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Heavy Metal Levels in Suspended Sediments, *Crassostrea gigas*, and the Risk to Humans

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Abstract Wild Pacific oyster (*Crassostrea gigas*) and sediment, both resuspended and suspended (RSS) samples (<53 μm), were collected over an 8-month period from a coastal estuary in Baynes Sound, BC, Canada. Stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) was used to determine, first, if RSS sediments was an important dietary source to *C. gigas* and, second, if so, whether it served as a significant dietary exposure route for Cd, Cu, Pb, and Zn. Although RSS sediments were high in trace metals such as Cd, Pb, Zn, and notably Cu, stable isotope signatures for oyster tissues did not correspond to those of RSS sediments. These results indicate that RSS sediments are not a significant source of metal to the oysters. In addition, we compared the potential risk to humans ingesting *C. gigas* with levels of Cd, Cu, Pb, and Zn from this study to the current Agency for Toxic Substances and Disease Registry (ATSDR) chronic oral minimal risk levels (MRLs) using current Canadian consumption guidelines of three oysters/week. Cu, Pb, and Zn levels were below MRL levels, with Cd exceeding the recommended 0.2 μg/kg/day MRL level fourfold. Total daily intake levels (TDI) for Cd were additionally calculated and either reached or exceeded the Food and Agriculture Organization/World Health Organization (FAP/WHO) reference dose of 1 μg/kg/day for Cd consumption in Canadians. Our results indicate that the current Canadian Cd consumption guidelines do not protect individuals consuming the maximum recommended levels of wild oysters.

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Introduction

Trophic transfer of metals is increasingly recognized as an important pathway for metal accumulation in marine invertebrates and fishes (Fisher and Reinfelder 1995; Wang and Fisher 1999). In suspension feeding marine bivalves such as *Crassostrea gigas*, both aqueous and dietary exposures represent dominant routes for metal uptake and accumulation. The quantitative significance of dietary metal exposure is species-specific and varies depending on the physicochemical and ecological conditions within an ecosystem (Bendell-Young 1999; Wang 2002). Marine intertidal habitats are extremely diverse and differ in their geography, hydrology, and the abiotic conditions that define each environment. Quantifying dietary metal uptake in *C. gigas* inhabiting these environments is difficult when food sources are numerous and highly variable.

The Pacific oyster *C. gigas* is a highly selective suspension feeder, ingesting a wide range of seston, including plankton, material resuspended from the ocean floor, sediments, and aggregates consisting of high-molecular-weight substances, detritus, fecal pellets, and microorganisms (Ward and Shumway 2004). As adults, this species is predominantly sessile and, therefore, needs to filter large volumes of water to consume sufficient amounts of particulate matter to grow, reproduce, and survive. Depending on the season and availability of food particles, sediment might constitute a significant portion of dietary uptake in *C. gigas* (Gray 1981; Thomson 1981). Therefore, any metals associated with sediment particles will also be ingested by the bivalve. The finest particle fraction of sediment (2−60 μm) is known to retain the highest proportion of metals and is preferentially ingested by many suspension feeding organisms (Selim and Amacher 1997; Tarradellas and Bitton 1997). *C. gigas* actively feeds on

these fine-sediment particles, causing an increased potential exposure to sediment-bound metals (Mudroch and MacKnight 1991; Siegel 2002). In fact, recent studies indicate extremely high levels of Cd in west coast cultured oysters (Cheng and Gobas 2007; Kruzynski 2004; Schallie 2001). The source of these high Cd levels remains unknown and is therefore of great economic and ecological concern. In the North Pacific, dissolved Cd levels are three to five times the levels found in the North Altantic (Bruland 1980). Metal sorption/uptake by phytoplankton and/or sediment and the subsequent transfer to filter-feeding bivalves, although as yet unmeasured, is highly likely (Thompson 2001).

In recent studies, stable carbon and nitrogen isotope analysis has been used to successfully identify bivalve food sources in marine and estuarine environments (Kang et al. 1999; Lorrain et al. 2002; Page and Lastra 2003). Application of this technique is based on the stable isotope composition of an animal being determined by the composition of its food (Michener and Schell 1994). Although there is a trend of enrichment of heavier isotopes with increasing trophic level, such metabolic isotopic fractionations occurring within animal bodies are measurable and predictable (Rau 1978). In addition, using a dual-isotope approach of carbon and nitrogen provides more precise information when there are two to three potential food sources being utilized by a consumer such as *C. gigas* (Currin et al. 1995).

Few studies exist that use combined stable isotope and trace metal analyses to distinguish food sources and dietary metal uptake in an aquatic consumer (Croteau et al. 2005; Quinn et al. 2003; Xu and Wang 2004). Hence, the objectives of this study were threefold: (1) to measure metal levels in resuspended and suspended sediments and relate these levels to wild *C. gigas*, (2) to confirm if resuspended and suspended (RSS) sediments serve as a primary dietary and metal source to wild *C. gigas* using stable isotope analysis, and, finally, (3) to determine if metal concentrations in wild *C. gigas* pose a risk for human consumption by exceeding the Agency for Toxic Substances and Disease Registry minimum risk levels (MRLs). The site (Deep Bay) used in this study is located along the west coast of British Columbia (BC), which has reportedly high levels of Cd (Kruzynski 2004). Deep Bay is within an area of high economic and ecological importance: Baynes Sound. This area currently houses over 50% of the shellfish aquaculture farms in BC and is the second most important waterfowl habitat in the province (Paynter 2002). Cd metal concentrations within west coast sediments have exhibited levels exceeding the interim sediment quality guidelines, causing justified concern for metal uptake in sediment-ingesting organisms (Kruzynski 2004). It is an ideal environment to examine metal uptake routes in wild oysters harvested for human consumption.

Materials and Methods

Study Area

Sediment traps were deployed and wild beach oysters collected from Deep Bay, BC, Canada from February to August 2004. Deep Bay is a cobble-stone tidal estuary located on the eastern coastline of Vancouver Island in Baynes Sound (123°N, 48°W). Baynes Sound is a shallow coastal channel that comprises about 8500 ha, fringed by protected bays, open foreshore, intertidal mud and sand flats, low-grade deltas, tidal estuaries, inshore marshes, and rocky shorelines (Paynter 2002). These rich productive habitats are a result of the combination of sheltered water, low-gradient tidal areas, fine substrates, and nutrient-rich freshwater input. The area is a critical staging, breeding, and wintering area for migratory birds. There are a number of native and exotic intertidal bivalves, resulting in a large increase in foreshore shellfish aquaculture development (Paynter 2002). Water, sediment, and biota contained within the waters adjacent to Baynes Sound have been historically sampled and metal levels quantified. Cd was identified as a metal of significant concern, accumulating to levels exceeding the Canadian MRL for the bivalve *M. balthica* (Thomas et al. 2003).

Collection of Resuspended and Suspended Sediments and Oysters

Unique intertidal sediment traps were designed for easy deployment at the low-tide line to collect large volumes (5–10 g wet weight) of RSS sediment on a monthly basis. Traps were constructed of a single schedule 40 grade PVC pipe that was capped at one end and left open at the other (Fig. 1). Traps were positioned parallel to the shoreline, 3 m from one another, at the 0.9 m tidal line. Each trap was submerged 15 in. into the sediment, open pipe end facing upward at a 90° angle from the sediment–water interface, completely burying the four arms. This upright vertical position allowed the trap to retain all particles during daily tidal fluxes and withstand strong wind and current conditions over the 3–4-week period.

A total of 10 traps were deployed in Deep Bay from January to August 2004, to include the winter, spring, and summer seasons. Sediment traps were sampled every 24–28 days, with salinity and temperature recorded at each sampling period. All collection and holding containers were acid-washed for 24 h in 10% HNO_3 to remove any sorbed metals. Trap contents were emptied into 3-L polypropylene beakers, transferred to 500-mL polypropylene bottles, and transported to the lab on ice within 6 h. Standard stainless-steel testing sieves #30 mesh (600 μm),

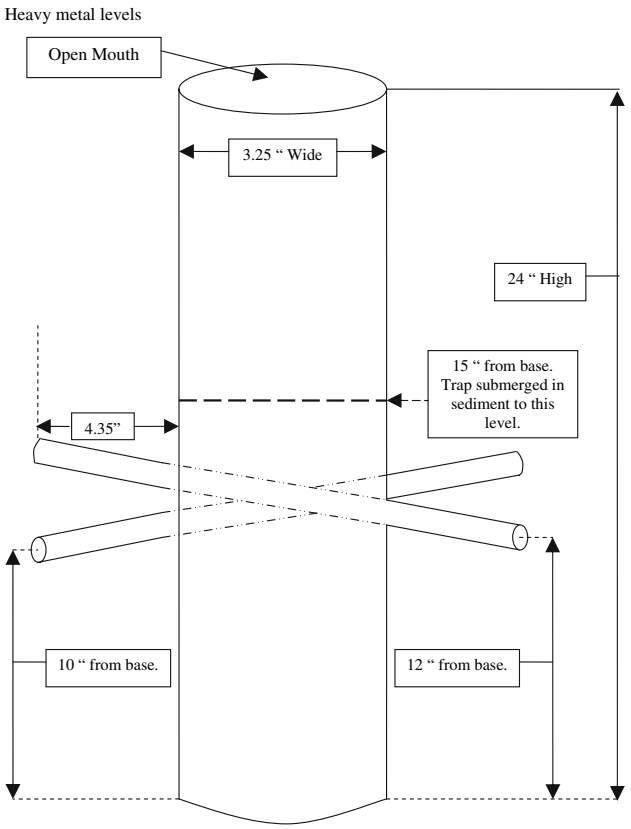

Fig. 1 Schematic of trap used to collect RSS sediments

#70 mesh (212 μm), and #270 (53 μm) were used to wet sieve sediments in their native waters. All particles below 53 μm were held at 4°C (to minimize microbial activity) for 24 h in clean 500-mL bottles. Supernatant waters were then removed by simple vacuum filtration, resulting in RSS sediment in ~40 mL of native water. The slurry was transferred to acid-washed 50-mL polypropylene test tubes and centrifuged at 3500 rpm for 20 min. Following centrifugation, the RSS sediment formed a semisolid pellet, and the remaining native water was decanted. Wet RSS sediments were weighed and placed in a 60°C oven for 48 h, and a dry weight was obtained. RSS sediments were homogenized using an acid-washed ceramic mortar and pestle and stored at room temperature in 25 ml acid-washed polypropylene jars. RSS sediment metal levels were reported as microgram per gram dry weight values.

Twelve wild beach oysters (10–15 cm in width) were removed from the same region where sediment collection occurred. Oysters were placed into plastic bags, held on ice, and transferred to the lab where they were frozen within 12 h of collection. For metal analyses, oysters were allowed to thaw just enough to use a stainless-steel shucker to pry open the two valves. All tissues were removed from the shell, with the digestive gland and stomach (digestive tissues) separated from the rest of the tissues (nondigestive tissues) using a stainless-steel scalpel. Digestive and nondigestive oyster tissues were placed into separate plastic weigh boats, weighed, and dried in a 60°C oven until a constant dry weight was obtained. Tissues were initially ground and homogenized using a Cuisinart mini-mixer. Samples were stored in sterile plastic whirl-bags prior to processing for acid digestion or stable isotope analysis. Finer-sized subsamples were obtained for acid-digestion and stable isotope analysis using a ceramic mortar and pestle.

Metal Analysis of Oyster Tissue

Oyster tissue metal levels (Cu, Cd, Pb, Zn) were measured using a Perkin-Elmer atomic absorption spectrophotometer. Oyster tissues were prepared for metal analysis according to the US Environmental Protection Agency Protocol 200.3 (McDaniel 1991). Subsamples of dried homogenized digestive tissues (digestive gland, stomach) (0.5 g) and remaining oyster tissue (1.0 g) were acid-digested using environmental-grade HNO_3 and H_2O_2 at 95°C in 50- and 125-mL Erlenmeyer flasks. Analytical efficiency was verified using the National Bureau of Standards reference Oyster–2 (1566b). The NBS standard was digested and analyzed using the same procedures as the oyster samples; measured values were always within 90% of the reported standard values for Cd, Cu, and Pb and 80% for Zn. Tissue metal levels were reported as microgram per gram wet weight values.

Metal Analysis of RSS Sediments

The RSS sediment metal levels (Cu, Cd, Pb, Zn) were measured using a Perkin-Elmer Elan 6000 inductively coupled plasma–mass spectrometer (ICP-MS). Sediment samples (0.50 g dry weight) were acid-digested in 1.5-mL environmental-grade HCl and 4.5 mL HNO_3 at room temperature for 1 h, then in a block digestor at 95°C for 2 h. Samples were cooled to room temperature, diluted to 50 mL with double-distilled (dd) H_2O, and held at 4°C until analysis. Analytical efficiency was verified using NBS marine sediment standard reference materials (MESS-3, PACS-4) and blanks; 2 NBS and 1 blank were used for every 10 digested samples. Standards and blanks were digested and analyzed using the same procedures as the RSS sediment samples; measured values were always within 95% of the reported standard values.

Stable Isotope Analysis

Stable isotope ratios of carbon and nitrogen were analyzed at the Stable Isotope Facility, University of Davis, California

using a Europa 20/20 continuous-flow isotope ratio mass spectrometer (CFIRMS). Ratios were compared to standard gases Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$ that were injected directly into the CFIRMS before and after the sample peaks. Values for $\delta^{13}C$ and $\delta^{15}N$ were calculated and reported using the standard delta (δ) notation in parts per thousand (‰) as follows:

$$\delta X = [(R_{sample}/R_{standard}) - 1\} \times 1000,$$

where X is $\delta^{13}C$ or $\delta^{15}N$ and R is the corresponding ratio $\delta^{13}C/^{12}C$ or $\delta^{15}N/^{14}N$. Replicate laboratory standards (sucrose $\delta^{13}C = -23.83$, SE = 0.01, and ammonium sulfate $\delta^{15}N = 1.31$, SE = 0.03) were analyzed before and after every 12 samples to determine the accuracy of $\delta^{13}C$ and $\delta^{15}N$ values.

Risk Characterization of Metals to Humans

The Agency for Toxic Substances and Disease Registry (ATSDR) has set chronic oral MRLs of 0.2 μg/kg/day for Cd, 10 μg/dL hematocrit for Pb, 0.3 mg/kg/day for Zn, and 0.14 mg/kg/day for Cu. Metal exposure levels (MRLs) were calculated based on the current Health Canada maximum recommended consumption rate of 12 oysters per month using the lowest seasonal combined oyster tissue levels as measured in this study: 2.38 ± 0.16 μg/g for Cd, 36.1 ± 5.15 μg/g for Cu, 0.61 ± 0.05 μg/g for Pb, and 414 ± 24 μg/g for Zn (Table 3). Due to the high levels of Cd detected in our samples, Cd total exposure was additionally calculated according to the US EPA model (Han et al. 1998; US EPA 1998), generating a Total Daily Intake Level (TDI) (Table 4). Four risk categories were used to quantify TDI levels: nonoyster consumer and nonsmoker, nonoyster consumer and smoker, oyster consumer and nonsmoker, and oyster consumer and smoker. MRLs were derived as follows:

$$\text{MRL} = [\text{metal oyster}]\ \mu g/g \times 3 \text{ oysters per week} \times 55 \text{ g}$$

$$= [\text{metal}]\ \mu g/\text{week}.$$

The average oyster wet weight for this study was 55 g ($n = 84$, SE = 1.2):

$$\text{MRL} = [\text{metal}]\ \mu g/\text{week}/7 \text{ days/week}/70 \text{ kg}$$

$$= [\text{metal}]\ \mu g/\text{kg/day}.$$

The Cd TDI was

$$\text{TDI} = (\text{SCE} \times \text{EF} \times \text{ED})/(\text{BW} \times \text{AT}),$$

where TDI is the total daily intake of Cd (μg/kg/day), ΣCE is the total combined Cd exposure from all sources (μg/person/day), EF is the exposure frequency (365 days/year), ED is the exposure duration (70 years), BW is the body weight (70 kg), and AT is the averaging time for exposure duration (ED × 365 days/year = 25,550 days). To derive the above ΣCE value, the following Cd exposure values were obtained from the Health Canada database (2006); air 0.09 (μg/person/day), water 0.036 (μg/person/day), soil 0.023 (μg/person/day), diet without oyster contribution 13.8 (μg/person/day), and cigarettes 3.7 (μg/person/day).

Data Analysis

All statistical analyses were done using JMP In 4.0.4 and Figure 2 was generated using SigmaPlot 2000. All data followed a Normal distribution; therefore, parametric analysis using one-way analyses of variance (ANOVAs)was applied to determine seasonal differences in metal levels in RSS sediments in digestive and nondigestive oyster tissues. Two-way ANOVAs were applied to determine significant variation between sediments in digestive and nondigestive tissues. Relationships between stable carbon and nitrogen isotope values for RSS sediment in digestive and nondigestive tissues were assessed using linear regression analysis. Relationships between RSS sediment or tissue metal levels and $\delta^{13}C$, $\delta^{15}N$ values were also assessed using linear regression analysis. Significance for all tests was accepted at 0.05.

Results

Metal Levels in *C. gigas* and RSS Sediments

Seasonal variations in metal levels occurred for RSS sediment in digestive and nondigestive tissues and were metal dependent. Cd, Pb, and Zn levels in digestive tissues and nondigestive tissues did not differ significantly on a seasonal

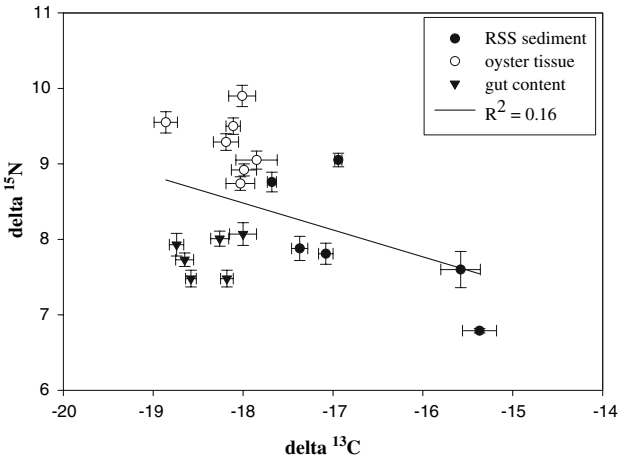

Fig. 2 $\delta^{13}C$ versus $\delta^{15}N$ for RSS sediments and wild oyster tissues (gut content/digestive tissue and oyster tissue/nondigestive tissues) from Deep Bay

Table 1 Cd, Cu, Pb, and Zn levels in digestive, nondigestive, total combined oyster tissues, and RSS sediments ± standard errors

Season	*n*	Cd wet weight (μg/g)	SE	Cu wet weight (μg/g)	SE	Pb wet weight (μg/g)	SE	Zn (wet weight (μg/g)	SE
Nondigestive tissue									
Winter	24	0.37	0.02	5.19	0.43	0.31	0.02	138	9.9
Spring	36	0.55	0.03	7.00	0.32	0.33	0.03	181	14.8
Summer	24	0.74	0.05	7.78	0.59	0.54	0.04	189	17.9
Digestive tissue									
Winter	23	2.01	0.16	31.1	4.83	0.31	0.04	291	23.3
Spring	36	1.96	0.09	46.8	2.62	0.58	0.28	248	17.9
Summer	24	2.28	0.11	58.2	4.04	0.58	0.18	304	24.0
Combined tissues									
Winter	23	2.38	0.16	36.3	5.15	0.61	0.05	431	29.7
Spring	36	2.51	0.11	53.8	2.84	0.63	0.05	414	23.8
Summer	24	3.02	0.15	66.1	4.46	1.12	0.18	478	27.6
RSS sediment		Cd dry weight	SE	Cu dry weight	SE	Pb dry weight	SE	Zn dry weight	SE
Winter	8	1.15+	0.17	398++	47.3	141++	25.2	185+	16.0
Spring	16	2.40+	0.06	115++	9.64	7.96	0.31	76.2	2.88
Summer	15	2.36+	0.06	430++	52.8	8.55	0.57	110	8.60

Note: + indicates a level exceeding Canadian Sediment Quality Guidelines (SQG), + + indicates a level exceeding SQG probable effect level

basis (one-way ANOVA, $p > 0.05$), whereas Cu levels in both tissues were significantly different ($p = 0.007$). RSS sediment metal levels were seasonally dependent ($p < 0.0001$), with Cd being higher in the spring and summer, Cu being higher in the winter and summer, and both Zn and Pb being highest in the winter. RSS sediment metal levels for Cd exceeded the sediment quality guidelines for all three seasons while exceeding Zn levels only the winter. Levels in Cu and Pb surpassed the SQG level and exceeding the sediment quality probable effect level for Cu in all three seasons and in the winter for Pb. (See Table 1)

δ^{15}N and δ^{13}C Signatures in *C. gigas* and Sediments

The δ^{13}C signatures in RSS sediments were enriched (less negative value) compared to either digestive or nondigestive tissues (Table 2). δ^{15}N signatures were higher for nondigestive tissues versus RSS sediment, but no clear trend was present for digestive tissues versus RSS sediment. The mean δ^{13}C signature was -18.4 ± 0.05 in digestive tissues, -18.1 ± 0.07 in nondigestive tissues, and -16.9 ± 0.11 in RSS sediment. The mean δ^{15}N signature was 7.78 ± 0.05 in digestive tissues, 9.26 ± 0.06 in nondigestive tissues, and 8.15 ± 0.12 in RSS sediment. Relationships between RSS sediment or tissue metal levels and δ^{13}C and δ^{15}N signatures were assessed using linear regression analysis (Fig. 2). No significant relationship was noted ($R^2 = 0.16$; $p > 0.05$). Linear regressions were also used to examine whether significant relationships existed between either δ^{13}C, δ^{15}N, or the δ^{13}C/ δ^{15}N ratio and Cu, Cd, Pb, and Zn for digestive and nondigestive tissues and for RSS sediments. No significant relationships were noted, with R^2 values ranging from 0.0 to 0.20.

Table 2 Stable isotope data for oyster nondigestive tissues, digestive tissues, and RSS sediments collected from Deep Bay over three seasons

Month	*n*	δN	SE	δC	SE	C/N ratio	SE
Nondigestive tissue							
Winter	16	9.77	0.11	−18.33	0.15	1.88	0.03
Spring	30	9.28	0.07	−18.05	0.10	1.95	0.02
Summer	20	8.83	0.06	−18.01	0.10	2.04	0.02
Digestive tissue							
Winter	10	7.93	0.15	−18.74	0.08	2.37	0.05
Spring	30	7.68	0.08	−18.25	0.07	2.38	0.03
Summer	20	7.87	0.07	−18.45	0.08	2.35	0.02
RSS sediment							
Winter	10	7.12	0.16	−15.46	0.14	2.18	0.04
Spring	22	8.89	0.09	−17.34	0.09	1.96	0.03
Summer	20	7.85	0.11	−17.25	0.07	2.21	0.03

Risk Characterization of Metals to Humans

Metal levels in combined oyster tissues were compared with the ATSDR chronic oral MRLs. Cd levels of 0.80 μg/kg/day

Table 3 ATSDR chronic oral MRLs for metal uptake compared with experimental levels from the current study

Metal	Recommended ATSDR MRL (μg/kg/day)	Experimental level (μg/kg/day)
Cd	0.20	0.80
Cu	140	12.2
Pb	10 (μg/dL)	0.21
Zn	300	139

Note: All levels were derived using the current Canadian consumption guidelines for humans consuming three oysters per week. The lowest possible mg/kg whole oyster tissue wet weight metal level was used to calculate the experimental level

exceeded the recommended MRL (0.20 μg/kg/day) fourfold, whereas Cu (12.2 vs. 140 μg/kg/day), Pb (0.21 vs. 10 μg/kg/day), and Zn (139 vs. 300 μg/kg/day) levels were well below their recommended MRLs (Table 3). Cd exposure levels (μg/person/day) for oyster consumer/nonsmoker (70 μg/person/day) and oyster consumer/smoker (73 μg/person/day) met or exceeded the FAO/WHO established Provisional Tolerable Daily Intake (PTDI) for Cd of 70 μg/person/day. Levels for nonoyster consumer/nonsmoker and nonoyster consumer/smoker were well below the recommended PTDI. Cd TDI's for the four risk categories were as follows (Table 4): nonoyster consumer/nonsmoker (0.2), nonoyster consumer/smoker (0.25), oyster consumer/nonsmoker (1.0), oyster consumer/smoker (1.05). TDI's either met or exceeded the recommended Cd MRL (0.2) for all four categories and either met or exceeded the FAO/WHO and US EPA limits (1.0) for acceptable risk in two of the four categories.

Discussion

Within the field of ecotoxicology, scientists are striving to develop more integrated levels of research, attempting to link contaminant levels with adverse biological effects within ecosystem food webs. Regulatory agencies are also recognizing the need for integrated levels of research that can be used to develop more realistic exposure guidelines. Deciphering routes of metal uptake in a diverse and dynamic marine intertidal environment is difficult but possible using an integrated research approach. Thus, our first two objectives were to quantify metal levels in RSS sediments and relate determined metal concentrations to *C. gigas* and to determine whether RSS sediments constituted a primary dietary and metal source to *C. gigas* using stable isotope analysis.

Stable carbon and nitrogen isotope ratios are increasingly used to provide time-integrated information about feeding relationships and energy flow through food webs (Cabana and Rasmussen 1994; Kling et al. 1992). Trophic structure and position is indicated by the level of δ^{13}C and δ^{15}N enrichment as you move up a food chain. Normally, an increase in trophic level causes an average increase of 1‰ in δ^{13}C signatures and 3‰ in δ^{15}N signatures (Fry and Sherr 1984). Surprisingly, we found that the expected enrichment in δ^{13}C signatures were not present in our study. Rather, δ^{13}C signatures were diluted, as RSS sediment was more enriched than digestive tissues by 1.5‰ and than nondigestive tissues by 1.2‰, suggesting that RSS sediments were not an important route of dietary metal exposure to the Pacific oyster.

The RSS sediments collected from Deep Bay had δ^{13}C signatures of −16.9‰. The average δ^{13}C range for estuarine surface sediments is normally between −14‰ and –24‰ (Fry and Sherr 1984). The δ^{13}C signatures for *C. gigas* in our study were within this range (−18‰) for both digestive and nondigestive tissues. If the RSS sediment was the main food source for *C. gigas*, then δ^{13}C signatures for RSS sediment and digestive tissues would be more similar than what was observed. *C. gigas* collected from varying estuarine environments normally have δ^{13}C signatures ranging from −16‰ to −24‰, with more enriched values indicative of oysters feeding on oceanic phytoplankton (Riera and Richard 1996). It is likely that the Deep Bay oysters ingested a higher percentage of oceanic phytoplankton versus RSS sediments, as indicated by the enriched δ^{13}C signature (−18‰) for both digestive and nondigestive tissues. This is difficult to confirm, as the average range of δ^{13}C in temperate marine phytoplankton is −18 to −24, overlapping considerably with δ^{13}C signatures for estuarine oysters (Gearing et al. 1984).

When δ^{15}N signatures for RSS sediments and oyster tissues were examined, the expected increase of 3‰ for *C. gigas* tissue was not observed. As estuaries have combined inputs of terrestrial and marine organic matter and

Table 4 Total Cd exposure (μg/person/day), Cd TDI (μg/kg/day), and the ATSDR chronic oral MRLs for Cd in humans, calculated using oyster Cd levels from the current study

	Nonoyster consumer and nonsmoker	Nonoyster consumer and smoker	Oyster consumer and nonsmoker	Oyster consumer and smoker
Cd exposure (μg/person/day)	14	17.7	70	73.7
Cd TDI (μg/kg/day)	0.2	0.25	1.0	1.05
Cd MRL (μg/kg/day)	0.2	0.2	00.2	0.2

terrestrial organic matter has a significantly lighter isotope signature, both $\delta^{13}C$ and $\delta^{15}N$ signatures were used to help identify trophic position (Riera and Richard 1996). Unfortunately, $\delta^{15}N$ signatures did not help clarify the dilution observed for oyster $\delta^{13}C$ signatures. Oyster digestive tissues were 0.37‰ less enriched for $\delta^{15}N$ than RSS sediment, whereas nondigestive tissues were 1.11‰ more enriched compared with RSS sediment. As a final approach, we used a linear regression model to examine whether a relationship existed between $\delta^{13}C$ and $\delta^{15}N$ signatures for RSS sediments in digestive and nondigestive tissues; no significant relationship was present.

Not surprisingly, metal levels in both digestive and nondigestive tissues did not correlate with those in RSS sediments when examined using a linear regression model. There were also no significant trends in $\delta^{13}C$, $\delta^{15}N$, or the $\delta^{13}C$/ $\delta^{15}N$ ratio and Cu, Cd, Pb, and Zn levels for either RSS sediments in digestive and nondigestive tissues. Recent studies examining metal assimilation in filter-feeding mussels also reported that suspended sediment metal levels were not good measures of dietary trace metal bioavailability (Rainbow 2002; Rainbow et al. 2004; Zachariadis et al. 2001). These studies were unable to infer that sediments constituted significant dietary components to the mussels, as without the application of stable isotope analysis, this correlation was not possible. In summary, stable isotope and metal analysis indicate no significant dietary relationship between RSS sediments and oyster tissues. We conclude that the RSS sediments collected in this study were not a predominant food or metal source to wild *C. gigas* inhabiting Deep Bay, BC, Canada.

The exposure risk to humans ingesting Deep Bay oysters contaminated with Cd, Cu, Pb, and Zn was calculated and compared to current consumption guidelines set by the Agency for Toxic Substances and Disease Registry (MRLs) and the FAO/WHO reference doses (ASTDR 1999; JECFA 2004). Levels of Cu, Pb, and Zn were well below the recommended guidelines, whereas levels of Cd were four times the recommended daily dose. To generate a more comprehensive exposure risk for Cd, we calculated a total TDI level using all likely additional sources of exposure (*i.e.*, air, soil, total food, smoking). Our results indicate that although the current recommended maximum oyster consumption rates is consistent with the FAO/WHO and US EPA limits for acceptable risk (1 μg/kg/day), it leaves little or no room for error or uncertainty. Furthermore, the Canadian Total Diet Study (1993–1999) lists fresh or frozen shellfish to contain 0.0153 mg/kg wet weight for the average Canadian, a much lower level than the Cd concentrations in BC cultured oysters and the wild oysters from this study. Cd-linked bone and kidney toxicities have been observed in people whose dietary Cd intakes were well within the FAO/WHO 1 mg/kg/day limits (Satarug and Moore 2004). Recent studies indicate that Cd exposure levels of 0.43–0.71 μg/kg/day demonstrate an increased risk of bone fracture, cancer, kidney dysfunction, and hypertension (Satarug et al. 2000, 2003). Thus, there is little to no safety margin between Cd exposure in the normal diet and exposure that could produce deleterious effects, particularly in persons consuming oysters on a regular basis.

Pacific oyster (*C. gigas*) aquaculture is an important shellfish industry in the province of British Columbia, with regular export to Asian and US markets. Since 1999, several shipments of BC and Washington state (USA) oysters have been rejected in Hong Kong, as a result of cadmium (Cd) levels in the organisms being higher than maximum tolerable limits (>2 mg kg^{-1}wet weight) (WHO 1989). During this same period, wild oysters from the east coast of Canada and France did not have levels exceeding the WHO maximum tolerable limits (Crispo 2001, Miramand et al. 2001). The recent application by the European Community of a 1-mg/kg wet weight import limit to bivalve mollusks and the current deliberation by FAO/WHO expert committee on food additives to adopt the same value pose significant threats to the wild and cultured shellfish export trade in the Pacific Northwest (BC, Washington, and Alaska) (WHO 1989). For BC shellfish growers to remain competitive, reestablish markets in Hong Kong, and initiate sales in the EU, this crucial Cd issue needs to be addressed.

The source of heavy metal contamination to sediments and oysters inhabiting the Deep Bay ecosystem remains unknown. Our research eliminates resuspended and suspended sediments as a significant dietary and metal source to *C. gigas*. To successfully implement remediation practices and diminish human consumption risks, the source of metal contamination and uptake pathways within the Deep Bay food web must be investigated. Addressing these concerns will essentially solve two social dilemmas: the economic and ecological ramifications of chronically high metal levels within this productive marine ecosystem.

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References

ATSDR (Agency for Toxic Substances, Disease Registry) (1999) Toxicological profile for cadmium. US Department of Health and Human Services, Atlanta, GA

Bendell-Young LI (1999) Application of a kinetic model of bioaccumulation across apH and salinity gradient for the prediction of

cadmium uptake by the sedimentdwelling chironomidae. Environ Sci Technol 33:1501–1508
Bruland KW (1980) Oceanographic distributions of cadmium, zinc, nickel, and copperin the North Pacific. Earth Planet Sci Lett 47:176–198
Cabana G, Rasmussen JB (1994) Modeling food chain structure and contaminantbioaccumulation using stable nitrogen isotopes. Nature 372:255–257
Cheng WW, Gobas FA (2007) Assessment of human health risks of consumptionof cadmium contaminated cultured oysters. Human Ecol Risk Assess 13(2):1–13
Crispo S (2001) Marine biogeochemistry of cadmium. In: Kruzynski GM, Addison R, Macdonald RW (eds) Proceedings of a workshop on possible pathways of cadmium into the Pacific oyster Crassostrea gigas as cultured on thecoast of British Columbia, Institute of Ocean Sciences, March 6–7, pp 41–42 Can Tech Rep Fish Aquat Sci 2405
Croteau MN, Luoma SN, Stewart AR (2005) Trophic transfer of metals alongfreshwater food webs: evidence of cadmium biomagnification in nature. Limnol Oceanol 50(5):1511–1519
Currin CA, Newell SY, Paerl HW (1995) The role of standing dead Spartinaalterniflora and benthic microalgae in salt marsh food webs: considerations basedon multiple stable isotope analysis. Marine Ecol Prog Ser 121:99–116
Fisher NS, Reinfelder JR (1995) The trophic transfer of metals in marinesystems. In: Tessier A, Turner DR (eds) Metal speciation and bioavailabilityin aquatic ecosystems. Wiley, Cheshester, pp. 363–406
Fry B, Sherr EB (1984) δ^{13}C measurements as indicators of carbon flow inmarine and freshwater ecosystems. Contrib Marine Sci 27:13–47
Gearing JN, Gearing PL, Rudnick DT, Requejo AG, Hutchins MJ (1984) Isotope variability of organic carbon in a phytoplankton-based, temperate estuary. Geochim Cosmo Acta 48:1089–1098
Gray JS (1981) The ecology of marine sediments. Cambridge University Press, Canbridge
Han BC, Jeng WL, Chen RY, et al. (1998) Estimation of target hazard quotients and potential health risks for metals by consumption of seafood in Taiwan. Arch Environ Contam Toxicol 35:711–720
Health Canada (2006) Average concentrations (ng/g) of trace elements in foods for Total Diet Study from 1993 to 1999. Available from http://www.hc-sc.gc.ca/fnan/surveill/total-diet/concentration/metal_conc_plomb_9399.e.html
JECFA (Food, Agriculture Organization and World Health Organization (FAO/WHO)) (2004) Safety evaluation of certain food additives and contaminants. 61st meeting of the Joint FAO/WHO Expert Committee on Food Additives. WHO Food Additives Series 52. International Programme on Chemical Safety, World Health Organization, Geneva
Kang CK, Sauriau P-G, Richard P, Blanchard GF (1999) Food sources of the infaunal suspension-feeding bivalve *Cerastoderma edule* in a muddy sand flat of Marennes-Oleron Bay, as determined by analyses of carbon and nitrogen stable isotopes. Marine Ecol Prog Ser 187:147–158
Kling GW, Fry B, O'Brien WJ (1992) Stable isotopes and planktonic trophicstructure in arctic lakes. Ecology 73:561–566
Kruzynski GM (2004) Cadmium in oysters and scallops: the BC experience. Toxicol Lett 148(3):159–169
Lorrain A, Paulet Y-M, Chauvaud L, Savoye N, Donval A, Saout C (2002) Differential δ^{13}C and δ^{15}N signatures among scallop tissues: implications for ecology and physiology. J Exp Marine Biol Ecol 275(1):47–61
McDaniel W (1991) Method 200.3 Sample preparation procedure for spectrochemical determination of total recoverable elements in biological tissues. Environmental Protection Agency, Cincinnati, OH, pp. 24–29
Michener RH, Schell DM (1994) Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) Stable isotopes in ecology and environmental science. Blackwell, Oxford, pp. 138–157
Miramand P, Ferchaud R, Pigeot J, Caurant F, Bustamante P, Guyot T (2001) Estimation of the Cd intake in the human dietary from the shellfish caught in the seashore of Charente-Maritime (France). Bordeaux Aquacult
Mudroch A, MacKnight DS (1991) CRC handbook of techniques for aquatic sediments sampling. CRC Press, Boston
Paynter R (2002) Baynes Sound coastal plan for shellfish aquaculture. Available from http://srmwww.gov.bc.ca/rmd/coastal/south_island/baynes/ (accessed October 10, 2004)
Page HM, Lastra M (2003) Diet of intertidal bivalves in the Ria de Arosa: evidence from stable C and N isotope analysis. Marine Biol 143:519–532
Quinn MR, Feng XH, Folt CL, Chamberlain CP (2003) Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. Sci Total Environ 317(1–3):73–89
Rau G (1978) Carbon-13 depletion in a subalpine lake: carbon flow implications. Science 201:901–902
Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 120:497–507
Rainbow PS, Failkowski W, Sokolowski S, Smith BD, Wolowicz M (2004) Geographical and seasonal variation of trace metal bioavailabilities in the Gulf of Gdansk, Baltic Sea using mussels (*Mytilus trossulus*) and barnacles (Balanusimprovisus) as biomonitors. Marine Biol 144:271–286
Riera P, Richard P (1996) Isotopic determination of food sources of Crassostreagigas along a trophic gradient in the estuarine Bay of Marennes-Oleron. Estuar Coastal Shelf Sci 42:347–360
Satarug S, Baker JR, Urbenjaol S, et al. (2003) A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Letters 137:65–83
Satarug S, Haswell-Elkins MR, Moore MR (2000) Safe levels of cadmium intake to prevent renal toxicity in human subjects. Br J Nutr 84:791–802
Satarug S, Moore MR (2004) Adverse health effects of chronic exposure to low level cadmium in foodstuffs and cigarette smoke. Environ Health Perspect 112(10):1099–1103
Schallie K (2001) Results of the 2000 survey of cadmium in B.C. oysters. In: Kruzynski GM, Addison R, Macdonald RW (eds) Proceedings of a workshop on possible pathways of cadmium into the Pacific oyster *Crassostrea gigas* as cultured on the coast of British Columbia, Institute of Ocean Sciences, March 6–7, 2001, pp 31–32 Can Tech Rep Fish Aquat Sci 2405
Selim HM, Amacher C (1997) Reactivity and transport of heavy metals in soils. CRC Lewis, New York
Siegel FR (2002) Environmental geochemistry of potentially toxic metals. Springer-Verlag, Berlin
Tarradellas J, Bitton G (1997) Soil ecotoxicology. CRC Lewis, New York
Thomas C, Bendell-Yound LI, Yin K, Harrison PJ, Ross L, Fenney T, Arvai JL, Levings CD (2003) Biogeochemistry of the intertidal area of the Fraser River estuary. Geol Surv of Can 567:1–24
Thompson P (2001) Cadmium in Pacific oysters. In: Kruzynski GM, Addison R, Macdonald RW (eds) Proceedings of a workshop on possible pathways of cadmium into the Pacific oyster *Crassostrea gigas* as cultured on the coast of British Columbia, Institute of Ocean Sciences, March 6–7, 2001, pp 54–56. Can Tech Rep Fish Aquat Sci 2405
Thomson RE (1981) Oceanography of the British Columbia coast. Department of Fisheries and Oceans, Sidney, BC, Canada
USEPA (1998) Human health risk assessment: Protocol for hazardous waste combustion facilities, Volume I. Peer review draft. Office of Solid Waste, Region 6, Multimedia Planning and Permitting

Division. Center for Combustion Science and Engineering, Washington, DC

Wang W-X (2002) Interactions of trace metals and different marine food chains. Marine Ecol Prog Ser 243:295–309

Wang W-X, Fisher NS (1999) Assimilation efficiencies of chemical contaminants in aquatic invertebrates: a synthesis. Environ Toxicol Chem 18(9):2034–2045

Ward JE, Shumway SE (2004) Separating the grain from the chaff: particle selection in suspension and deposit-feeding bivalves. J Exp Marine Biol Ecol 300:83–130

WHO (1989) Evaluation of certain food additives and contaminants (thirty-third report of the Joint FAO/WHO expert committee on food additives). WHO Technical Report Series No. 776. WHO, Geneva

Xu Y, Wang W-X (2004) Silver uptake by a marine diatom and its transfer to the coastal copepod *Acartia spinicauda*. Environ Toxicol Chem 23(3):682–690

Zachariadis GA, Anthemidis AN, Caniou I, Stratis JA (2001) Determination of lead, cadmium and mercury in surface marine sediments and mussels. Int J Environ Anal Chem 80(3):153–166