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Element concentrations in shell of *Pinctada margaritifera* from French Polynesia and evaluation for using as a food supplement

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Abstract

Element concentrations in shell of *Pinctada margaritifera* (black-lip pearl oyster) from Manihi, French Polynesia, were measured with Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES). The respective average concentrations were: calcium (Ca) 396.4 mg/g, sodium (Na) 5.536 mg/g, magnesium (Mg) 2.136 mg/g, strontium (Sr) 890.6 ppm, iron (Fe) 67.89 ppm, aluminum (Al) 45.74 ppm, phosphorus (P) 27.19 ppm, boron (B) 12.17 ppm, manganese (Mn) 2.308 ppm, copper (Cu) 1.050 ppm, zinc (Zn) 0.7180 ppm; and nickel (Ni), chromium (Cr), mercury (Hg), arsenic (As), cadmium (Cd), lead (Pb), and vanadium (V) were below detection limits with ICP-AES.

The above concentrations were normalized and compared to the safety standards for human consumption determined by regulatory agencies of United Nations (UN), the European Union (EU), and the United States (US). Element concentrations detected in this study were all lower than the safety standards promulgated by regulatory agencies. These findings suggest that shells of *P. margaritifera* from Manihi, French Polynesia, do not raise any significant health concerns for human consumption. The shell of *P. margaritifera* thus potentially represents an important natural source for calcium-fortified foods, calcium supplement, and even for potential osteogenesis applications.

Keywords: *Pinctada margaritifera*; ICP-AES; Trace metal; Human consumption

1. Introduction

Pinctada margaritifera is a pearl oyster known for producing black pearls. It is reasonable to hypothesize that the calcium carbonate-rich shell of *P. margaritifera* may be a source of calcium for human consumption; actually, several calcium supplements made from 'oyster shell powder' are commercially available, e.g. 'Os-Cal' from GlaxoSithKline (US), 'Hi-Calcium from oyster shell' from Eckerd (US), 'Oyster Ca' from Vitasan (The Netherlands), and 'Nature Made Oyster Shell Calcium' from Otsuka Pharmaceutical (Japan).

Calcium carbonate has been widely studied for its protective effects against bone-related illnesses in intervention studies. A UK study of calcium intake and physical activity demonstrated that calcium supplementation significantly increased size-adjusted bone mineral content in adolescent girls ($n = 144$) (Stear, Prentice, Jones, & Cole, 2003). A Danish clinical trial demonstrated that calcium carbonate and vitamin D supplementations provided effective prevention of osteoporotic fractures in elderly community dwelling residents ($n = 9{,}605$) (Larsen, Mosekilde, & Foldspang, 2004). Calcium carbonate prepared from

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molluscan shell has also been tested for its effects on bone mineral density. A Japanese clinical trial using heated oyster shell powder along with heated seaweed in a women cohort ($n = 58$) shows beneficial effects on trabecular bone density (Fujita, Fujii, Goto, Miyauchi, & Takagi, 2000).

Osteoporosis with a hallmark of skeletal fragility is a major health problem of morbidity and mortality (Power et al., 1999), especially in an aging population (Blank & Bockman, 1999). Thus, an adequate amount of calcium intake from childhood to the end of the life span is critical for the formation and retention of a healthy skeleton.

Other studies in recent years on nacre powder prepared from *Pinctada* molluscan shell, including *Pinctada margaritifera*, showed bone morphogenesis in implantation into bone tissue in mammals (Berland, Delattre, Borzeix, Catonne, & Lopez, 2005; Cognet et al., 2003; Currey, Zioupos, Davies, & Casino, 2001; Rousseau et al., 2003, 2005). Despite these studies, the mechanism underlying the interaction between nacre and bone at the cellular level has yet to be elucidated. Use of molluscan shell as a food supplement may invoke concerns about heavy metals, e.g. arsenic, cadmium, mercury, and lead. Numerous publications have demonstrated that an intake of those heavy metals damages organs and tissues and may have other toxic effects to health (for a review, Bridges & Zalups, 2005). The Food and Agriculture Organization (FAO), World Health Organization (WHO), European Union, and the US agencies have specified heavy metal allowable levels for human consumption and exposure (EEC, 2001, 2005; FAO/WHO, 2003; NAS, 1997, 1998, 2000, 2001, 2004; PROPOSITION 65, 2005; USFDA, 1993).

In this study, we sampled *P. margaritifera* from Manihi, French Polynesia, and measured the baseline element concentrations in shells of *P. margaritifera* for validating applications of these shells as a natural source for calcium-fortified foods, calcium supplementation, and even for potential osteogenesis applications.

2. Materials and methods

2.1. Reagents and materials

Deionized, double distilled water (ddH_2O) was prepared with the Milli-Q apparatus (Millipore, Billerica, MA, USA). Bottles and vials used for sample preparations were made of polypropylene, polyethylene, or polystyrene, and were leached in 1 M HCl (trace metal grade) at ambient conditions for at least 24 h. Trace metal grade nitric acid and hydrochloric acid were from J.T. Baker (Phillipsburg, NJ, USA) and 0.2 μ PTFE Acrodisc CR 13 syringe filters were from Pall Corporation (East Hills, NY, USA). ModBlock digestion system, multi-element standards, and single-element standards were purchased from CPI International (Santa Rosa, CA, USA).

2.2. ICP-AES

The ICP-AES experiments were carried out with Thermo Jarell Ash IRIS Advantage/1000 Radial ICAP Spectrometer (Thermo Electron Corp., Waltham, MA, USA) with Charge Injection Device (CID) detector.

Elements selected for concentration determination were primarily based on the regulatory standards issued by authoritative regulatory agencies (EEC, 2001, 2005; FAO/WHO, 2003; NAS, 1997, 1998, 2000, 2001, 2004; PROPOSITION 65, 2005; USFDA, 1993). Wavelength lines were adjusted for the best sensitivity of the element carrying out ICP-AES. The selected wavelength lines (nm) were: Al (309.27), As (189.042), B (249.7), Ca (317.933), Cd (228.8), Cr (267.716), Cu (224.754), Fe (238.204), Hg (194.227), Mg (285.213), Mn (257.6), Na (589.5), Ni (231.604), P (213.618), Pb (220.3), Sr (346.4), V (292.4), and Zn (206.2), respectively.

2.3. Sample collection and preparation

Pinctada margaritifera shells were randomly collected at pearl farms inside the lagoon of Manihi, French Polynesia, during February, 2002. Manihi Atoll is located 502 km northeast of Tahiti main island, lagoon area is 165 km^2, opening index (percentage of channel width against total coral rim) is 8%, and seawater temperature ranges 26.5–30 °C (Pouvreau & Prasil, 2001). Collected shells were farmed for pearling with subsurface long line systems that obviates shells' growth at seabed (Haws, 2002).

A sample of 20 oysters was collected, with sizes ranging 15–18 cm in dorsoventral measurement. One piece of shell from each oyster was selected. A total of 20 pieces of shells were washed with ddH_2O, and microorganisms were brushed off, and then air dried in hood. Each shell was then submerged in liquid nitrogen for 10 min, followed by applying sheer forces with a hammer. Crushed shell debris was washed with ddH_2O, followed by grinding into powder with an aluminum mortar and pastel in liquid nitrogen. Homogenized shell powder (0.2 g) from each sample shell was subject to acid digestion in 10 ml of concentrated nitric acid/hydrochloric acid (1:1, v/v), followed by heating with ModBlock digestion system at 80 °C for 20 min. Dissolved shell samples were diluted with ddH_2O to 1/100×, 1/200×, 1/500×, 1/1000×, and 1/2000×, respectively. An aliquot of diluted sample solution was then followed by syringe filtration prior to analyzing with ICP-AES. A multi-element standard solution was freshly prepared from purchased stock solutions for ICP-AES analysis.

2.4. Statistical analysis

Sample data were analyzed using SPSS 13 (SPSS Inc., Chicago, IL, USA), and the results were reported in mean ($n = 20$) and standard deviation (SD). For ICP-AES measurement of each diluted sample, a total of 10 raw data points per element were collected and calculated for mean

($n = 10$) and SD. Raw data points were treated as outliers if they were outside 2SD range.

3. Results

The element concentrations of *P. margaritifera* are shown in Table 1. Heavy metals, including arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), and vanadium (V) were below the detection limit (BDL) of ICP-AES, suggesting these trace elements may range within one to two digits of ppb levels or lower.

Manganese (Mn), zinc (Zn), and iron (Fe) had significant skewness of 2.19, 2.15, and 1.80, respectively (Fig. 1). These positively skewed distributions are indicative that the ambient seawater is non-contaminated (Baldwin, Maher, Kleber, & Krikowa, 1996; Lobel, Mogie, Wright, & Wu, 1982, 1991, 1992). The observation of skewness in the analysis is consistent with the environment of Manihi Atoll.

Table 1
Concentrations of elements in shells of *Pinctada margaritifera* ($n = 20$) determined by ICP-AES

Element	Shell of *Pinctada margaritifera*			Ambient seawater means ± SD (ppm)
	Means ± SD (ppm)	Skewness	Kurtosis	
Ca	396,493 ± 6,009	0.54	0.17	511.3 ± 26.4
Na	5,536 ± 300	−0.29	−0.20	10,127 ± 100
Mg	2,136 ± 816	0.43	0.04	1,331 ± 12
Sr	890.6 ± 104.9	−1.13	0.99	5.293 ± 0.043
Fe	67.89 ± 94.52	1.80	2.64	0.023 ± 0.012
Al	45.74 ± 3.20	0.28	−0.04	2.626 ± 0.415
P	27.19 ± 12.04	1.03	0.97	0.023 ± 0.006
B	12.17 ± 1.09	1.01	0.51	3.866 ± 0.071
Mn	2.308 ± 1.181	2.19	5.85	BDL[b]
Cu	1.050 ± 0.316	1.32	2.53	BDL
Zn	0.718 ± 0.545	2.15	5.40	BDL
Ni	0.660 ± 0.168[a]	−0.12	0.68	0.013 ± 0.012
Hg	0.221 ± 0.03[a]	0.34	−0.62	BDL[b]
Cr	BDL	–	–	BDL
As	BDL[b]	–	–	BDL[b]
Cd	BDL[b]	–	–	BDL[b]
Pb	BDL[b]	–	–	BDL[b]
V	BDL[b]	–	–	BDL

Means of element concentrations in seawater ($n = 1$) were determined with raw data points ($n = 10$) directly from ICP-AES detection. Results of the Kolmogorov–Smirnov test on element concentrations of the shell showed that concentrations of Ca, Na, Mg, Sr, and Al conformed to normal distributions ($P > 0.2$), and concentrations of Fe, P, B, Mn, Cu, and Zn deviated from the normal distributions ($P \leqslant 0.2$).
BDL: Below Detection Limit; As, 7 ppb; Cd, 0.6 ppb; Cr, 10 ppb; Cu, 0.4 ppb; Hg, 57 ppb; Mn, 18 ppb; Pb, 9 ppb; and Zn, 1 ppb.
[a] Concentration is below the detection limit (i.e. virtually undetectable); measurement data were consistent across various dilutions during ICP-AES detection.
[b] Raw data were negative.

4. Discussion

The shell of *P. margaritifera* represents the body part potentially to be used as a calcium source and a therapeutic agent, and therefore, the soft body tissue is not focus and is excluded in this study. For contemporary operational practices and economic considerations, only the shell part, not the whole body of oyster, is utilized at the post-pearling, e.g. shell powder containing nacre as a base ingredient for cosmetic cream (Haws, 2002).

We compared the element composition in *P. margaritifera* with that in *P. fucata* (Table 2). *P. fucata* (Japanese pearl oyster) is known for producing Akoya pearls. Shells from these two species share similar element compositions, except for aluminum (Al), manganese (Mn), and zinc (Zn). The levels of these elements in *P. fucata* are much higher than that in *P. margaritifera*; particularly, the values of the bioaccumulation factor (BAF) of iron (Fe) and aluminum (Al) in *P. fucata* are 2.1×10^3 and 4.9×10^5 fold higher than those in *P. margaritifera*, respectively.

The differences may be attributable to influential factors that govern the incorporation of trace metals during molluscan shell formation (Wilbur, 1972). Huanxin, Lejun, and Presley (2000) summarized these influential factors, which include differences in (1) taxonomies and ontogenetic stages, (2) mineralogy (biomineralization) of the shell, (3) salinity and temperature of different water environments,

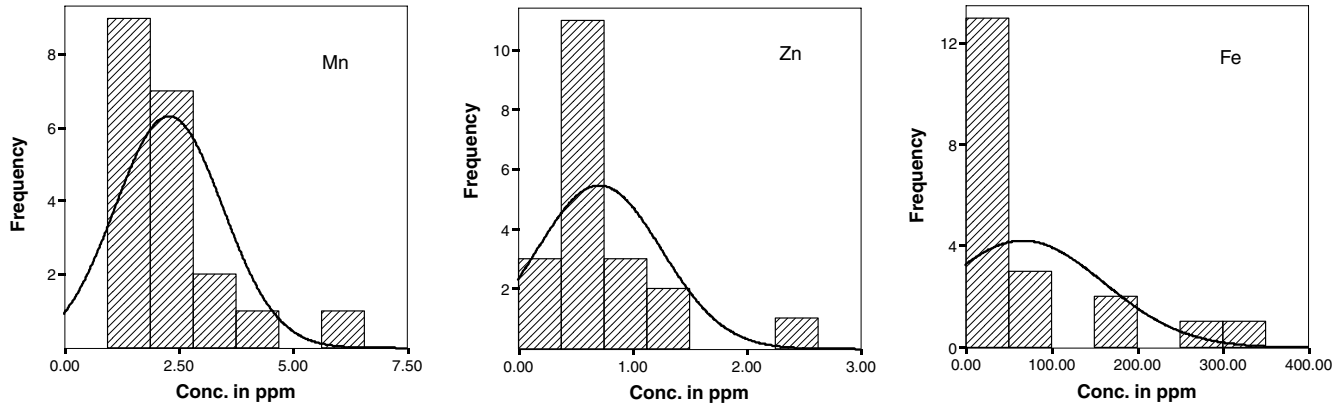

Fig. 1. Histograms of skewed distributions ($n = 20$). Significant skewnesses for Mn, Zn, and Fe, were 2.193, 2.153, and 1.803, respectively. The solid line represents the normal curve. Normality test (Kolmogorov–Smirnov) shows K–S values for Mn, Zn, and Fe were 0.225 ($P = 0.009$), 0.226 ($P = 0.008$), and 0.250 ($P = 0.002$), respectively.

Table 2
Comparison of element concentrations with *Pinctada fucata*

Element	Shell of *Pinctada margaritifera*		Shell of *P. fucata*[c]	
	ppm	BAF	ppm	BAF
Ca	3.96×10^5	7.75×10^2	3.56×10^5	8.64×10^2
Na	5,536	0.54	5,310	0.47
Mg	2,136	1.60	2,700	2.10
Sr	890	1.68×10^2	1,030	1.32×10^2
Fe	67.8	2.92×10^2	18	6.13×10^5
Al	45.7	17.41	258	8.60×10^6
P	27.1	1.18×10^3	NR	–
B	12.1	3.14	NR	–
Mn	2.30	BDL(SW)	37	1.85×10^6
Cu	1.05	BDL(SW)	6.5	4.33×10^4
Zn	0.71	BDL(SW)	7.6	5.13×10^4
Ni	0.66[a]	50.98	NR	–
Hg	0.22[a]	BDL(SW)	NR	–
Cr	BDL	BDL(SW)	NR	–
As	BDL[b]	–	NR	–
Cd	BDL[b]	–	NR	–
Pb	BDL[b]	–	NR	–
V	BDL[b]	–	NR	–
K	NR	–	116	0.29

BDL(SW): Element concentration in seawater was below the detection limit.
NR: Not reported, detection did not perform.
BAF = $(\text{ppm}_{\text{element in shell}})/(\text{ppm}_{\text{element in ambient seawater}})$, and the equation is drawn by definition (Willman et al., 1999).
BDL and footnote symbols of a and b, refer Table 1.
[c] Fujino et al. (1999) used 'concentration ratio', and 'bioaccumulation factor' was used in this study.

and (4) concentrations of various metal elements in ambient waters due to both seasonal and environmental variations. Hence, trace metal concentrations in shells of mollusk species vary to a wide range even if they belong to the same taxon. The differences further suggest that genotypic and environmental variations should be weighed in food safety assessment.

Another possibility is that the element composition of ambient seawater for the *P. fucata* study was compiled from literature data, rather than from the specific surveyed site. Though the relative composition of seawater is constant for all ocean waters, surface seawater composition varies subtly because of differences in both geographical locations and seasonality of sea currents.

It is well known that major composition of shell is calcium carbonate in the form of aragonite and calcite; Weiss, Tuross, Addadi, and Weiner (2002) have shown that the molluscan shell formation undergoes mineral morphology during its growth. As for the aim of this study, changes of mineral morphology maintain the same composition of calcium carbonate in shell and the digestion of calcium carbonate in gastric acid when consumed by humans. Hence, the mineral morphology of calcium carbonate is unlikely to affect the digestion mechanism in the human body.

5. Conclusion

Minerals listed in Dietary Reference Intakes (NAS, 1997, 1998, 2000, 2001, 2004), such as boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), phosphorus (P), zinc (Zn), and sodium (Na) were detectable in shells of *P. margaritifera*. Chromium (Cr), nickel (Ni), and vanadium (V) were below the detection limits with ICP-AES. However, fluorine (F), iodine (I), molybdenum (Mo), selenium (Se), silicon (Si), and sulfur (S) were not established in this study. Calcium constitutes about 40% of the total weight of the shell; the abundance of calcium suggests the economic value of using shells as a food supplement.

According to the Dietary Reference Intakes, NAS determines 2.5 g/day of calcium intake as the tolerable upper intake level (UL). Based on Tables 1 and 3, the intake amounts of other elements based on 2.5 g of calcium (Ca)

Table 3
Hazardous metal elements and safety levels set by regulatory agencies

Element	Shell of *Pinctada margaritifera* (ppm)	Maximum levels in foodstuffs[e]		Maximum levels for intake/exposure[e]		
		USFDA[a] (μg/g)	EU[b] (mg/kg)	FAO/WHO[c] (μg/kg/wk)	California Proposition 65[d] (μg/day)	
					A	B
As	BDL	110	–	–	10	–
Cd	BDL	5	1.0	7	0.05	4.1
Hg	0.22	–	0.5	1.6	–	–
Pb	BDL	6.3	1.0	–	15	0.5

[a] USFDA lead levels of concern in molluscan bivalves is set for adults 18–44 of age (USFDA, 1993).
[b] EU: Maximum level is set mg/kg wet weight for bivalve molluscs (EEC, 2001, 2005).
[c] Provisional tolerable weekly intake (PTWI) (FAO/WHO, 2003). FAO/UN unit is μg per kg of body weight per week; average body weight is assumed 65 kg for males and 55 kg for females at a low activity level (WHO, 1985).
[d] California Proposition 65: Part A. No significant risk levels (NSRLs) adopted in regulation for carcinogens, μg/day. Part B. Maximum allowable dose levels (MADLs) adopted in regulation for chemicals causing reproductive toxicity, μg/day (PROPOSITION 65, 2005).
[e] Because of the complexity of compounds, food types, technology innovations, policy-making structures, public opinions, and interest groups, regulations on food safety vary from regulatory agency to regulatory agency, and no international harmonization on safety standards for foods has been reached across nations. Some regulatory agencies determine safety levels based on concentrations in foods and some agencies determine standards based on maximum intake levels by humans.

was normalized. It is noteworthy that the normalized intake amounts of elements detected in this study were all under UL determined by NAS, indicating that the shell sample collected meets the safety standards for human consumption.

With regard to the levels of essential hazardous metals, e.g. arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), their concentrations in the shells were lower than the maximum levels in foods determined by the USFDA and the EU, and were also lower than the allowable intake levels determined by FAO/WHO (Table 3). Further, a very stringent standard of 'California Proposition 65' (PROPOSITION 65, 2005) was applied to evaluate the safety of maximum intake levels; such element concentrations in shell of *P. margaritifera* were all under regulated levels (Table 3). Hence, shells of *P. margaritifera* from Manihi Atoll are likely not to pose as a health hazard and potentially represent an important natural source for calcium-fortified foods, calcium supplements, and even for potential osteogenesis applications.

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