

Shell of abalone *Haliotis diversicolor supertexta* can eliminate waterborne zinc biokinetically

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Abstract

Dietary and nondietary accumulation of waterborne zinc (Zn) by shell of abalone *Haliotis diversicolor supertexta* was studied to determine if abalone shell can accumulate and eliminate Zn biokinetically. Shell of *H. diversicolor supertexta* rapidly accumulated Zn at microgram per gram concentrations during a 7-d uptake period for dietary and nondietary exposure to 1 µg ml⁻¹ Zn seawater. Depuration half-lives were 7.22 and 15.40 d for dietary and nondietary exposure, respectively. The uptake rate constants were 5.12 and 4.84 ml g⁻¹ d⁻¹, respectively, for dietary and nondietary exposure. The depuration rate constants were 0.048 and 0.10 d⁻¹, respectively for dietary and nondietary exposure. Results from this study showed that the shell of *H. diversicolor supertexta* accumulated Zn and that it reflected the composition of the seawater in which the abalone lived. This suggested that the shell of *H. diversicolor supertexta* can be used as a bioindicator or can act as a receptor to biokinetically eliminate heavy metals from aquatic food webs.

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1. Introduction

The accumulations of heavy metal in some marine organisms, such as mollusks and algae, have been suggested as indicators of heavy metal contamination in aquatic ecosystem (Zatta et al., 1992; Karez et al., 1994; Walsh et al., 1995; Han et al., 1997). Many mollusks and algae have wide distributions, extensive populations, sedentary nature and the ability to accumulate contaminants. Monitoring those bioaccumulators for heavy metals is useful as an ideal contamination index in aquatic ecosystems (Burdin and Bird, 1994; Walsh et al., 1994; Uno et al., 1997).

Abalone are common gastropod mollusks that inhabit the coastal reefs in tropical and subtropical areas (Hahn, 1989). The herbivorous gastropod, *Haliotis diversicolor supertexta*, is the most abundant abalone species in Taiwan. The aquaculture of *H. diversicolor*

supertexta is one of the important aquatic products in Taiwan (Chen, 1984, 1989). *H. diversicolor supertexta* prefers red algae, *Gracilaria* spp., which yield the best growth of the abalone (Chen, 1989; Singhraiwan and Doi, 1993). Because of economic considerations, the seaweed *Gracilaria tenuistipitata* var. *liui* has been selected to be the major forage for culturing *H. diversicolor supertexta*.

The accumulation of heavy metals in mollusks has been mainly studied from the content of soft tissues (Lautie et al., 1988). Although Bertine and Goldberg (1972) and Walsh et al. (1995) noted that heavy metals could be accumulated in soft tissue and calcareous shells of mollusks, yet the biokinetic characteristics of the shell is poorly known. Chen (1989) reported that the shell coloration of *H. diversicolor supertexta* differed according to the algae consumed: it was green after consuming *Chlorophyceae* and brown after consuming *Rhodophyceae*. It seems conceivable that this abalone utilizes the deposition process of new shell material to relocate bioaccumulated recalcitrant chemicals from the metabolically active soft tissues to the relatively inert shell

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Nomenclature

BCF	bioconcentration factor	k_1	uptake rate constant for zinc of abalone shell
C_s	zinc concentration in abalone shell	k_2	depuration rate constant for zinc of abalone shell
C_w	dissolved zinc concentration in water	n	sample size
F	the critical value tests for homogeneity	p	the probability tests for homogeneity
LT ₅₀	median tolerant time	r^2	coefficient of determination
SE	standard error from the mean	$t_{1/2}$	chemical half-life
T	the time when depuration begins		

material. Karez et al. (1994) indicated that there was a relative lack of information for contaminated regions in tropical and subtropical areas regarding the ability of abalone shell to accumulate heavy metals through non-dietary and dietary processes in aquatic environments.

An ideal bioindicator should be sedentary, abundant and exhibit longevity. It should be also easy to collect, able to accumulate pollutants and provide sufficient tissue for contaminant analysis. *H. diversicolor supertexta* meets all of these conditions. Therefore, abalone shell can be considered as an ideal biomaterial for environmental monitoring. Most of *H. diversicolor supertexta* are used for seafood in Taiwan restaurants. The shells of *H. diversicolor supertexta* can be obtained at minimal cost from processing wastes. Belcher et al. (1998) indicated that mollusk shell was a microlaminate composite of mineral and biopolymers with exceptional regularity, and with a strength far exceeding that of the crystals themselves in that the calcium carbonate inorganic phase of the shell contributes 98% of the shell mass. For example, the shell of *Haliotis rufescens*, the red abalone, is composed of two different polymorphs of calcium carbonate, calcite and aragonite (Belcher et al., 1996).

Zinc (Zn) is an essential micronutrient found at high levels in the algae and in the tissues of fish/shellfish (Hogstrand et al., 1998; Genter and Lehman, 2000). If waterborne Zn levels are elevated, however, toxicity can occur and have severe effects on the health of *H. diversicolor supertexta*, which will reduce market prices and cause closure of abalone farms. Previous investigations indicated that Zn was detected in many rivers and that maximum Zn concentrations in contaminated aquacultural waters were reported to range from 60 to 300 $\mu\text{g l}^{-1}$ in different areas of Taiwan (Lee et al., 1996). At these levels, Zn specifically disrupts calcium uptake by the gills of fish (Hogstrand et al., 1996; Galvez et al., 1998), leading to hypocalcemia, which may end with the death of the fish within a few days, depending on the Zn concentration.

This paper details a study to examine if shell of *H. diversicolor supertexta* exposed to waterborne Zn from dietary and nondietary routes via the alga *G. tenuistipitata* var. *liui* and ambient water, respectively, can accumulate and eliminate Zn biokinetically in a controlled

environment. To our knowledge, this study is the first to examine experimentally both the uptake and elimination of Zn in the shell of *H. diversicolor supertexta*.

2. Methods

2.1. Experimental setup

Viable *H. diversicolor supertexta* and *G. tenuistipitata* var. *liui* were collected for the laboratory exposure experiments from Toucheng located in northern Taiwan, because this was the most Zn-contaminated area in Taiwan (Chen, 1989). The abalone and algal samples initially were washed in seawater to remove epiphytes and kept at 4 °C during transfer to the laboratory. Abalone with average shell length of 4.03 ± 0.78 cm were selected for the experiments. The algal samples selected were mature, whole and healthy. A total of 200 abalone were transferred into four aquatic tanks of approximately 54 l, containing 50 l of artificial seawater. In order to imitate the environment of the abalone farms, the abalone were held in baskets. Each tank contained 10 baskets. Four abalone per basket were used for analysis. To be sure that at least four abalone would be alive at the end of the experiment, we put one extra abalone in each basket. Dissolved oxygen was maintained near saturation by aeration throughout the experiment. The temperature and salinity were maintained at 25 ± 1.5 °C and 35‰ under constant illumination (Yang and Ting, 1986). The pH value remained fairly constant during the experiment (7.75 ± 0.24). The abalone were fed daily with *G. tenuistipitata* var. *liui*. The average Zn concentration in *G. tenuistipitata* var. *liui* obtained from Toucheng abalone farm was measured to be 91.04 ± 33.10 $\mu\text{g g}^{-1}$ (mean \pm SE, $n = 9$). The abalone were acclimatized for two weeks before they were exposed to Zn.

In two tanks, Zn (ZnCl_2) was added to the seawater. In one tank the abalone were fed with algae, and in the other tank, the abalone were kept without food. The Zn level was determined by a preliminary experiment exposing abalone to different Zn concentrations (0.25, 0.5, 1, 2, 4, and 6 ppm). The tolerance (LT₅₀) of abalone

at ≤ 1 ppm Zn was longer than 21 days. Therefore, the abalone were exposed to $1 \mu\text{g ml}^{-1}$ Zn for 7 days in this experiment. The abalone were reared in the contaminated environment for 7 days uptake, then transferred to clean seawater and reared for 7 days of depuration. To examine if starvation affects Zn depuration in abalone shell, the same procedure with abalone was followed over 14 days using the other two tanks, but without Zn in the seawater.

Abalone and water samples were collected at day 0, 1, 2, 4, and 7, starting from the day that those organisms were exposed to the contaminated seawater and from the day the organisms were transferred to clean seawater. Each time one basket was taken along with 500 ml water out of each tank. From this basket four abalone were collected. Because preliminary observations showed that *H. diversicolor supertexta* only feeds at night, and has an empty gut in the evening, we collected the abalone at night to make sure the contents of gut would not influence the results. The experiments in the four tanks, described above, were repeated again. The water samples were fixed with 5 ml 1 N HNO_3 , and the samples of abalone were stored in the dark at -20°C until they were analyzed.

2.2. Chemical analysis

The abalone shells were freeze-dried overnight, and then ground into a fine powder in a grinder (Tai-Hsiang S36-89). A 500 mg portion of the ground samples was digested in 10 ml of 65% concentrated HNO_3 overnight at room temperature. The resulting solution was evaporated and redissolved in 0.1 N HCl. A Perkins-Elmer model 5100PC atomic absorption flame spectrophotometer (Perkins-Elmer, Shelton, Connecticut, USA) equipped with an HGA-300 graphite furnace atomizer was used to analyze Zn. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference materials (DORM-2, Dogfish Liver-2-organic matrix, NRC-CNRC, Canada). Recovery rates ranged from 95% to 97% and the levels of detection were $0.5 \mu\text{g Zn/g}$ shell and $5 \mu\text{g Zn/l}$ water.

2.3. Data analysis

Uptake and depuration rate constants were determined by fitting concentration data to the integrated form of the kinetic rate equation for constant dietary/nondietary Zn exposure, using iterative nonlinear regression,

$$C_s(t) = C_s(t=0) + C_w \frac{k_1}{k_2} (1 - e^{-k_2 t}), \quad (1)$$

where C_s is the Zn concentration in abalone shell ($\mu\text{g g}^{-1}$), C_w , the dissolved Zn concentration in water

($\mu\text{g ml}^{-1}$), k_1 , the uptake rate constant for Zn of shell ($\text{ml g}^{-1} \text{d}^{-1}$), and k_2 , the depuration rate constant for Zn of shell (d^{-1}). The bioconcentration factor (BCF) is calculated from the equation $\text{BCF} = k_1/k_2$.

As the first-order one-compartment model assumes that k_2 is not a function of Zn concentration in shell, k_2 is often determined by depurating contaminated organisms in uncontaminated water and determining k_2 directly in that test organism. Therefore, after the abalone are transferred to clean water and diet tank, respectively, the depuration rate constant (k_2) can be calculated by the linear regression of log-transformed shell Zn concentrations on depuration time (days) as $\ln C_s(t) = \ln C_s(t=T) - k_2 t$ where T is the time when depuration begins. Depuration half-life ($t_{1/2}$) was calculated as $\ln 2/k_2$. Variances in k_2 values derived from two methods were tested for homogeneity using an F -test. Values were then compared using t -test. All curve fittings were performed using the nonlinear regression option of the Statistica[®] software (StatSoft, Tulsa, OK, USA). Statistica[®] was also used to calculate the coefficient of determination (r^2) and to perform all statistical comparisons.

3. Results and discussion

The nonlinear regression resulting from the best fits of Eq. (1) to uptake and depuration concentration data for dietary and nondietary exposure is shown in Fig. 1. The regression equations to exposure concentration data are: For dietary exposure: (1) uptake phase: $C_s(t) = 14.4e^{-0.048t} + C_w(106.58)(1 - e^{-0.048t})$ ($r^2 = 0.99$) and (2) depuration phase: $C_s(t) = 40.5/e^{-0.096t}$ ($r^2 = 0.32$); and for nondietary exposure: (1) uptake phase: $C_s(t) = 14.4e^{-0.10t} + C_w(48.36)(1 - e^{-0.10t})$ ($r^2 = 0.80$) and (2) depuration phase: $C_s(t) = 32.67/e^{-0.045t}$ ($r^2 = 0.62$). The experimentally determined biokinetic parameters for the Eq. (1) describing the Zn bioaccumulation process in the shell of *H. diversicolor supertexta* exposed to Zn from dietary and nondietary routes are summarized in Table 1. A simple, well-established, one-compartment uptake-depuration model in Eq. (1) was thus fitted by the nonlinear technique to the 14 d exposure data and the average coefficient of determination (r^2) was 0.68 (Table 1). Estimates of k_2 were also determined from the depuration phases. All of these regressions were significant ($p < 0.05$), with coefficient of determination values ranging from 0.32 to 0.62 (Table 1).

The BCF of abalone shell in the uptake phase (107.56 ml g^{-1}) was greater than that in the depuration phase (53.33 ml g^{-1}) during dietary exposure, whereas the result was opposite in the nondietary exposure (Table 1). Analysis of variance showed that the uptake rate constant of shell of *H. diversicolor supertexta* in dietary exposure was not different from that of nondietary

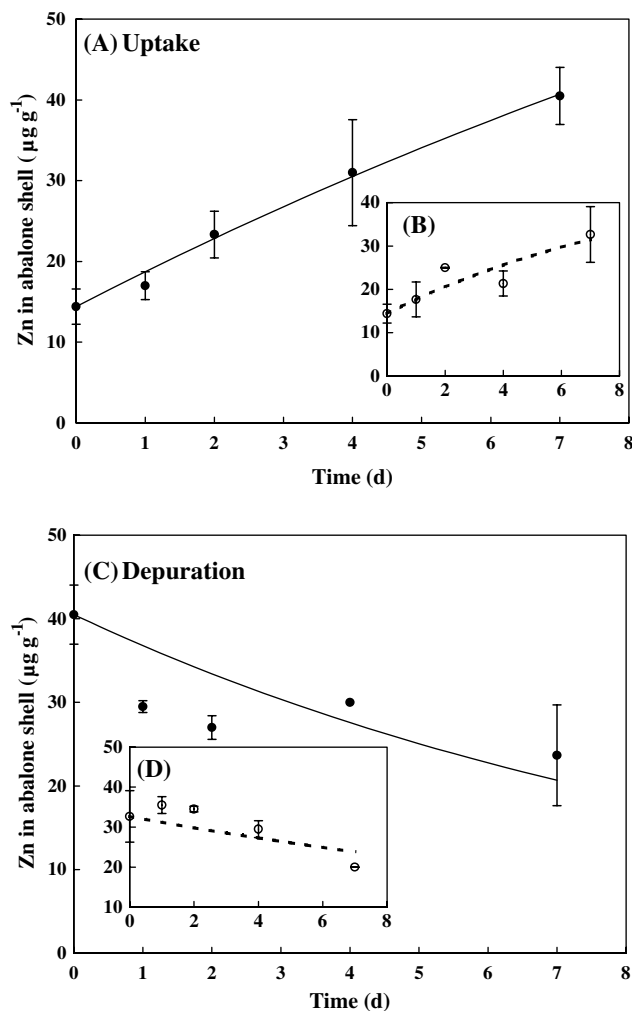


Fig. 1. Uptake ((A) and (B)) and depuration ((C) and (D)) of Zn by shell of *H. diversicolor supertexta* during a 14-d dietary and nondietary exposure. The measurements are shown with symbols (●): dietary exposure; (○): nondietary exposure and the model fittings are shown in lines (—): dietary exposure; (---): nondietary exposure. The error bars show one standard error from the mean ($n = 4$).

exposure ($F = 0.015$, $p > 0.05$). There were also no significant differences between dietary and nondietary exposure regarding BCFs ($F = 0.543$, $p > 0.05$). The

results from the experiments without Zn indicated that Zn concentrations in abalone shell in dietary exposure were not significantly different from those in nondietary exposure ($F = 0.46$, $p > 0.05$), suggesting that the effect of starvation can be overlooked during the experimental exposure period.

There was some accumulation of Zn in control abalone shell ($14.4 \mu\text{g g}^{-1}$ at day 0 in Fig. 1) due to the unavoidable presence of trace amounts at the abalone farm. During dietary exposure, the abalone shell continued to accumulate Zn in the uptake phase, leading to slower depuration rate (0.048 d^{-1}) compared to that in the depuration phase (0.096 d^{-1}), whereas the absence of algae in nondietary exposure resulted in a higher depuration rate in the uptake phase compared to that in the depuration phase (Table 1). The half-life of the uptake phase in dietary exposure (14.44 d) was larger than that in nondietary exposure (6.93 d). In the depuration phase, the half-life in nondietary exposure (15.4 d) was larger than that in dietary exposure (7.22 d).

The results demonstrated that shell of *H. diversicolor supertexta* accumulated considerable amounts of Zn from seawater. The quantity of heavy metals in shell of mollusks clearly reflects the quantity of the heavy metals in the aquatic environment where mollusks grow (Bertine and Goldberg, 1972; Walsh et al., 1995), suggesting that the shell of *H. diversicolor supertexta* can potentially be a useful bioindicator for the bioaccumulation of Zn in artificial and natural environments. The percent difference between uptake rate constants in dietary and nondietary exposure was 5.5%, indicating that Zn in abalone shell was from ambient water and not from the algae. A similar conclusion was reported by Amiard-Triquet et al. (1987) where they demonstrated that Zn levels in algae-grazing mollusks, *Gibbula umbilicalis* and *Littorina littorea*, were not different from Zn levels in a brown alga, *Fucus serratus*, which was the food species of the mollusks.

Bertine and Goldberg (1972) and Walsh et al. (1995) demonstrated that heavy metals were usually higher in the soft parts than those in the solid shell of clams, mussels and shrimps. The presence of contaminants in

Table 1

Bioaccumulation parameters (mean \pm 1 SE) for Zn calculated from *H. diversicolor supertexta* shell dietary/nondietary exposure data

	Dietary		Nondietary	
	Uptake phase	Depuration phase	Uptake phase	Depuration phase
k_1 ($\text{ml g}^{-1} \text{d}^{-1}$) ^a	5.12 ± 1.18		4.84 ± 1.51	
k_2 (d^{-1})	0.048 ± 0.044	0.096 ± 0.028	0.10 ± 0.033	0.045 ± 0.02
BCF (ml g^{-1}) ^b	106.58	53.33	48.36	107.56
$t_{1/2}$ (d) ^c	14.44	7.22	6.93	15.40
r^2 ^d	0.99	0.32	0.80	0.62

^a Uptake rate constant (k_1) and depuration rate constant (k_2) calculated from Eq. (1), whereas at depuration phase k_2 calculated using the model \ln concentration = a + b (time) fitted to depuration concentration data.

^b Bioconcentration factor calculated from the equation: $\text{BCF} = k_1/k_2$.

^c Depuration half-life calculated from $t_{1/2} = \ln 2/k_2$.

^d Coefficient of determination.

the shell of *H. diversicolor supertexta* may represent an effective and sensitive means to assess contamination in the soft tissue of this organism and to monitor the aquatic ecosystems. Based on these characteristics, the Zn levels in shell of *H. diversicolor supertexta* indicate the Zn levels in water. Walsh et al. (1995) suggested that although the Zn in abalone shell was less than in soft tissue, the shell was still useful as an indicator, yet the amount of Zn in shell was proportional to the concentration in soft tissue. Results from this study suggest that a certain fraction of the elimination of Zn may be due to biotransformation and not just a physical diffusion-related elimination process.

4. Conclusions

This study has provided biokinetic data for uptake and elimination of Zn by shell of *H. diversicolor supertexta*, which are essential for developing predictive models of Zn accumulation in field aquacultural ecosystems. Results from this study imply that abalone shell could act as a toxic waste sink to remove waterborne heavy metals from the metabolically active tissue and therefore effectively eliminate these metals from the food chain in that the relocation of the contaminants to the shell represents an effective detoxification mechanism. The use of abalone shell as a bioindicator may be useful for determining the extent of biotransformation in aquatic food webs, as an essential component of risk assessment of heavy metals.

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