace metals are classified as toxic substances for marine organism since nonbiodegradable trace metals such as cadmium and mercury are accumulated in the tissues of marine organisms and the elevated levels of trace metals can cause adverse effects on the growth, breeding, and metabolism of marine organisms (Bryan 1979; Turoczy et al. 2001; Kim and Yoon 2011). Especially, Cd and Hg are accumulated at high levels in crabs compared to other marine organisms (fish and shellfish) due to the binding to metallothionein in tissues (mainly hepatopancreas) of crabs (Chen et al. 2005; Reed et al. 2010; Mok et al. 2014). In addition, these metals are transferred to high tropic levels through the food chain and eventually could be threatened to the human health through the consumption of contaminated seafood (Wang 2002; Tsuchiya et al. 2008; Qiu et al. 2011).

Crabs (Decapoda, Crustacea) are widely distributed in deeper open sea as well as the shallow coastal ocean. The human and industrial activities associated with urbanization and industrialization increased over the last few decades. The potential for metal pollution of benthic crabs in coastal ocean is very high relative to other marine organisms because benthic crabs with restricted mobility mainly reside on the bottom sediment which contains high metal concentrations compared to any other medium in coastal environment and can accumulate high levels of trace metals in their tissues both from water, sediment, and prey (Chen et al. 2005; Reed et al. 2010; Chiarelli and Roccheri 2014). Therefore, trace metal concentrations in benthic crustaceans reflect the status of the pollution of marine environment, and benthic crabs in some coastal oceans have been used as biomonitoring indicators of metal pollution in marine environment (Jewett and Naidu 2000; Burger et al. 2002; Reed et al. 2010; Julshamn et al. 2015). However, the accumulation of trace metals in tissues of deep sea crab species is less well known (Turoczy et al. 2001; Kim and Yoon 2011; Perry et al. 2015).

Chionoecetes crab is a large, long-lived, and deep sea crab, and five species of *Chionoecetes* crabs live mainly in deep cold waters of the North Pacific Ocean including the East/Japan Sea (EJS), the Okhotsk Sea, the Bering Sea, adjacent to the Aleutian Islands, and the Kamchatka Peninsula and the northwest Atlantic Ocean (from Greenland south to Casco Bay, Maine) (Jadamec et al. 1999). In these *Chionoecetes* crab species, two species of *Chionoecetes* crabs, beni-zuwai crab (*Chionoecetes japonicus*) and snow crab (*Chionoecetes opilio*), are distributed in the EJS, a marginal sea of the northwestern Pacific Ocean (Chun et al. 2009). *C. opilio* occurs along the continental shelf at depths ranging from 200 to 450 m, while *C. japonicus* is observed along the continental slope at depths ranging from 400 to 2000 m in the bottom sediment (Yosho and Hayashi 1994; Kon 1996; Jadamec et al. 1999).

Crabs are one of most preferred seafood products in the whole world. *Chionoecetes* crab (especially, *C. japonicus*) is a representative commercial species caught in the EJS and is consumed in the neighboring country, such as Korea, Russia, and Japan, either as boiled or steamed cooked product. Generally, mainly the muscle tissue of legs and claws of the *Chionoecetes* crabs is consumed; however, sometimes the hepatopancreas of *Chionoecetes* crabs is also consumed in Korea. According to Statistics Korea, the production of *Chionoecetes* crabs has gradually increased from ~25,000 t in the 1990s to ~40,000 t in 2010's in Korea (KMOF 2015). However, Korea during the last 23 years has been discharging various kinds of liquid and solid wastes at two dumping sites of EJS located within the habitat of *Chionoecetes* crabs (KMOF 2004; Song et al. 2015). Recently, as the consumption of *Chionoecetes* crabs increases, Koreans have become increasingly aware of the safety of *Chionoecetes* crabs.

In this study, we investigated the concentrations of eight trace metals (As, Cd, Co, Cu, Fe, Hg, Pb, and Zn) in tissues of *Chionoecetes* crabs collected in the EJS. The primary purpose of our study is to evaluate to what degree these *Chionoecetes* crabs are contaminated with these selected trace metals. Potential human health risk assessment is also conducted to evaluate whether *Chionoecetes* crab consumption presented any potential hazard to humans.

Materials and methods

Sample collection and analytical methods

Five male beni-zuwai crabs and snow crabs were collected from each of the four and three fishing ports, respectively, along the eastern coast of Korea in March 2011 (Fig. 1).

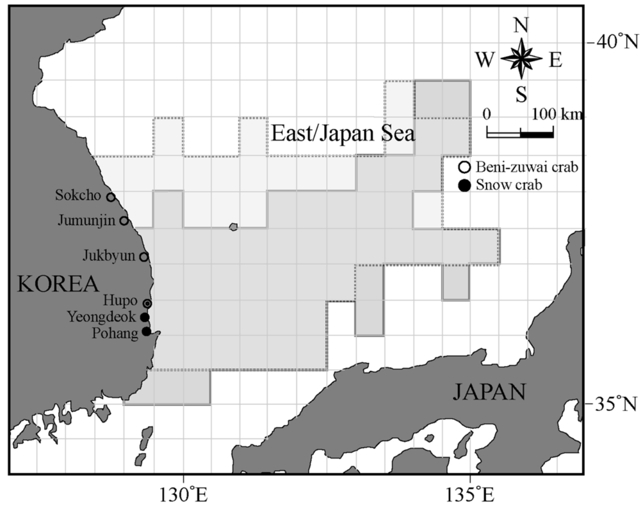

Fig. 1 A map showing the sampling locations for analyzing the trace metals in the tissues of *Chionoecetes* crabs caught from East/Japan Sea waters. The *dotted* and *solid lines* represent the habitats of beni-zuwai crab and snow crab, respectively

These ports were the representative distributing centers of *Chionoecetes* crabs. After collection, *Chionoecetes* crabs were kept alive in a cool box filled with ice and were transferred to the laboratory within 2 days. In the laboratory, these crabs were stored in a freezer at −20 °C and were euthanized by chilling. Before the crabs were dissected, crab sizes were measured. Crab sizes (carapace length, carapace width, body thickness, and weight) of these *Chionoecetes* crabs collected at each sampling site are shown in Table 1.

The trace metals in the tissues of *Chionoecetes* crabs were measured by an analytical procedure modified from Kim et al. (2011). Firstly, *Chionoecetes* crabs were washed with deionized water (DIW) passed through a Milli-Q water purification system (Elix 5, Millipore, USA) and were dissected using a precleaned ceramic knife over a clean ceramic chopping board. Muscle samples in body, claw and leg, and hepatopancreas samples were taken from each individual and then were freeze-dried at −80 °C with a vacuum freeze dryer. The dried tissues were pulverized and homogenized with a mixer mill in a jar.

Approximately 1.0 g of the powdered tissue samples was placed in an acid-cleaned 60-mL digestion vessel (Teflon, Savillex Corp., USA) with 10 mL of nitric acid (HNO_3, Suprapur grade, Merck, Germany) and then was settled at room temperature for 3 h. After prereaction, the tissue samples were digested with lids at 80 °C for 7 h and were dried by heating the vessel without lids at 100 °C for 3 h. This procedure was repeated until only a negligible amount of white residue remained, and the residues were dissolved in 80 mL of 2% HNO_3. The solutions were filtered using a filter paper (Advantec, 5C, 110 mm), and the filtered solutions were made up to 100 mL with 2% HNO_3 into a 100-mL volumetric flask. The As, Cd, Cu, Co, Fe, Pb, and Zn concentrations except Hg in the solution were measured by using an inductively coupled

Table 1 The sample number and crab size of *Chionoecetes* crabs caught from the East/Japan Sea waters

Species	Sampling site	Sample number	Crab size			
			Carapace length (cm)	Carapace width (cm)	Body thickness (cm)	Weight (g)
Beni-zuwai crab (*Chionoecetes japonicus*)	Sokcho	5	10.7–12.0 (11.1 ± 0.6)	10.0–11.1 (10.6 ± 0.4)	4.3–4.7 (4.5 ± 0.2)	332–498 (400 ± 57)
	Jumunjin	5	11.0–12.7 (11.8 ± 0.8)	10.4–12.2 (11.3 ± 0.8)	4.5–4.9 (4.7 ± 0.2)	429–552 (497 ± 53)
	Jukbyeon	5	12.0–12.5 (12.2 ± 0.2)	11.4–11.9 (11.6 ± 0.2)	4.9–5.4 (5.1 ± 0.2)	570–640 (601 ± 25)
	Hupo	5	11.7–12.4 (12.0 ± 0.3)	11.3–11.8 (11.5 ± 0.2)	4.5–5.3 (4.9 ± 0.3)	472–592 (520 ± 44)
Snow crab (*Chionoecetes opilio*)	Hupo	5	10.2–10.9 (10.7 ± 0.3)	10.1–10.7 (10.3 ± 0.2)	4.6–4.9 (4.7 ± 0.1)	242–350 (284 ± 44)
	Yeongdeok	5	10.1–11.1 (10.4 ± 0.4)	9.3–10.5 (9.8 ± 0.4)	4.6–4.9 (4.6 ± 0.1)	209–302 (248 ± 34)
	Pohang	5	10.2–11.0 (10.4 ± 0.3)	9.9–10.5 (10.1 ± 0.3)	4.5–4.9 (4.7 ± 0.2)	240–257 (250 ± 7)

plasma–mass spectrometer (ICP-MS, ELAN DRC-e, PerkinElmer, USA). Approximately 0.1 g of homogenized dry tissues was used for the Hg analysis. Total Hg concentrations in the dried tissue samples were measured directly without pretreatment using an automated mercury analyzer (DMA 80, Milestone, Italy).

The accuracy and precision of the analytical procedures were checked using certified reference material (CRM). DOLT-4 (dogfish liver, National Research Council Canada) for trace metals except Hg and ERM-CE278 (mussel, European Commission—Directorate General Joint Research Centre) for Hg was used as CRM. These reference materials were treated and measured with the same procedures as the sample, and the mean recovery of each metal was 118% for As, 81% for Cd, 94% for Co, 95% for Cu, 86% for Fe, 102% for Hg, 87% for Pb, and 91% for Zn. In this study, the concentrations of trace metal are expressed as micrograms per gram wet weight (hereafter μg/g).

Potential risk assessment

In order to evaluate the harmful effect of Korean health by *Chionoecetes* crab consumption, the potential risk assessments were performed for the muscle of *Chionoecetes* crabs. In this study, we used the provisional maximum tolerable daily intake (PMTDI) of Cd, Cu, Fe, Hg, and Zn suggested by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (WHO 2016) and reference dose (RfD) recommended by the US Environmental Protection Agency (Onsanit et al. 2010; USEPA 2016a). Here, PMTDI for Cd and Hg was calculated from the provisional tolerable monthly intake (PTMI) and provisional tolerable weekly intake (PTWI) established by the JECFA, respectively.

Firstly, the estimated daily intake (EDI) was calculated and then was compared with PMTDI. The EDI (μg/kg·bw/day) depends on the metal concentrations in *Chionoecetes* crabs and the amount of *Chionoecetes* crab consumption, which was calculated using Eq. (1):

$$\mathrm{EDI} = (\mathrm{C_{crab}} \times \mathrm{DC_{crab}})/\mathrm{BW} \tag{1}$$

where C_{crab} is the mean concentration of trace metals in the muscle of *Chionoecetes* crabs (μg/g ww), DC_{crab} is the daily consumption of *Chionoecetes* crabs by Korean adults (g/day), and BW is the mean body weight (kg) of Korean adults.

We also assessed the health risk for the consumption of *Chionoecetes* crabs using the target hazard quotient (THQ). THQ indicates the ratio between exposure and the reference dose (RfD) and is based on the USEPA Human Health Risk Assessment approach (Copat et al. 2013; USEPA 2016b). This assessment approach provides useful and valuable information about the safety of consuming marine products including fish and shellfish (Mok et al. 2014). THQ can be expressed by Eq. (2):

$$\mathrm{THQ} = (\mathrm{EF} \times \mathrm{ED} \times \mathrm{C_{crab}} \times \mathrm{DC_{crab}})/(\mathrm{RfD} \times \mathrm{BW} \times \mathrm{ET}) \tag{2}$$

where EF is the exposure frequency (from 52 days/year for people who eat crab once a week to 365 days/year for people who eat crab seven times a week), ED is the exposure duration (about 80 years as the average lifetime of adults in Korea), and ET is the average exposure time for noncarcinogens and is equal to EF × ED. Therefore, THQ was simply calculated by dividing the RfD suggested by USEPA (2016a) by the EDI as below (Eq. (3)):

$$\mathrm{THQ} = \mathrm{EDI}/\mathrm{RfD} \quad (3)$$

On the other hand, the value of THQ less than 1 can be interpreted to be no obvious risk.

Statistical analysis

The Shapiro–Wilk test was used to assess normality of metal concentrations in tissues of *Chionoecetes* crabs. The Student's *t* test and the Mann–Whitney *U* test were also performed to identify any significant differences in metal concentrations between species or tissues of *Chionoecetes* crabs. Statistical significance was set at $p < 0.05$. All statistical analysis of data was carried out using SPSS version 11.0 software package for Windows (SPSS, Chicago, IL, USA).

Results and discussion

Accumulation pattern of trace metals in tissues of *Chionoecetes* crabs

The concentrations of trace metals in the muscle and hepatopancreas of *Chionoecetes* crabs caught from the EJS waters are shown in Table 2. The trace metal concentrations in muscle of *C. japonicus* decreased in the order As > Zn > Cu > Fe > Hg ≥ Cd ≥ Co > Pb, and the decreasing order has statistically significant difference except Cd ($p < 0.05$). The trace metal concentrations in muscle of *C. opilio* decreased in the order As, Zn > Cu > Fe > Hg, Cd, Co > Pb, and the decreasing order has statistically significant difference ($p < 0.05$) although no significant differences were found in the concentrations between As and Zn or among Cd, Co, and Hg.

Based on the overall metal concentrations in muscle tissue, the accumulation patterns of trace metals in muscles of *C. japonicus* and *C. opilio* are very similar to each other although the habitat depth of two *Chionoecetes* crabs from the EJS waters is different. The highest and lowest metal concentrations in the muscle were As and Pb, respectively, which imply that As relative to other metals is selectively absorbed very well in the muscle of two *Chionoecetes* crabs. This trend is similar to the accumulation pattern of metals in the muscle of stone crab (*Menippe mercenaria*) from South Carolina coastal waters (Reed et al. 2010).

The trace metal concentrations in hepatopancreas of *C. japonicus* decreased in the order Cu, Fe > As > Zn, Cd > Co > Hg > Pb, and the decreasing order has statistically significant difference ($p < 0.05$) although no significant differences were found in the concentrations between As and Zn or between Hg and Pb. The trace metal concentrations in the hepatopancreas of *C. opilio* decreased in the order Fe > Cu > As, Zn > Cd > Co > Pb, Hg, and the decreasing order has statistically significant difference ($p < 0.05$) although no significant differences were found in the concentrations between As and Zn or between Hg and Pb.

On the basis of the metal concentrations in hepatopancreas tissue, the accumulation patterns of trace metals in the hepatopancreas of *C. japonicus* and *C. opilio* are very similar with statistically significant difference. The highest metal concentrations were Fe or Cu, while the lowest metal concentrations were Hg or Pb. This indicates that Fe or Cu relative to other metals is selectively absorbed very well in the hepatopancreas of two *Chionoecetes* crabs. These results of *Chionoecetes* crabs in the EJS were similar to those of stone crabs in the South Carolina coastal area (Reed et al. 2010).

Comparison of metal concentrations in tissues of *Chionoecetes* crabs

Generally, trace metals are heterogeneously distributed among tissues in the crab (Nissen et al. 2005; Reed et al. 2010). The concentrations of trace metals in the tissues of *C. japonicus* and *C. opilio* showed statistically significant difference ($p < 0.05$) between muscle and hepatopancreas (Table 2). The average concentrations of As, Hg, and Zn in *C. japonicus* were relatively higher in muscle than in hepatopancreas. This result is consistent with that in tissues of intertidal burrowing crab (*Scylla serrata*) found from estuaries on the eastern coast of Australia (Mortimer 2000). In *C. opilio*, there was no significant difference between muscle and hepatopancreas in the levels of As, Hg, and Zn. However, the average concentrations of Cd, Co, Cu, Fe, and Pb were much higher in hepatopancreas than in muscle in both species. Especially, the concentrations of Cd and Fe in hepatopancreas were one or two orders of magnitude higher than those in muscle. The higher concentrations of trace metals in the hepatopancreas relative to other tissues are due to detoxification function of the hepatopancreas (Rainbow 2007; Reed et al. 2010).

Inter-species difference for the average concentrations of trace metals in muscle and hepatopancreas of two *Chionoecetes* crabs showed a statistically significant difference ($p < 0.05$) (Fig. 2). The average concentrations of As, Cu, Hg, and Zn in muscle were higher in *C. japonicus* than in *C. opilio*, while the average Pb concentrations were about two times of magnitude higher in *C. opilio* than in *C. japonicas* (Fig. 2a). Similarly, the average concentrations of As, Cu, and Hg in hepatopancreas were relatively higher in *C. japonicus* than in *C. opilio*, while the average Fe concentrations were higher in *C. opilio* than in *C. japonicas* (Fig. 2b). These results appear to be due to the difference of prey organisms and uptake (or excretion and detoxification) rate for each metal between two *Chionoecetes* crab species because it has become appreciated that uptake of trace metals through diet could be

Table 2 Concentrations of trace metals (As, Cd, Co, Cu, Fe, Hg, Pb, and Zn) in two tissues of *Chionoecetes* crabs caught from the East/Japan Sea waters

Species	Tissue	Sampling site	Concentration (μg/g wet weight)							
			As	Cd	Co	Cu	Fe	Hg	Pb	Zn
Beni-zuwai crab (*Chionoecetes japonicus*)	Muscle (*n* = 20)	Sokcho	9.6–30.7 (19.6 ± 8.3)	0.027–0.358 (0.151 ± 0.142)	0.061–0.098 (0.082 ± 0.019)	5.2–18.3 (8.9 ± 5.6)	1.21–4.43 (3.28 ± 1.21)	0.080–0.215 (0.128 ± 0.053)	0.001–0.013 (0.005 ± 0.005)	10.8–26.2 (16.8 ± 6.2)
		Jumumjin	16.1–28.5 (20.3 ± 4.8)	0.022–0.074 (0.046 ± 0.022)	0.031–0.061 (0.039 ± 0.013)	1.3–5.2 (2.8 ± 1.7)	0.79–2.09 (1.39 ± 0.47)	0.102–0.222 (0.168 ± 0.049)	ND–0.073 (0.021 ± 0.030)	10.1–15.4 (12.0 ± 2.1)
		Jukbyeon	27.8–54.9 (37.5 ± 11.0)	0.059–0.463 (0.166 ± 0.170)	0.070–0.170 (0.115 ± 0.038)	3.3–12.6 (8.5 ± 3.5)	1.78–3.28 (2.45 ± 0.55)	0.115–0.301 (0.193 ± 0.069)	0.005–0.041 (0.021 ± 0.013)	14.1–22.4 (19.0 ± 3.6)
		Hupo	20.1–31.1 (24.0 ± 4.4)	0.031–0.344 (0.125 ± 0.125)	0.079–0.193 (0.127 ± 0.042)	7.7–10.9 (9.2 ± 1.4)	2.16–3.52 (2.76 ± 0.49)	0.095–0.159 (0.111 ± 0.027)	0.004–0.010 (0.006 ± 0.002)	12.9–17.9 (15.3 ± 1.9)
		Mean ± SD	25.3 ± 10.2a,*	0.12 ± 0.13ef	0.09 ± 0.04f	7.4 ± 4.2c	2.5 ± 1.0d	0.15 ± 0.06e,*	0.01 ± 0.02 g	15.8 ± 4.4b,*
	Hepatopancreas (*n* = 20)	Sokcho	7.9–17.9 (10.7 ± 4.1)	0.5–2.6 (1.4 ± 0.7)	0.104–0.184 (0.145 ± 0.032)	6.5–36.6 (16.5 ± 12.0)	26.6–41.1 (33.9 ± 6.0)	0.088–0.162 (0.126 ± 0.031)	0.015–0.052 (0.040 ± 0.015)	3.5–8.7 (5.3 ± 2.1)
		Jumumjin	12.4–31.7 (23.3 ± 8.4)	3.0–17.5 (9.9 ± 5.3)	0.070–0.305 (0.179 ± 0.095)	40.8–90.5 (67.4 ± 21.6)	18.8–59.8 (31.5 ± 17.1)	0.074–0.131 (0.105 ± 0.028)	0.042–0.193 (0.082 ± 0.063)	6.3–12.7 (10.6 ± 2.7)
		Jukbyeon	18.7–29.9 (22.5 ± 4.8)	6.5–13.9 (10.1 ± 2.8)	0.107–0.213 (0.172 ± 0.044)	31.6–65.7 (46.1 ± 13.1)	19.8–44.4 (35.6 ± 9.5)	0.125–0.155 (0.142 ± 0.013)	0.022–0.061 (0.034 ± 0.015)	6.1–13.5 (10.7 ± 2.9)
		Hupo	13.4–21.9 (17.4 ± 3.2)	3.4–13.1 (8.9 ± 3.9)	0.136–0.346 (0.251 ± 0.076)	29.9–143 (78.5 ± 42.2)	38.9–91.6 (61.1 ± 21.0)	0.055–0.098 (0.068 ± 0.018)	0.025–0.047 (0.037 ± 0.008)	5.2–7.7 (6.3 ± 1.0)
		Mean ± SD	18.5 ± 7.2b	7.58 ± 4.96c,*	0.19 ± 0.07d,*	52.1 ± 33.6a,*	40.5 ± 18.2a,*	0.11 ± 0.04e	0.05 ± 0.04f,*	8.2 ± 3.3c
Snow crab (*Chionoecetes opilio*)	Muscle (*n* = 15)	Hupo	2.7–18.3 (9.1 ± 5.9)	0.009–0.180 (0.055 ± 0.071)	0.014–0.097 (0.043 ± 0.032)	0.9–6.5 (2.9 ± 2.1)	0.29–3.36 (1.08 ± 1.29)	0.029–0.070 (0.056 ± 0.016)	ND–0.022 (0.007 ± 0.008)	2.9–18.9 (9.0 ± 6.1)
		Yeongdeok	7.9–24.8 (14.4 ± 7.3)	0.032–0.215 (0.081 ± 0.075)	0.049–0.078 (0.067 ± 0.012)	4.7–8.4 (5.9 ± 1.5)	2.09–4.37 (2.76 ± 0.92)	0.025–0.069 (0.040 ± 0.018)	0.025–0.062 (0.046 ± 0.015)	8.4–18.1 (13.0 ± 4.3)
		Pohang	9.0–78.2 (32.7 ± 26.4)	0.018–0.265 (0.137 ± 0.095)	0.047–0.228 (0.114 ± 0.072)	2.9–9.2 (5.0 ± 2.6)	1.16–3.53 (2.48 ± 0.97)	0.033–0421 (0.177 ± 0.149)	0.008–0.028 (0.019 ± 0.008)	10.1–28.6 (15.6 ± 7.5)
		Mean ± SD	18.7 ± 18.3a	0.09 ± 0.08d	0.07 ± 0.05d	4.6 ± 2.4b	2.1 ± 1.2c	0.09 ± 0.10d	0.02 ± 0.02e	12.5 ± 6.3a
	Hepatopancreas (*n* = 15)	Hupo	7.5–19.3 (13.3 ± 4.5)	1.5–3.7 (2.3 ± 0.9)	0.147–0.366 (0.246 ± 0.079)	8.9–53.3 (28.5 ± 16.4)	68.1–129 (101 ± 30.1)	0.035–0.046 (0.041 ± 0.005)	0.017–0.036 (0.026 ± 0.007)	8.3–15.3 (10.7 ± 3.0)
		Yeongdeok	3.9–13.1 (7.9 ± 4.0)	2.0–6.2 (4.2 ± 1.9)	0.090–0.237 (0.157 ± 0.062)	21.3–68.7 (39.0 ± 18.8)	34.7–116 (75.5 ± 38.6)	0.016–0.057 (0.032 ± 0.017)	0.109–0.253 (0.177 ± 0.058)	4.1–7.8 (5.6 ± 1.4)
		Pohang	16.0–21.5 (18.8 ± 2.3)	2.1–12.4 (5.3 ± 4.1)	0.234–0.338 (0.284 ± 0.037)	16.8–34.3 (24.4 ± 6.9)	18.8–104 (50.8 ± 38.2)	0.056–0.093 (0.077 ± 0.016)	0.033–0.067 (0.048 ± 0.013)	10.1–17.1 (12.5 ± 2.7)
		Mean ± SD	13.3 ± 5.7c	3.94 ± 2.76d,*	0.23 ± 0.08e,*	30.6 ± 15.2b,*	75.8 ± 39.4a,*	0.05 ± 0.02f	0.08 ± 0.08f,*	9.6 ± 3.8c

Different letters and asterisk (*) marks in the mean ± SD row indicate statistically significant differences ($p < 0.05$) between trace metals in each tissue of crab and between muscle and hepatopancreas in each crab, respectively

ND not determined

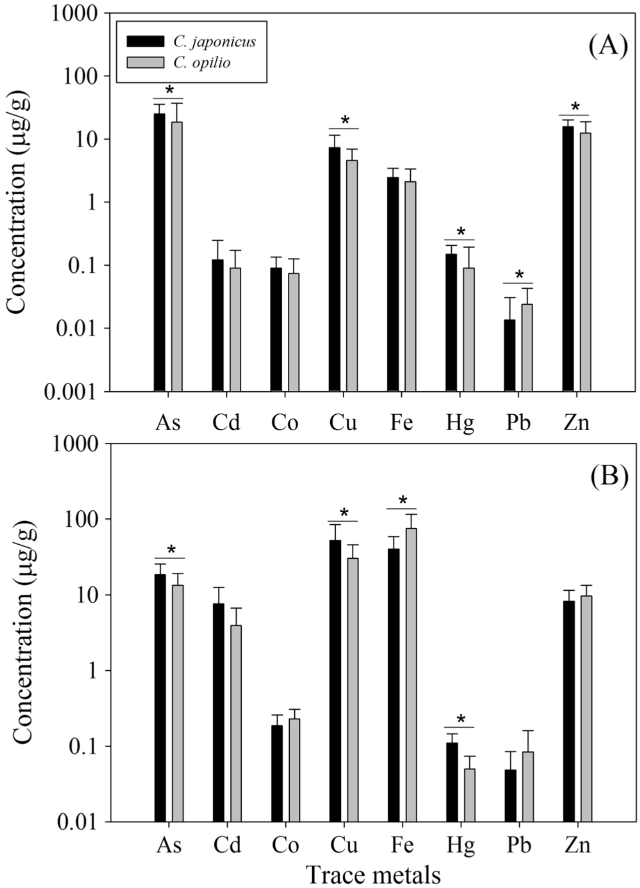

Fig. 2 Comparison of average concentration between beni-zuwai crab and snow crab for trace metals (As, Cd, Co, Cu, Fe, Hg, Pb, and Zn) in **a** muscle and **b** hepatopancreas. The *error bar* and *asterisk mark* represent the standard deviation of metal concentrations and significant difference ($p < 0.05$), respectively

the major source of metals for many aquatic invertebrates (Wang 2002; Rainbow 2007).

Comparing the concentrations of trace metals in the muscle and hepatopancreas of other crabs from various oceanic environments in the whole world (Table 3), the average concentration of As in muscle of *C. japonicus* was higher than that of horseshoe crab from USA, red king crab from Barents Sea, and stone crab from USA, and that of *C. opilio* was higher than that in other studies of snow crab, but lower than that of horseshoe crab and stone crab from the USA (references in Table 3). The average concentrations of Co in muscle of two *Chionoecetes* crabs were higher than those of rock crab from Taiwan and stone crab from USA, while those of Fe in muscle of two *Chionoecetes* crabs were lower than those of rock crab from Taiwan and stone crab from USA (references in Table 3). The average concentrations of Cd, Cu, and Zn in muscle of two *Chionoecetes* crabs are much lower than those of king crab from Australia and blue crab of Yellow Sea, the continental shelf located in the western sea of Korea (references in Table 3). However, the average concentrations of Cd, Hg, and Pb in muscle of two *Chionoecetes* crabs are much higher than those of red king crab from Norway (references in Table 3).

The average concentrations of As, Co, Cu, Fe, and Zn in hepatopancreas of two *Chionoecetes* crabs were much lower than those of stone crab from USA (references in Table 3). The average concentration of Cd in hepatopancreas of *C. japonicus* was higher than that of king crab from Australia and stone crab from USA, while that of *C. opilio* was lower than that of king crab from Australia and stone crab from USA (references in Table 3). The average concentration of Hg in hepatopancreas of *C. japonicas* was similar to that of king crab from Australia, and that of *C. opilio* was similar to that of stone crab from USA (references in Table 3). The average concentration of Pb in hepatopancreas of *C. japonicas* was lower than that of stone crab from USA, while that of *C. opilio* was higher than that of stone crab from USA (references in Table 3). These results appear to be associated with the difference in ecological needs, feeding, and metabolic activities among different crab species and various pollution sources such as urban and agricultural activities, electrical power station, and international shipping activities.

Potential risk assessment by the consumption of *Chionoecetes* crabs

Some metals, such as Cu, Fe, and Zn, are present in high concentration as the essential element in the human body because these metals play an important role in the metabolism and immunity system of the human body (Prashanth et al. 2015). However, excessively high metal concentrations can adversely affect the human body. In addition, the toxic metals, such as Cd, Hg, and Pb, can act as a hazard factor to human health although a small amount of these metals remain in the human body (Bilandžić et al. 2011; Mok et al. 2014). Therefore, we assessed the potential health risk of trace metals by the consumption of two *Chionoecetes* crabs caught from the EJS. Here, we only used the concentrations of metal in muscle tissue of two *Chionoecetes* crabs to evaluate the health risk assessment since most Korean people only consume the muscle tissue of *Chionoecetes* crabs.

Firstly, the metal concentrations (except Co and Fe) in the muscle of two *Chionoecetes* crabs were compared with the values of the regulatory limit set by each country for ensuring the human health and the safety of marine products (references are shown in Kim and Han (1999) and Mok et al. (2014)). The As and Cu concentrations in muscles of two *Chionoecetes* crabs were in the range of 2.7–78.2 µg/g (mean 22.5 ± 14.4 µg/g) and 0.9–18.3 µg/g (mean 6.2 ± 3.7 µg/g), respectively. Although the standards of As and Cu for crustaceans are not yet established in the whole world, the As and Cu concentrations in all samples were much lower than the regulatory limits (86 µg/g and 20 µg/g) of As and Cu for mollusk and shellfish applied in USA and UK, respectively.

Table 3 Comparison of average concentrations for trace metal (As, Cd, Cr, Cu, Hg, Pb, and Zn) in the tissues of marine crabs throughout the whole world

Species (scientific name)	Location	Concentration (μg/g wet weight)								Reference
		As	Cd	Co	Cu	Fe	Hg	Pb	Zn	
Muscle										
Beni-zuwai crab (*Chionoecetes japonicus*)	East/Japan Sea, Korea		0.12		12.0		0.13	0.012	52.2	Mok et al. (2014)
	East/Japan Sea, Korea	25.3	0.12	0.09	7.4	2.5	0.15	0.014	15.8	This study
Blue crab (*Portunus trituberculatus*)	Yellow Sea, Korea		0.71		14.8		0.04	0.009	52.8	Mok et al. (2014)
Horseshoe crab (*Limulus polyphemus*)	Eastern coast, USA	17.4	0.04				0.06	0.041		Burger et al. (2002)
King crab (*Pseudocarcinus gigas*)	Southeastern coast, Australia		0.43		14.0		0.25		63.0	Turoczy et al. (2001)
Red king crab (*Paralithodes camtschaticus*)	Barents Sea, Norway	10.0	0.02				0.04	<0.01		Julshamn et al. (2015)
Rock crab (*Thalamita crenata*)	Dapeng Bay, Taiwan		0.04	0.02	8.0	3.9			32.0	Chen et al. (2005)
Snow crab (*Chionoecetes opilio*)	White Rose, Canada	6.5	0.09		4.1		0.09		24.2	Whitford (2003)
	East/Japan Sea, Russia	7.9	0.05		2.5				10.4	Kim and Yoon (2011)
	East/Japan Sea, Korea	10.7	0.02		6.0				11.0	Kim and Yoon (2011)
	East/Japan Sea, Korea	14.5	0.09	0.06	4.3	2.0	0.07	0.024	11.4	This study
Stone crab (*Menippe mercenaria*)	South Carolina coast, USA	18.9	0.02	0.02	15.1	13.1	0.08	0.003	70.0	Reed et al. (2010)
Hepatopancreas										
Beni-zuwai crab (*Chionoecetes japonicus*)	East/Japan Sea, Korea	18.5	7.6	0.19	52.1	40.5	0.11	0.048	8.2	This study
King crab (*Pseudocarcinus gigas*)	Southeastern coast, Australia		5.6		13.0		0.14		27.0	Turoczy et al. (2001)
Snow crab (*Chionoecetes opilio*)	East/Japan Sea, Korea	11.2	2.4		12.9				8.9	Kim and Yoon (2011)
	East/Japan Sea, Korea	13.3	3.9	0.23	30.6	75.8	0.05	0.084	9.6	This study
Stone crab (*Menippe mercenaria*)	South Carolina coast, USA	23.8	4.6	0.39	308	93.0	0.06	0.063	46.4	Reed et al. (2010)

In addition, for arsenic, nontoxic arsenobetaine is the most common As species in marine organisms and inorganic As, the most toxic form, has generally been found in very low levels in marine organisms, such as in red king crab from the Barents Sea (Julshamn et al. 2015).

The Cd concentrations in muscles of two *Chionoecetes* crabs were in the range of 0.009–0.463 μg/g (mean 0.109 ± 0.110 μg/g). The Cd concentrations in all samples were below the regulatory limits (0.5 to 1.0 μg/g) of Cd established in the European Commission and Korea. Although it is well known that Cd is accumulated in very high levels in hepatopancreas of crab than in muscle, high Cd concentrations above the regulatory limit (0.5 μg/g) set by the European Commission in muscle (claw) of some crab (e.g., edible crab, *Cancer pagurus*) have been found in Northern Europe such as Norway, Scotland, and the English channel (Maulvault et al. 2012; Wiech et al. 2017), which have raised concern about the safety of crab consumption. However, the Cd concentrations in the muscle of *Chionoecetes* crabs are much lower than those in other crabs from Europe and thus the muscle of *Chionoecetes* crabs is much less of a problem about food safety.

The Pb concentrations in the muscles of two *Chionoecetes* crabs were in the range of ND–0.073 μg/g (mean 0.018 ± 0.018 μg/g). The Pb concentrations in all samples were below the regulatory limits (0.5 to 1.0 μg/g) of Pb established in the European Commission and Korea. The Hg concentrations in muscles of two *Chionoecetes* crabs ranged from 0.025 to 0.421 μg/g (mean 0.125 ± 0.084 μg/g), which were relatively lower than the regulatory limits (0.5 to 1.0 μg/g) set by many countries including Australia and New Zealand, USA, and the European Commission. The Zn concentrations in muscles of two *Chionoecetes* crabs ranged from 2.9 to 28.6 μg/g (mean 14.4 ± 5.5 μg/g), which were much lower than the regulatory limits (50 μg/g) established in UK. Therefore, the concentrations of all metals in muscles of two *Chionoecetes* crabs in the EJS were lower than the standards set by many countries around the world.

As mentioned in the "Potential risk assessment" section, the EDI and THQ are calculated by using Eqs. (1) and (3), respectively, and the values of the calculated EDI and THQ are shown in Table 4. Here, we assessed the potential health risk for only five metals (Cd, Cu, Fe, Hg, and Zn) measured in this study since the previously established PTWIs of Pb and

Table 4 Hazardous level of trace metal (Cd, Cu, Fe, Hg, and Zn) through muscle consumption of *Chionoecetes* crabs in Korea

Species	Trace metal	Average concentration (μg/g)	Daily consumption[a] (g/day)	EDI[b] (μg/kg·bw/day)	PMTDI[c] (μg/kg·bw/day)	Hazardous level (%)	RfD[d] no. (μg/kg/day)	Target hazard quotient (THQ)	Daily maximum permissible intake (g/day)
Beni-zuwai crab									
(*Chionoecetes japonicus*)	Cd	0.12		0.00002	0.83	0.0023	1	0.00002	~440
	Cu	7.36		0.00115	500	0.0002	–	–	~4400
	Fe	2.47	0.01	0.00039	800	0.0000	–	–	~20,800
	Hg	0.15		0.00002	0.57	0.0041	–	–	~240
	Zn	15.8		0.00247	300	0.0008	300	0.00001	~1220
Snow crab									
(*Chionoecetes opilio*)	Cd	0.09		0.00055	0.83	0.0659	1	0.00055	~600
	Cu	4.60		0.02803	500	0.0056	–	–	~7000
	Fe	2.11	0.39	0.01286	800	0.0016	–	–	~24,500
	Hg	0.09		0.00055	0.57	0.0960	–	–	~410
	Zn	12.5		0.07617	300	0.0254	300	0.00025	~1550

[a] Daily consumption of *Chionoecetes* crabs reported by the Korea National Health and Nutrition Examination Survey (KMOHW 2013)

[b] EDI: Estimated daily intake calculated from the average concentration of trace metal in *Chionoecetes* crab muscle, daily *Chionoecetes* crab consumption, and the mean body weight(bw, ~64 kg) of Korean adults (KMOHW 2015)

[c] PMTDI: Provisional maximum tolerable daily intakes recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO 2016). PMTDI for Cd and Hg were calculated from the provisional tolerable monthly intake (PTMI) and provisional tolerable weekly intake (PTWI) established by the JECFA, respectively

[d] RfD: Reference dose suggested by the Integrated Risk Information System (IRIS) of the US Environmental Protection Agency (USEPA) (USEPA 2016a)

As for the human health protection are withdrawn in the 79th JECFA meeting held in 2014 and the PTWI for Co is absent.

On the basis of the average concentrations of trace metals in the muscle of *C. japonicus* and *C. opilio* in this study (average concentrations are in Table 4), the daily consumption of *C. japonicus* (0.01 g/day) and *C. opilio* (0.39 g/day) for Korean adults reported by the Korea National Health and Nutrition Examination Survey (KMOHW 2013), and the mean body weight (~64 kg) in Korean adults based on the Korean Health Statistics 2014 (KMOHW 2015), the EDI values of Cd, Cu, Fe, Hg, and Zn for *C. japonicus* and *C. opilio* are calculated to be approximately $0.02–2.47 \times 10^{-3}$ and $0.55–76.2 \times 10^{-3}$ μg/kg·bw/day, respectively (Table 4). The calculated EDI values for all metals in muscles of *C. japonicus* and *C. opilio* were below 0.1% of the PMTDI established by the JECFA (WHO 2016), and the hazardous level decreased in the order Hg > Cd > Zn > Cu > Fe. On the other hand, the EDI values for the essential elements (Cu, Fe, and Zn) in muscle of two *Chionoecetes* crabs were one or two orders of magnitude higher than those for the toxic metals (Cd and Hg), implying that the muscle of *Chionoecetes* crabs contributes to the dietary intake of Cu, Fe, and Zn and is a useful source for these elements.

Generally, the THQ proposed by USEPA has been used widely in the whole world for the risk assessment of trace metals in seafood consumption including fish and shellfish (Storelli 2008). In this study, we also used the THQ to assess the health risk to Korean adults by the consumption of two *Chionoecetes* crabs caught from the EJS as another criterion. Here, we assessed the potential health risk for only two metals (Cd and Zn) in this study since RfD values for As (total), Co, Cu, Fe, Hg (total), and Pb were not assessed or estimated by USEPA in the present.

The RfD values (USEPA 2016a) and the calculated THQ results are shown in Table 4. The THQ values of Cd and Zn in the muscle of *C. japonicus* are estimated to be approximately 0.02×10^{-3} and 0.01×10^{-3}, respectively. The THQ values of Cd and Zn in the muscle of *C. opilio* are estimated to be approximately 0.55×10^{-3} and 0.25×10^{-3}, respectively. The THQ values of Cd and Zn in muscles of two *Chionoecetes* crabs were much less than 1.0. This implies that Cd and Zn intakes through the consumption of two *Chionoecetes* crabs caught from the EJS do not pose an appreciable hazard to Korean adults. Although this result has a high uncertainty because of a small amount of sample, the metal concentrations in two *Chionoecetes* crabs of this study are similar to or lower than those in *Chionoecetes* crab previously reported around the eastern sea of Korea (Mok et al. 2014).

As above shown in the results of health risk assessments, *Chionoecetes* crabs caught from the EJS do not have an adverse impact on the Korean health. However, Korean intake of *Chionoecetes* crabs (maximum ~460 g/day) is large at one time during the catch period although the daily average consumption of *Chionoecetes* crabs (0.01–0.39 g/day) is very small (KMOHW 2013). In addition, as demands for *Chionoecetes* crabs increase recently, a large amount of *Chionoecetes* crabs are imported from other countries (mainly Russia) and potential exposure opportunities of Koreans for metals by consumption of *Chionoecetes* crabs are gradually increasing. Therefore, in this study, we calculated the daily maximum permissible intake for *Chionoecetes* crabs using the average concentrations of trace metals in muscle of *C. japonicus* and *C. opilio*. Based on the average concentrations in the muscles of two *Chionoecetes* crabs, PMTDI, and THQ, the daily maximum permissible intakes of *C. japonicus* and *C. opilio* were estimated to be approximately 240 and 410 g/day, respectively (Table 4). The estimated values were relatively lower than the maximum intake of Korean (~ 460 g/day) for *Chionoecetes* crabs (KMOHW 2013). Thus, the appropriate consumption for *Chionoecetes* crabs is necessary to protect the Korean health in the future.

Conclusion

The concentrations of trace metals (As, Cd, Co, Cu, Fe, Hg, Pb, and Zn) were measured in muscle and hepatopancreas of two *Chionoecetes* crabs (*C. japonicus* and *C. opilio*) caught from the EJS. The highest metal concentrations were As in muscle and Fe in hepatopancreas, while the lowest concentration was Pb in both tissues. Inter-tissues and inter-species difference for the metal concentrations in two *Chionoecetes* crabs showed a statistically significant difference ($p < 0.05$). The concentrations of Cd, Co, Cu, Fe, and Pb in two *Chionoecetes* crabs were significantly higher in hepatopancreas than in muscle, and the concentrations of As, Cu, and Hg in both tissues were relatively higher in *C. japonicus* than in *C. opilio*. Based on the evaluation results of the potential health risk using regulatory limits, EDI, and PTDI, *Chionoecetes* crabs caught from the EJS do not have an adverse impact on the Korean health, and the daily maximum permissible intakes of *C. japonicus* and *C. opilio* were estimated to be approximately 240 and 410 g/day, respectively. For the Korean health protection, the appropriate regulation for intakes of *Chionoecetes* crabs is necessary in the future.

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