Effect of body size and food quality on the assimilation of ^{65}Zn and ^{110m}Ag in Bloody Cockles (*Anadara senilis*) from Ghana

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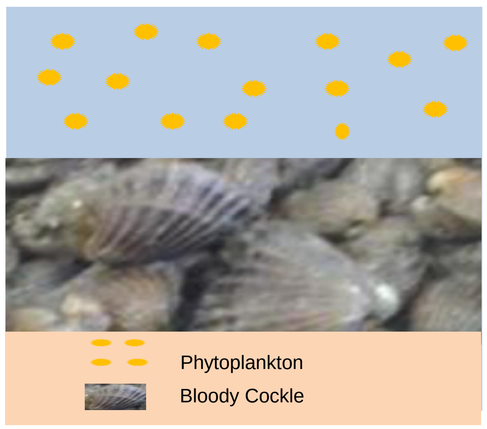
Introduction

Phytoplanktons are considered a primary source of nutrition for most herbivore feeding bivalves. In aquatic systems however these phytoplankton cells can bioconcentrate metals of varying properties from its ambient environment and subsequently transfer these metals to their consumers.

However, the relative importance of the efficiency of assimilation in bivalves is metal-specific and could be characterized by external environmental conditions and internal biological processes such as salinity, temperature, food composition and body size.



In Ghana and other coastal waters along the Gulf of Guinea, the "bio-sentinel" species, bloody cockle (*Anadara senilis)* has been assumed as a bioindicator as a result of their cosmopolitan abundance in this region. Meanwhile only few studies have considered the metal accumulation potential of this species let alone the biokinetics of metal uptake required for realistic interpretation of biomonitoring data.

The study therefore employed radiotracers of ^{65}Zn and ^{110m}Ag to investigate the effect of body size and food sources on the bloody cockle (*Anadara senilis*). These two metals are of environmental and public health significance in most coastal habitats. Information on assimilation and retention in response to these conditions is vital for the development of predictive bioaccumulation model for this species.

Moreover, with concerns raised about the safety of seafood, the assimilated metals in the bloody cockle harvested as food for human consumption in Ghana was further examined to estimate potential threats posed to predators and humans through dietary ingestion. This was studied using two approaches: subcellular fractionation which quantifies the proportion of assimilated metals associated with soluble cytosolic fraction available for trophic transfer and human *in vitro* digestion simulation that estimates the bioaccessible fraction (i.e the extractable fraction resulting from human digestive processes).

Experimental Protocol

Bloody cockles of shell length and body weight for small (28.0±1.9 mm, 12.0±1.8 g) and big (39.0±3.1 mm, 27.6±4.9 g) sizes respectively were sampled from Narkwa lagoon in Ghana. Samples were maintained in an aerated seawater at temperature and salinity of 27^0C and 38‰ respectively at the IAEA Environment Laboratories in Monaco where experimental work was carried out.

Radiolabelling of phytoplankton and feeding experiment

Skeletonema costatum (*SKE*) and *Isochrysis galbana* (*ISO*) radiolabelled for five days

Filtration and resuspension

↓

Cockles fed for 2 hr

↓

28 Days Depuration

↓

$$A_t = A_{0s}e^{-k_{es}t} + AE$$

Experimental design of *in vitro* human digestion simulation

Grilled Cockle

↓

Saliva
pH 6.8, 5 min

↓

Gastric Juice
pH 1.3, 2 hrs

↓

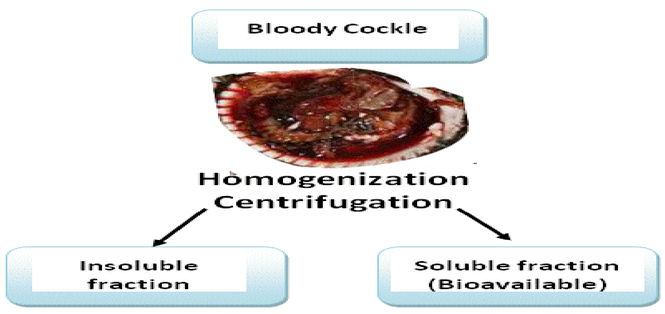
Duodenal Juice + Bile+ HCO_3
pH 8.1, 2 hrs

↓

Centrifuge, 5 min
Pellet and supernatant (bioaccessible fraction) separated

Schematic diagram of *in vitro* digestion simulation model

Subcellular fractionation of metals

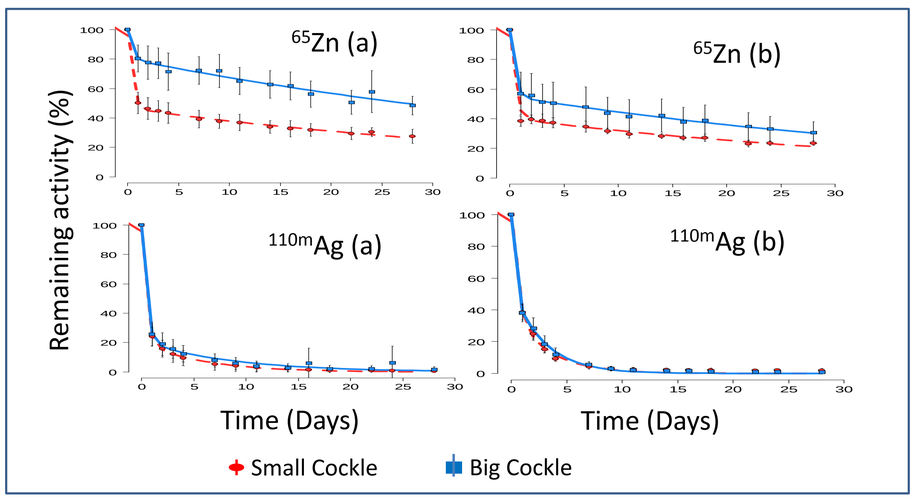


Differential centrifugation diagram for subcellular fractionation

Instrumental Analysis

All experimental samples were radioanalyzed using a high-resolution γ-spectrometer system composed of five Germanium – N or P type–detectors (EGNC 33-195-R, Canberra® and Eurysis®).

Results and Discussion



Whole body depuration kinetics of ^{65}Zn and ^{110m}Ag in small and big sized bloody cockle (*Anadara senilis*) following 2 hr feeding on radiolabelled phytoplankton (a) *Isochrysis galbana* and (b) *Skeletonema costatum;* remaining activity (%) ± SD, n=6.

➢Depuration of radiotracers best described by a double exponential kinetics with initial rapid egestion of unassimilated metals.

➢High retention of ^{65}Zn, an essential element than ^{110m}Ag which has no biological function.

Assimilation efficiency (AE, %) and biological half-life ($T_{b1/2}$, d) of ^{65}Zn and ^{110m}Ag in two sizes of bloody cockles (*Anadara senilis*) fed radiolabelled *Isochrysis galbana* and *Skeletonema costatum;* ASE: asymptotic standard error; R^2: determination coefficient.

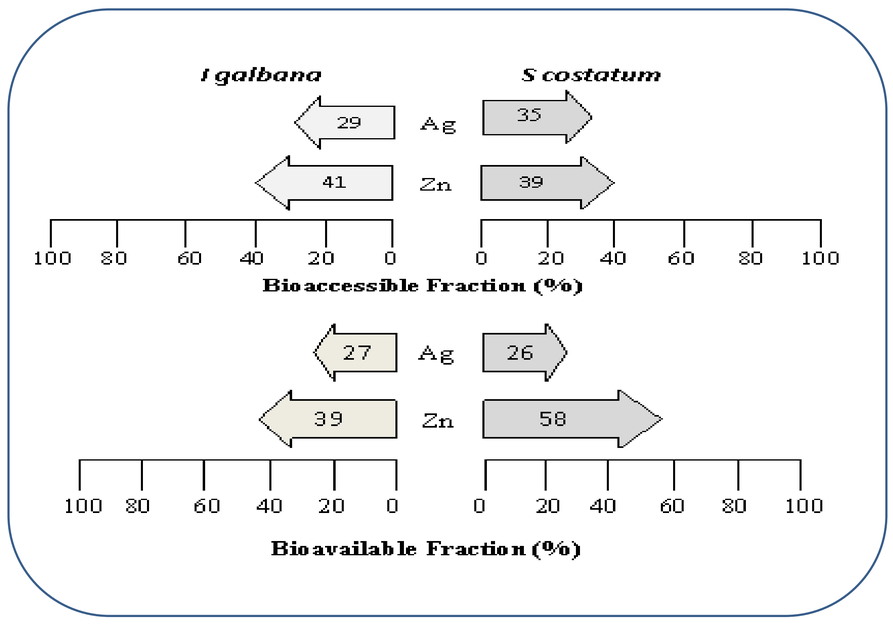
Isotope	Food type	Cockle Size	AE±ASE	$T_{b1/2}$±ASE	R^2
^{65}Zn	*I galbana*	Small	46.5±1.5**	34.5±4.1**	0.962
		Big	80.1±2.5**	40.2±5.3**	0.833
	S costatum	Small	40.2±0.8**	30.2±1.9**	0.993
		Big	55.3±2.7**	32.2±5.6**	0.867
^{110m}Ag	*I galbana*	Small	20.5±2.53**	4.0±0.6**	0.992
		Big	21.0±3.6**	6.0±1.5 **	0.979
	S costatum	Small	38.9±17.5*	2.3±0.7*	0.997
		Big	54.4±2.4**	2.0±0.1**	0.995

Significance of estimated parameters: *p<0.05, **p<0.01

➢For both food types, big sized cockles assimilated the metals at higher efficiency than small sized cockles.

➢ Whereas ^{65}Zn assimilation was higher when ingested via *I galbana* than *S costatum,* ^{110m}Ag assimilation efficiency was the opposite.

➢ The variable AE and biological half-life of trace metals based on body size and food composition provides an indication of the biological difference and specific nature of metal digestion in marine bivalves.



Proportion of metals associated with (a) bioaccessible fraction and (b) trophically (bio) available metals.

➢The fraction of Zn and Ag bioaccessible to humans through consumption of grilled cockles from both contaminated food sources was less than 45%

➢Using differential centrifugation to estimate the bioavailability from cytosolic fraction of the cockle revealed a further lower degree of trophic transfer from the assimilated metals although Zn was relatively higher than Ag.

Conclusion

➢The bloody cockles have demonstrated the ability to appreciably assimilate the studied metals from ingested food sources.
➢Food quality and body size appears to have a profound effect on Zn but to a lesser extent on Ag assimilation and retention which suggest the diversified strategies of the cockle in handling the essential and nonessential metals.
➢A consistent reflection of low levels of Ag in the cytosolic fraction and human digestion simulation in contrast to Zn further suggests that this metal may not be biologically available for trophic transfer.

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