Heavy Metals Bioaccumulation Pattern of Bivalve *Geloina* spp., and Crustaceans *Penaeus marguensis* and *Scylla serrata* from the Southern Central Java Ocean Margin, and Its Consumer's Safety

Sri Noegrohati
Faculty of Pharmacy, Gadjah Mada Univerity, Jogyakarta, Indonesia, 55281
E-mail: srinoegrohati@yahoo.com

ABSTRACT

Aquatic organisms exposed to low level natural heavy metals pollutant tend to accumulate in their tissue by means of metabolic and biosorption processes. However, the influence of external environment to heavy metal bioavailability and internal organism characteristics to bioaccumulation processes remain uncertain. In this work, the influence of Cu, Zn, Cd and Hg bioavailability, feeding strategies and heavy metals homeostasis regulations or detoxification mechanisms to bioaccumulation pattern in aquatic bioindicators were studied.

Only Hg in Segara Anakan water samples was determined, while Cu, Zn and Cd were not detected. Sediment samples of Segara Anakan indicated higher heavy metals level than those of Serang river mouth, but only Hg concentration in sediment samples of Segara Anakan which is significantly higher than those of Serang river mouth, while Cd was not detected. The trend of heavy metals bioconcentration ratio of filter feeder Geloina spp and planktonic grazer Penaeus marguensis follows the desorption coefficient, i.e. Zn>Hg>Cu, but the bioconcentration trend in Scilla serrata, which is macrobenthos predator, is Hg>Zn>Cu.

The exposed Cu was not accumulated by Geloina spp., but accumulated at highest concentration in the exoskeleton of Penaeus marguensis and cartilage of Scilla serrata. Highest accumulation concentration of Zn and Hg were determined in meat, and lower concentrations were determined at other tissues. The extent of heavy metals bioaccumulation in Geloina spp. and Scilla serrata reflect the pollution of the surrounding environment, but the extent of heavy metal bioaccumulation in Penaeus marguensis were influenced more by its physiological status. Since the environmental exposure of Hg in Segara Anakan estuary was relatively high, pregnant women from this region should consider the potential hazard of Hg to their fetus when consuming aquatic organism of higher trophic level such as estuarine crabs.

INTRODUCTION

Trace metals find their way into the environment by natural (e.g., volcanic eruptions and erosion of rocks) and anthropogenic means. Metals are nonbiodegradable, and therefore, tend to accumulate in the environment, especially in the sediments (Tessier and Campbell, 1990; Birch et al., 1996). Aquatic organisms exposed to low level natural heavy metals pollutant tend to accumulate in their tissue by means of metabolic and biosorption processes. The bioaccumulation studies of metals by the tissues led to the adoption of the bioindicator concept (Langston and Spence, 1995). The heavy metal levels measured in bioindicators assumed to be representing an integrated value of the mean ambient load that is biologically available. However, the influence of external environment to heavy metal bioavailability and internal organism characteristics to bioaccumulation processes remain uncertain.

Trace metals may be taken up by aquatic organisms mainly from solution and from food. Abiotic key factor that influence the bioavailability and toxicity is metals distribution between the sediment and the aqueous phase. In the ocean margin environment, the sorption-desorption equilibrium mainly determined by salinity (Noegrohati, 2005). The undisturbed K_D of heavy metals Cu, Zn Cd and Hg in brackish water determined in this study were between $10-10^3$, indicating of relatively more metals present in aqueous phase, and therefore, more metals are taken up from solution compared to food (Luoma, 2002). When these metals are taken up by organism, the metals bonded in sediment will immediately replace the taken up metal form through sorption-desorption equilibrium. Therefore, sediment analysis may be more useful to detect metal pollution problems and sources.

Inside the organisms, internal factors such as metals distribution among cytosol, detoxified forms and tissues or organelles (lysosome and related vesicles), also influence metal bioavailability. Due to the constant exposure to metals, sophisticated detoxification processes have developed. Detoxified metal forms occur as conjugates (e.g in granules within tissues) or associated with metal-specific protein systems (metallothioniens, MTs). MTs bind specifically to Cu, Zn, Cd and Hg ions and, therefore, can be considered regulatory molecules for essential metals (Cu and Zn) or detoxification proteins for non essential metals (Cd and Hg). These bonded heavy metals were then incorporated into lysosomes. After accumulated in lysosomes, they are excreted out of the cell and transported into the integument epithelia. The mantle epithelial cells of bivalves accumulate Cd, Cu, Fe, Hg and Zn in lysosomes. Metal ions become incorporated into the crystalline structure of the shell by replacing Ca in the carbonate complex; therefore the shell serves as a storage matrix for toxic metals. These mechanisms ensure continuous metal uptake. However, metals bioaccumulation pattern in organisms at the same location differ between different species and individuals due to species-specific ability/capacity to regulate or accumulate trace metals (Reinfelder et al., 1997; Otchere et al., 2003).

The bioindicators used in this study are those lead double lives, sometimes swimming and some times burrowing in the sediments, i.e. estuarine bivalves *Geloina* spp. and estuarine crustaceans: penaeid shrimps (*Penaeus marguensis*) and estuarine crabs (*Scilla serrata*). These organisms are widely used as bioindicators of aquatic pollution. They have different feeding habit: *Geloina* spp. is a sessile epibenthic filter feeder; therefore they may integrate over time various water qualities (Day et al, 1989). Penaeid shrimps are higher in trophic level since their natural food is phytoplankton, benthic algae, detritus, and zooplankton. While estuarine crab is the highest trophic level bioindicator in this study, they are macrobenthos predator, deposit feeding, and scavenging dead organisms (Day et al, 1989). Differences in feeding strategy may significantly affect the bioaccumulation of heavy metals (Lee *et al.* 2000).

In this work, the influence of feeding strategies and heavy metals regulations or detoxification mechanisms to bioaccumulation pattern of heavy metals Cu, Zn, Cd and Hg in *Geloina* spp., penaeid shrimps and estuarine crabs will be evaluated. The obtained data will be used for safety assessment of seafood consumers.

MATERIALS AND METHODS

Study area

Two sites of Southern Central Java ocean margin were chosen for this study. The first site, Segara Anakan Estuary, which scavenges water borne heavy metals influx from Citanduy river. These heavy metals were originated from weathered rock of Gunung Galunggung eruption. The second site is Serang river mouth, around 150 km from Segara Anakan, which is expected to represent an ocean margin environment without natural pollutions from volcanic area.

Field sampling

The samples of water, sediment and biological samples: *Geloina* spp., penaeid shrimps (*Penaeus marguensis*) and estuarine crab (*Scilla serrata*).of Segara Anakan were collected at the same time at May 2004. Similar samples were collected from the mouth of Serang river at June 2004.

Water and surficial sediment samples were taken at lowest tide. The water samples were acidified with nitric acid to pH less than 2. The upper 5 cm sediment layer was collected and packed in a polyethylene bag. Penaeid shrimps were wrapped in a polyethylene bag and stored in an ice chest at -4°C, and transported into the laboratory as soon as possible. *Geloina* spp. and estuarine crab samples were scrubbed with water at the sampling sites. All of the samples were transported into the laboratory as soon as possible in wet condition

Analytical methods

The quality of reagents used in this study was pro analysis. The analytical instruments for Cu, Zn and Cd determination were Flame/Zeeman Atomic Absorption Spectrophotometer Hitachi Model 180-60/80, with air-acetylene flame. The resonance line used for Cu, Zn and Cd determination were 324,8 nm, 213,8 nm, and 228,8 nm, and the limit of detections were 0,181 mg/mL, 0,033 mg/mL and 0,059 mg/mL respectively.

Determination of mercury was done in Perkin Elmer AAS model 3400 system, equipped with a continuous cold vapor generator connected to an electrically heated quartz tube atomizer, at resonance line of 253,7 nm and limit detections of 4,12 ng/mL.

The analytical procedure for saline water was based on the EPA method 200.12 (Creed and Martin, 1997), while sediment and biota were based on the Standard Method for Examination of Water and Wastewater of APHA, AWWA, WEF, 1992..

Samples preparation

Water

Heavy metals were extracted into $CHCl_3$ after ammonium pyrolydine dithiocarbamate (APDC) complexation at pH 4, and then re-extracted into aqueous HNO_3 solution pH 2. The aqueous extract was then subjected into the appropriate AAS.

Sediment

Sediment samples were wet digested with 3:2 mixture of HNO_3:HCl at 80°C, the obtained clear solution, was than subjected into the appropriate AAS

Biota (Texas Parks and Wildlife Department (TPWD), 2003).

Geloina spp samples were determined its volume individually. To drain the shell liquor, the *Geloina* spp were frozen until they open up slightly and open the shell with a precleaned knife and the weighed individually. Cut the muscle from the upper shell and cut away the meat. Both, the meat and shells were weighed individually. The nutrition index of *Geloina* spp. was quantified as weight of meat/volume. The separated meat and shells were homogenized.

Penaeid shrimps samples were weighed individually, and then measured its length from the tip of the rostrum to the tip of the telson. The nutrition index of penaeid shrimps was quantified as weight/length. The separated meat and shells were homogenized.

Estuarine crab samples were weighed individually and measured its total width of the carapace from the tip of one lateral spine to the tip of the opposite lateral spine. They were anesthetized on ice prior being sacrificed. The nutrition index of estuarine crab was quantified as meat weight/ total width of the carapace. Meat, cartilage and a section of dorsal carapace from above the pericardium were dissected and homogenized.

All homogenized tissues were kept frozen prior to analysis. A portion of homogenized tissues were separated for dry weight determinations, and other portions were wet digested as in sediment samples to obtain clear solution and then subjected into the appropriate AAS.

Data Processing

The Cu, Zn, Cd and Hg concentration of all abiotic and biotic samples were quantified using standard addition methods.

The heavy metal bioaccumulation of each tissues in organisms are quantified as concentration ratio to sediment as their heavy metal source, while the extent of heavy metals accumulated in the organisms are described as the ratio of total heavy metal concentration in organisms to sediment concentration.

Evaluations were carried out at 95% confidence level.

RESULT AND DISCUSSION

Quality control

Ideally, calibration standards solution should have similar composition with the samples solution to be analyzed. The complexity of the matrix of seawater, sediments and tissues samples may cause chemical and spectral interferences which can be minimized by the standard addition method. Therefore, to overcome all possible interferences, standard addition method is applied in quantification of all samples. The result of the analytical procedure validations, including limit of quantitation (LOQ), linear range, correlation coefficient and recovery are presented in table 1. The results showed that the analytical procedure is valid and reliable with an overall 95 % confidence level.

Table 1. Validations of analytical procedures for heavy metals determinations in several environmental samples, including limit of quantitation (LOQ), linear range, correlation coefficient and recoveries

Samples Water	Heavy metals	LOQ mg/L or mg/kg	Linearity up to mg/L or mg/kg	Correlation Coefficient	Recovery %
	Cu	0,051	~0,510	0,997	88 - 90
	Zn	0,113	~0,129	0,798	80 - 90
	Cd	0,024	~0,471	0,998	98 - 106
	Hg	0,011	~0,048	0,992	78 - 102
Sediment	Cu	5,03	~84,06	0,993	88 - 103
	Zn	2,01	~16,13	0,966	85 - 105
	Cd	3,59	~22,18	0,974	71 - 78
	Hg	0,11	~2,36	0,999	95 - 101
Geloina spp					
meat	Cu	1,97	~32,75	0,998	98 - 120
	Zn	2,89	~31,66	0,949	101
	Cd	0,80	~16,36	0,998	97 - 103
	Hg	0,014	~1,01	0,998	99
shell	Cu	4,31	~64,71	0,997	88 - 103
	Zn	5,92	~33,41	0, 947	106
	Cd	0,71	~31,52	0,999	93 - 106
	Hg	0,39	~3,20	0,999	100 - 103
Penaeus marguensis					
meat	Cu	4,48	~37,49	0,988	80 - 111
	Zn	1,15	~21,60	0,991	97 - 115
	Cd	0,29	~16,01	0,999	95 - 103
	Hg	0,37	~1,04	0,899	99
shell	Cu	11,56	~124,23	0,980	77 - 104
	Zn	3,39	~24,33	0,954	90 - 140
	Cd	0,72	~31,60	0,999	96 - 105
	Hg	1,36	~3,83	0,971	93 - 128
Scilla serrata					
meat	Cu	2,98	~58,30	0,995	95 – 100
	Zn	5,18	~100,94	0,917	111 - 133
	Cd	0,44	~16,28	0,999	97 – 102
	Hg	0,42	~7,29	0,932	99
carapace	Cu	10,91	~92,01	0,983	74 - 109
	Zn	2,08	~15,65	0,982	97 - 112
	Cd	0,48	~31,60	0,999	85 - 104
	Hg	0,65	~4,26	0,956	99 - 104
cartilage	Cu	29,21	~275,86	0,892	89 - 135
	Zn	8,61	~80,85	0,899	113
	Cd	0,70	~31,69	0,999	94 - 102
	Hg	1,21	~11,66	0,782	100

Heavy metals distribution in abiotic system

In the ocean margin ecosystem, where fresh water and seawater are mixed, sedimentation occurred rapidly. All metals influx from continental streams are natural constituents of the environment, tend to accumulate in the sediments (Tessier and Campbell, 1990; Birch et al., 1996). However, sediments are not only functioning as heavy metal scavenger, but also as one of potential sources for heavy metals to the ecosystem (Noegrohati, 2005, b). The sediment texture was heavy clay with organic matter ranging from 2,65% to 4,55%, indicating of relatively high organic matter.

The average salinity at the sampling time was 23,8 ‰. In saline water condition, Cd and Hg cations form chlorocomplexes which prefer to be in water phase, while Cu and Zn cations are more retained in the sediment due to organocomplex formation.

No heavy metals could be detected in water samples, except Hg in water samples of Segara Anakan estuary (Table 2). Similarly, higher level of Hg was also observed in sediment samples of Segara Anakan (P 0,05). No significant difference was observed for Cu and Zn concentration in both sediment samples, and Cd could not be detected in sediments of both sampling sites.

The trend of sediment concentration in both sampling sites were Cu>Zn>Hg>Cd, which is in agreement with the adsorption coefficient (K_D) pattern obtained by Noegrohati (2005,b).at salinity 25‰.

Table 2. Heavy metals distribution in abiotic system of Segara Anakan Estuary and Serang River mouth

Heavy metals	Water Samples		Sediment Samples	
	Segara Anakan Estuary	Serang River mouth	Segara Anakan Estuary	Serang River mouth
	mg/L	mg/L	mg/kg	mg/kg
Cu	n.d.	n.d.	36,27±1.68	31,50±3,90
Zn	n.d.	n.d.	9,76±0,67	9,74±1,32
Cd	n.d.	n.d.	n.d	n.d
Hg	0,047±0,00□	n.d.	0,80±0,04	0,35±0,07

Heavy Metals Bioaccumulation

Bioaccumulation starts with the uptake of chemicals from the environment. In the field, exposure, bioavailability, and bioaccumulation are linked. Since much higher level of heavy metals were determined in sediments (table 2), the appropriate bioindicators should be those lead double lives, some times swimming and sometimes burrowing,in the sediment, such as estuarine bivalves *Geloina* spp.; estuarine crabs (*Scilla serrata*) and penaeid shrimps (*Penaeus marguensis*). These bioindicators are exposed to both waterborne and particulate metals.

Under such circumstances, if one particular metal form is taken up from the exposure solution, the external equilibrium will immediately adjust to replenish the metal form that has been taken up. The metals mobility within the sediment column and its relative strength of the association of metals with suspended particulate matter and sediment, are quantified as desorption coefficient, ($1/K_D$ x1000). In undisturbed condition, which is closer to the real condition in the field, the desorption coefficient of Cu, Zn and Hg were 0,1, 2,5 and 0,2, and its exchangeable fraction were 0,11%, 6,40%, and 0,92% respectively (Noegrohati, 2005, b). Similar trend of lower Cu exchangeable fraction, 0.86%, and higher for Zn, 35.09%, were observed at disturbed condition in Masan Bay, Korea, by Kwon and Lee (2001)

Cu and Zn are essential heavy metals; therefore these heavy metals are tightly homeostasis regulated leading to stable levels throughout their life span, while non essential Cd and Hg were less or not regulated (Bustamante and Caurant, 2002, Noegrohati, 2006, c). For that reason, Cd and Hg have been reported to accumulate at very high levels (Furness, 1993; Aguilar and Borrell, 1996). Evans *et al.* (2000) and Se-Jong Ju and H. R. Harvey (2002) reported that Cd and Hg are accumulated by crustaceans in proportion to environmental exposure, on the contrary, no correlation between their tissue concentrations and their levels in ambient seawater or sediment for Cu and Zn. However, since Cd was not detected in abiotic system, the heavy metals studied were Cu, Zn and Hg.

Physiological status of Bioindicators

Bioindicators integrate pollutant in their body with time, therefore, the extent of heavy metal accumulation will be determined by their residence time. Several physiological factors such as molting, age and animal size, may significantly influence the levels of metals in bioindicators. Since the protective shell may shed off in regular intervals (molting), their physiological status was quantified by the nutrition index, but their residence time was approached by their size.

The nutrition index of *Geloina* spp. of Segara Anakan estuary was not significantly different from those of Serang river mouth. They are 0,62±0,19 and 0,63±0,05 respectively. The size of *Geloina* spp. samples, quantified as volume, from Serang river was significantly larger (P 0.05) than those from Segara Anakan estuary, they are 77,6±11,2 mL and 38,0±22,8 mL respectively. These data indicate that *Geloina* spp. of Serang river mouth may integrate heavy metal for longer period than those from Segara Anakan estuary

The nutrition index of penaeid shrimps of Serang river mouth, 0,61±0,09 was relatively higher than those of Segara Anakan estuary 0,40±0,13, as well as their length, 10,9±1,6 cm to 8,6±1,6 cm. Even though these data are not significantly different (P 0.05), they indicate that penaeid shrimps of Serang river mouth may integrate heavy metal for longer period than those from Segara Anakan estuary

The nutrition index of estuarine crab of Segara Anakan estuary was not significantly different from those of Serang river mouth. They are 4,2±1,2 and 3,9±0,2 respectively, similarly are their width, 9,6±1,6 cm and 10,9±1,2 cm. These data indicate the same time integration for estuarine crab in both sampling site.

Heavy metals bioaccumulation in bioindicators

The moisture content of the individual tissues differed from one another and to some extent also from one individual to the next, therefore the analytical results of heavy metals concentrations were reported on dry weight basis (table 3). The significance test was carried out at P 0,05. The extent of heavy metals accumulated in bioindcators is described as bioconcentration ratio, which is the ratio of heavy metal concentration in tissues or organisms to heavy metal concentration in sediments (table 4).

Since only very small proportions of copper occur in the water phase, less than 0,051 μg/mL (table 1), Cu was not detected in *Geloina* spp., neither in meat nor in exoskeleton samples. However, Cu was detected in penaeid shrimps and estuarine crab in increasing manner following their position in trophic level. It is interesting to note that highest Cu concentration was observed in exoskeleton of penaeid shrimp and cartillage of estuarine crabs. As expected, due to longer time integration, Cu concentrations in meats, exoskeleton of penaeid shrimp and cartilage of estuarine crab from Serang river mouth were significantly higher than those of Segara Anakan. However, only bioconcentration ratios of penaeid shrimp showed similar trend with its external exposure. Due to the longer time in integrating Cu into their body, the bioconcentration ratio of Penaeid shrimp from Serang river mouth was higher than those of Segara Anakan estuary.

Zn has the highest desorpton coefficient, therefore, more Zn are in the aqueous phase, therefore Zn concentration in meat of filter feeder *Geloina* spp. was relatively high and no significant difference were observed between the two sampling sites, indicate of strong homeostasis regulation. Similar condition was observed in penaeid shrimp and estuarine crab, except that in estuarine crab, it was followed by cartilage concentration, not exoskeleton concentration. These concentrations increase with trophic level. The bioconcentration ratios did not follow the

Table 3. Heavy Metals Concentrations (dry weight basis) in Tissues of *Geloina spp.*, *Penaeus marguensis* and *Scylla serrata* Samples from Segara Anakan Estuary (S.A.) and Serang River Mouth (Serang), and their Differences

		Geloina spp.			*Penaeus marguensis*			*Scylla serrata*		
Cu		S.A.	Serang	Difference	S.A.	Serang	Difference	S.A.	Serang	Difference
	Meat	n.d.	n.d.		23,7±0,8	30,2±3,6	sig.	88,2±3,3	121,1±8,4	sig.
	Exoskeleton	n.d.	n.d.		60,5±3,8	108,7±3,6	sig.	28,3±3,6	27,4±1,7	not sig.
	cartillage							212,2±9,7	137,7±8,4	sig.
Zn										
	Meat	76,1±3,4	78.3±1,5	not sig.	48,9±1,6	69,9±4,4	sig.	303,3±5,8	319,6±34,0	not sig.
	Exoskeleton	13,4±1,0	8,2±2,3	sig.	15,2±1,1	19,5±0,7	sig.	6,6±0,7	8,6±1,3	not sig.
	cartillage							71,8±2,9	40,7±9,0	sig.
Hg										
	Meat	1,04±0,02	0,58±0,10	sig.	1,37±0,52	1,04±0,30	not sig.	21,92±0,46	9,09±0,41	sig.
	Exoskeleton	0,96±0,20	0,66±0,14	not sig.	0,97±0,45	1,39±0,79	not sig.	1,40±0,22	0,59±0,34	not sig.
	cartillage							10,23±0,40	3,31±0,64	sig.

* sig.: significant

Table 4. Heavy metals tissues or organism bioconcentration ratio of Geloina spp., Penaeus marguensis and Scylla serrata Samples from Segara Anakan Estuary (S.A.) and Serang River Mouth (Serang)

Heavy Metals	Desorption Coefficient.	Tissues	*Geloina spp.*		*Penaeus marguensis*		*Scylla serrata*	
			SA	Serang	SA	Serang	SA	Serang
Cu	0,1	Meat	n.d.	n.d.	0,65	0,96	2,43	3,84
		Exoskeleton	n.d.	n.d.	1,67	3,45	0,78	0,87
		Cartillage					5,85	4,37
		Organism			1,38	2,65	3,58	3,27
Zn	2,5	Meat	7,79	8,02	5,00	7,66	31,07	32,80
		Exoskeleton	1,37	0,84	1,56	1,20	0,67	0,88
		Cartillage					7,35	4,18
		Organism	2,62	2,14	2,55	3,67	12,62	11,73
Hg	0,2	Meat	1,31	0,73	1,72	2,96	27,47	25,98
		Exoskeleton	1,20	0,82	1,22	3,98	1,75	1,68
		Cartillage					12,82	9,48
		Organism	1,22	1,84	1,36	3,65	14,34	12,24

external environment, possibly due to the homeostasis regulation within bioindicators.

Again, among the bioindicators, highest Hg concentration was determined in meat samples, followed by exoskeletons, while in estuarine crab, it was followed by cartilage and then carapace. The tissue concentrations of Hg in Segara Anakan estuary were significantly higher than those of Serang river mouth, due to higher Hg concentrations in their external environment. Similar trend of increasing Hg concentrations with increasing trophic level was also observed. The highest and similar meat concentration ratios in both sampling sites indicate that there are Hg detoxification mechanisms. However, higher bioconcentration ratios were observed in *Geloina* spp. and estuarine crab from Segara Anakan estuary following its higher in Hg external source, but not in penaeid shrimp.

Due to their feeding strategies, bioconcentration ratio of *Geloina* spp. (filter feeder) and penaeid shrimp (phyto and zooplankton) followed the pattern of heavy metal's desorption coefficient, i.e. Zn>Hg>Cu (Noegrohati, 2005,b), but different pattern was observed in estuarine crab, which is macrobenthos predator, i.e. Hg>Zn>Cu

Consumer's safety

Seafood is the main protein source for local residences of Segara Anakan estuary and Serang river mouth. On the other hand, aquatic organisms in these ocean margin regions are constantly exposed to discharge from the continental stream and those precipitated and deposited in sediments. Aquatic organisms exposed to low level heavy metals as natural pollutants originated from weathering processes of volcanoes debris, will accumulate in their bodies and transferred to higher trophic level organisms at higher concentration.

Metals concentrations in organism at the same location differ between different species and individuals due to species-specific ability/capacity to regulate or accumulate trace metals (Reinfelder et al., 1997; Otchere et al., 2003). Due to the trophic levels of bioindicators in this study, it was observed that bioconcentration ratio of *Geloina* spp. and penaeid shrimps are lower than estuarine crab. As the consequence of homeostasis regulation, most of the exposed Cu is accumulated in the exoskeleton of penaeid shrimp and cartilage of estuarine crabs, while Zn is mostly accumulated in meat of these bioindicators. Cu and Zn are important micro-nutrients and part of some enzymes central to the body's function. National Research Council, USA (1989) recommended the uptake of Cu 1,5 – 3 mg/day and Zn 12 mg/day.

For that reason, seafood consumption is also recommended as the source for essential metal.

Hg is mostly accumulated in the edible part of these bioindicators, and only a prt which is accumulated in exoskeleton and catrtilage. Despite that there are detoxification mechanisms for toxic non-essential heavy metals, this study and others found that seafood is the major source of Hg in the diet (National Food Authority, 1994, Noegrohati, 2005,a). It is generally assumed that over 90% of Hg in seafood is in the form of methylmercury, which is accumulated and can induce neurological changes in adults. In pregnant women, it can be transferred into the developing fetus through placental barrier, and after birth, through the mother milk to the infants, which may induce alterations in the normal development of the brain of infants. Therefore, Joint FAO/WHO expert committee on food additives (2003) considered a PTWI (Provisional tolerable weekly intake) of 1.6 μg/kg bw, is sufficient to protect the developing fetus, the most sensitive subgroup of the population.

The indirect exposure through food can be calculated through food basket study by

Exposure = ε(C of each intake media x intake rate of each media)

According to GEMS/Food regional diets of Far Eastern diet (2003), the consumption of crustaceans (fresh/frozen) is 2.3 g/persom/day, and mollusk including cephalopods is 4.0 g/person/day. Assuming the local residents consume the same amount of *Geloina* spp., penaeid shrimps and estuarine crabs, seafood contributions to weekly intake of Hg will be 9%, 5% and 110% respectively for residents of Segara Anakan estuary, and 6%, 3% and 53% respectively for residents of Serang river mouth regions.

These data indicate that seafood originated from Serang river mouth and certain seafood from Segara Anakan estuary, i.e. Gelloina spp. and penaeid shrimps are safe for pregnant women, but it is better that pregnant women recognized the potential hazard of consuming estuarine crabs from Segara Anakan estuary. Similar suggestion has been provided for Mugil spp. and *Geloina* spp. consumption in dry season (Noegrohati, 2005).

CONCLUSION

For aquatic organisms, sediment in ocean margins is the source of heavy metals to be accumulated in their bodies; therefore, the level of heavy metals in sediment is a better predictor for heavy metal pollution problems. Cu and Zn determined in sediment samples of Segara Anakan were indicating a higher level than those of Serang river mouth, but the level of Hg in water and sediment samples of Segara Anakan were significantly higher than those of Serang river mouth, while Cd was not detected in sediment and water samples of both sampling sites. No Cu, and Zn were not detected in water samples, only Hg could be determined in Segara Anakan water samples.

Heavy metals distribution and bioaccumulation pattern in organisms not only influenced by environmental sorption desorption characteristics, but also by physiological status, feeding strategy, biochemistry and capacity to accumulate heavy metals in their body.

The trend of heavy metals bioconcentration ratio of filter feeder *Geloina* spp and planktonic grazer penaeid shrimps follows the desorption coefficient, i.e. Zn>Hg>Cu, but the trend in estuarine crab, which is macrobenthos predator, is Hg>Zn>Cu.

The exposed Cu was not accumulated in *Geloina* spp., but accumulated at highest concentration in the exoskeleton of penaeid shrimp and cartilage of estuarine crabs. Highest accumulation concentration of Zn and Hg were determined in meat, and lower concentrations were determined at other tissues. These distribution patterns may be due to Cu and Zn homeostasis regulations and Hg detoxification mechanisms.

The extent of bioaccumulation in *Geloina* spp. and estuarine crab reflects the pollution of the surrounding environment, but the extent of bioaccumulation in penaeid shrimps were influenced more by its physiological status. Since the environmental exposure of Hg in Segara Anakan estuary was relatively high, pregnant women from this region should consider the potential hazard of Hg to their fetus when consuming higher trophic level aquatic organisms such as estuarine crabs.

ACKNOWLEDGEMENT

Thanks are due to Dr. Narsito, professor in inorganic chemistry, Department of Chemistry, Gadjah Mada University for his constant encouragement and valuable scientific discussions, Dr. Jurgen Saeger, expert of BCEOM French Consulting Group, for his valuable support, to R. Asih, Mulyanti, Y. I. Listyana, S.W. Lestari, and Sanjayadi for their assisstance in carrying out these works

REFERENCES

Aguîlar, A., and Borrell, A., 1996, Marine mammals and pollutants: An annotated bibliography, in: "Fundacio pel Desenvolupament Sostenible", ed.. Rafica Dueto, Cornella, 251 p.

Birch L, Hanselmann, K.W. and Bachofen, R., 1996, Heavy metal conservation in Lake Cadagno sediments: Historical records of anthropogenic emissions in a meromictic alpine lake. *Wat. Res.* **30** (3) 679-687.

Boyd, C. E., 1989, Water Quality Management and aeration in Shrimp Farming, Fisheries and Allied Aquacultures Departmental, Series No 1, Alabama Agricultural Experiment Station, Auburn University, Alabama, USA,

Bustamante, P. and Caurant, F., 2002, Metal speciation in cephalopods: implications for bioaccumulation in marine top predators, in CIESM, 2002, Metal and radionuclides bioaccumulation in marine organisms, CIESM Workshop Monographs n°19, 128 pages, Monaco *www.ciesm.org/publications/ Ancona02.pdf*

Creed, J.T. and T.D. Martin, 1997, Determination of trace elements in marine waters by stabilized temperature graphite furnace atomic absorption, EPA 22, National exposure research laboratory office of research and development,. US EPA, Cincinnati, OH.

Day, J. W. Jr, Hall, C. A.S., Kemp, W.M., and Yanez-Arancibia, A., 1989, Estuarine ecology, John Willey & Sons

Evans, D.W., Kathman, R.D, and Walker, W.W., 2000, Trophic accumulation and depuration of mercury by blue crabs (*Callinectes sapidus*) and pink shrimp (*Penaeus duorarum*), *Mar. Environ. Res.*, 49: 419-434.

Furness R.W., 1993, Birds as monitors of pollutants, pp. 86-143. in: "Birds as monitors of environmental change",. eds Furness and Greenwood, Chapman & Hall, London.

Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme, *2003,* GEMS/Food regional diets: regional per capita consumption of raw and semi-processed agricultural commodities, GEMS/Food, FOOD SAFETY ISSUES, Rev. ed.

Joint FAO/WHO Expert Committee on Food Additives, 2003, Sixty-first meeting, Rome, 10-19 June 2003, JECFA /61/SC

Ju, S. and Harvey, H. R., 2002, Effects of Temperature and Heavy Metals on Extractable Lipofuscin in the Blue Crab, *Callinectes sapidus,* Journal of the Korean Society of Oceanography, Vol. 37, No. 4, JKSO-37-44.pdf

Langston, W.J. and Spence, S.K., 1995. Biological factors involved in metal concentrations observed in aquatic organisms. *In:* Tessier and Turner eds., Metal Speciation and Bioavailability in Aquatic Systems, John WIiley & Sons, New York. pp. 407-478

Lee, B.G, Grimscom, S.B., Lee J.S., Choi, H.J., Koh, C.H., Luoma, S.N., and Fisher N.S., 2000, Influences of dietary uptake and reactive sulfides on metal bioavailability from aquatic sediments, Science 287: 282-284.

Luoma, S. N., 2002, Processes affecting trophic transfer and resultant effects of metals : implications for monitoring metal pollution in the sea, in CIESM, 2002, Metal and radionuclides bioaccumulation in marine organisms, CIESM Workshop Monographs n°19, 128 pages, Monaco <www.ciesm.org/ publications/ Ancona02.pdf>

Martin, J.M and Windom, H.L., 1991, Present and future roles of Ocean Margin in Regulating Marine Biogeochemical Cycles of Trace Elements, in "Ocean Margin Processes in Global change", ed. RFC Mantoura, J.M. Martin and R. Wollast, Physical, Chemical and earth Sciences Research Report, John Willey & Sons.

Noegrohati, S., 2005, a, Assessment of Possible Indirect Risk of Naturally Occurring Mercury and Cadmium through *Mugil* Spp. and *Geloina* spp. Consumption in Segara Anakan Estuarine Ecosystem, Indo.J.Chem., Vol 5, No. 2, 135-142

Noegrohati, S., 2005, b, Sorption-desorption Characteristics of Heavy Metals and their Availability from the Sediment of Segara Anakan Estuary, Indo. J. Chem., Vol 5, No. 3, 236-244

Noegrohati, S., 2006, c, Bioaccumulation Dynamics of Heavy Metals in Oreochromis Nilotycus: Predicted through a bioaccumulation model constructed based on Biotic Ligand Model (BLM),

Otchere, F. A., 2003, Heavy metals concentrations and burden in the bivalves (*Anadara (Senilia) senilis, Crassostrea tulipa* and *Perna perna*) from lagoons in Ghana: Model to describe mechanism of accumulation/excretion, African Journal of Biotechnology Vol. 2 (9), pp. 280-287, Available online at *http://www.academicjournals.org/AJB*

Otchere, F.A., Joiris, C., and Holsbeek, L., 2003, Mercury in the bivalves *Anadara (Senilis) senilis, Perna perna* and *Crassostrea tulipa* from Ghana,. Sci. Total Environ. 304:369-375.

Reinfelder J.R., Wang W.X., Luoma S.N. and Fisher N.S., 1997. Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams, and mussels. *Marine Biology*, 129: 443-452.

Riedel, G.F., Abbe G.R. and Sanders, J.G., 1998, Temporal and Spatial Variations of Trace Metal Concentrations in Oysters from the Patuxent River, Maryland, Estuaries Vol. 21, No. 3, p. 423-434

Soto, M., Marigómez, I., and Cancio, I., 2005, Biological aspects of metal accumulation and storage, downloaded from zoxcauri @lg.ehu.es

Tessier, A., and Campbell, P.G.C., 1990, Partitioning of trace metals in sediments and its relationship to their accumulation in benthic organisms, in "Metal Speciation in the Environment": eds JAC Broekaert, S Grucer and F Adams, Springer, Berlin. pp 545-569.

Texas Commission on Environmental Quality, 2003, Chapter 7: Tissue Sample Collection, Guidelines for Tissue Sampling in Texas, Texas Parks and Wildlife Department (TPWD), rg_415_chapter 7_223594.pdf