

## BIOACCUMULATION DYNAMICS OF HEAVY METALS IN *Oreochromis nilotycus*: PREDICTED THROUGH A BIOACCUMULATION MODEL CONSTRUCTED BASED ON BIOTIC LIGAND MODEL (BLM)

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### ABSTRACT

In estuarine ecosystem, sediments are not only functioning as heavy metal scavenger, but also as one of potential sources for heavy metals to the ecosystem. Due the capability of aquatic organisms to accumulate heavy metals, there is possibility of heavy metals to exert their toxic effect towards the organisms and other organisms positioned in higher trophic level, such as fish, and further to human beings. To understand the different processes of heavy metal bioaccumulation in a dynamic manner, a bioaccumulation model is required. Since bioaccumulation starts with the uptake of chemical across a biological membrane, the bioaccumulation model was constructed based on Biotic Ligand Model (BLM). The input for the model was determined from laboratory scale simulated estuarine ecosystem of sediment-brackish water (seawater:Aqua® 1:1) for determining the heavy metal fractions in sediments; simulated *Oreochromis nilotycus* – brackish water (fish-water) ecosystem for determining the rate constants; simulated fish-water-sediment ecosystem for evaluating the closeness between model-predicted and measured concentration, routes and distribution within specific internal organs. From these bioaccumulation studies, it was confirmed that the internalization of metals into the cells of gills and internal epithelias follows similar mechanisms, and governed mostly by the waterborne or hydrophilic heavy metals. The level of hydrophilic heavy metals are determined by desorption equilibrium coefficients,  $1/K_D$ , and influenced by salinity. Physiologically, the essential Cu and Zn body burden in *O. nilotycus* are tightly homeostasis regulated, shown as decreasing uptake efficiency factor,  $E_w$ , at higher exposure concentrations, while non essential Cd and Hg were less or not regulated. From the distribution within specific internal organs, it was revealed that carcass was more relevant in describing the bioaccumulation condition than liver. It is clear that every heavy metal has its own bioaccumulation dynamics, depend to the metal studied and environmental conditions, however the obtained parameters are applicable to bioaccumulation of Cd and Hg in natural estuarine ecosystem of Segara Anakan, Central Java.

**Keywords:** heavy metal, estuarine, bioaccumulation, model, dynamics.

### INTRODUCTION

In nature, aquatic organisms are constantly exposed to metals from geochemical processes and anthropogenic activities. Metals exist in a variety of physical and chemical forms in the water column and in the bottom sediments. In estuarine ecosystems, it was confirmed that sediments are not only functioning as heavy metal scavenger, but also as one of potential sources for heavy metals to the ecosystem [1].

Fish residing in contaminated ecosystem are able to take up appreciable amounts of available heavy metal through the gill and the gastrointestinal tract, bioaccumulated and exert their toxic effect towards the organisms and other organisms positioned in higher trophic level. Heavy metals bioaccumulation influenced by physicochemical aspects, i.e. the dynamic nature of metal speciation, membrane passage properties and its bioavailability; and physiological aspects, i.e. distribution within specific tissues, biotransformation and excretion.

Bioaccumulation starts with the uptake of chemical across a biological membrane. Gill is the key interface

for the uptake of waterborne metal from water. Waterborne metals may enter the gill by facilitated cation transport, by facilitated anion transport and by passive diffusion of lipophilic metal forms, followed by rapid binding to intracellular ligands such as metallothioneins (MTs). Biotic Ligand Model (BLM) [2], assumed the available form to be transported across the cell membrane, is the free metal ion ( $M^{2+}$ ). Therefore, the importance of the free-metal ion activity as a predictor of metal bioavailability remains indisputable. Under such conditions, all labile diffusive species will contribute to metal uptake.

Waterborne metal is relatively easy in approaching a biological membrane; since there is no advective and/or diffusive limitation in transporting heavy metals from the bulk solution to the gill surface by the mechanisms [3]. More limitation is encountered in the bioavailability of heavy metal associated with surface of particles, such as biogenic particles (fecal pellets or phytodetritus), or abiotic particulate matters (clay and sediment's organic matter). In this case, equilibration between dissolution-precipitation, and

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sorption-desorption equilibrium between sediments and water phase controlled the dissolved levels of metals in aquatic environment [2].

Since the epithelial membrane in the gut is naturally rich in transport systems, it seems logical that the metal uptake across respiratory gill and gut epithelia are similar. Base on this reason, Kamunde *et al.*, [4] studied the uptake of waterborne and dietary  $^{64}\text{Cu}$  exposures, and found no significant difference in specific activities for waterborne and dietary  $^{64}\text{Cu}$ . This data confirmed that the internalization of metals into the cells of gills and gut epithelias are governed mostly by the free metal ion ( $\text{M}^{2+}$ ).

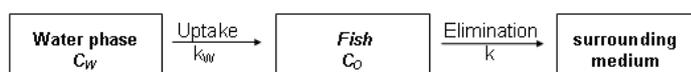
Inside an organism, metals are transported by blood and distributed into different compartments. The main target organ for metal accumulation in fish is the liver, and the remaining free metal-protein complex can be reabsorbed by the circulatory system to be re-distributed. Another organ that sometimes can exhibit strong metal accumulation in fish is the carcass [5]. Heavy metal accumulation in carcass is of great importance in human health because it can produce poisoning in fish consumers. Therefore, it is important to understand the heavy metal accumulation dynamics in fish due to chronic sublethal heavy metal exposure resembles those in the environment.

The objective of this work is to describe and predict the bioaccumulation using a bioaccumulation model. The heavy metals studied are Cu and Zn which represent the essential hard cations, and the nonessential soft cations: Cd and Hg.

### Theoretical Considerations In Bioaccumulation Model

Fish are exposed to waterborne and particulate heavy metal including suspended matter, sediment particles and food sources. A mechanistic understanding of the processes of heavy metal bioaccumulation in fish requires coupling of the different processes in a dynamic manner, involved the dynamics of exposure to uptake, compartmentalization, accumulation and excretion. For that reason, modeling rates and routes of heavy metal in the uptake and accumulation will be useful for understanding the framework of key processes in bioaccumulation.

Based on the BLM [2] and the study of Kamunde *et al.* [4], heavy metals uptake in fish are assumed to be mostly from waterborne exposure. The bioaccumulation dynamics of waterborne heavy metals can be described as follow:



Assuming the uptake of heavy metals from the water phase follows a first order kinetics, the rate of decrease of the amount of the heavy metals would be linearly proportional to the amount of that remaining in water phase, with a proportionality factor of  $k_W$ , which could be expressed mathematically as:

$$\frac{d[C_W]}{dt} = -k_W[C_W] \quad (1)$$

Kinetically, the factor of  $k_W$  is the rate constant for the first order uptake. The concentration of metal (mg/L) in the water phase  $[C_W]$  at a given time  $t$  (days) could be obtained by integrating equation (1), with a condition that at  $t=0$ ,  $[C_W] = [C_W]_0$  to give:

$$[C_W] = [C_W]_0 e^{-k_W t} \quad (2)$$

In order to calculate the amount taken up by the organisms, an uptake efficiency factor,  $E_W = V_W F$  (in  $L \text{ org}^{-1} \text{ day}^{-1}$ ), is required. This parameter describes the volume of water phase passing through the biological membrane  $V_W$ , multiplied by the fraction of metal taken up by organisms,  $F$ . Therefore, the amount of heavy metal taken up by organisms from water phase is equal to  $C_{W,0} E_W e^{-k_W t}$ .

Simultaneously, excretion of the heavy metal occurs with an excretion rate of linearly proportional to the concentration of heavy metal present in the organisms (fish),  $[C_f]$  (in  $\text{mg kg}^{-1}$ ), in the form of  $d[C_f]/dt = k[C_f]$ , with  $k$  = the rate constant for the first order excretion. Assuming that before exposure, no heavy metal was present in the organisms, the general mathematical expression for the rate of heavy metal in the organisms could be obtained by subtracting the rate of excretion from the rate of metal taken up by organisms from the water phase,  $k_W C_{W,0} E_W e^{-k_W t}$  as follows

$$\frac{d C_f}{dt} = k_W C_{W,0} E_W e^{-k_W t} - k \quad (3)$$

The metal concentration in the organism,  $[C_f]$  at a given time,  $t$  could be obtained by integrating equation (3) to give:

$$C_f = \frac{k_W [C_W]_0 E_W}{(k_W - k)} (e^{-k t} - e^{-k_W t}) \quad (4)$$

The main purpose in developing model for bioaccumulation in this study is to quantify the parameters related to bioaccumulation of heavy metals in fish. In that case, the following conditions were considered:

1. For short term exposure, the excretion of the heavy metal could be effectively negligible to that for the heavy metal uptake resulting in a simpler form equation (4):

$$C_f = [C_W]_0 E_W (1 - e^{-k_W t}) \quad (5)$$

Therefore the first order rate constant for the uptake of heavy metals,  $k_w$ , could be evaluated by the method of residuals, and further, the uptake efficiency could be calculated [6].

2. After long exposure,  $t \rightarrow \infty$ , when the accumulation capacity of the organism has been reached, the second term of equation (4) would be much smaller than that of the first term. Consequently, equation (4) could be simplified in the form of

$$C_f = [C_w]_0 E_w e^{-k t} \quad (6)$$

The first order rate constant for the excretion of heavy metals  $C_f$ ,  $k$ , could be evaluated by plotting of  $\ln[C_f]$  versus  $t$ , and the initial concentration of heavy metals could also be calculated from the slope [6].

3. Quantification of bioaccumulation due to waterborne exposure as bioconcentration factor, BCF, should be estimated at steady state ( $t \rightarrow \infty$ ), where  $dC_f/dt = 0$ . At steady state, the concentration in fish and in water phase are at the equilibrium concentration,  $[C_f]_{eq}$  and  $[C_w]_{eq}$  respectively, and the rate of uptake is equal to the rate of excretion, and the BCF could be estimated as follow:

$$BCF = [C_f]_{eq} / [C_w]_{eq} = E_w k_w / k \quad (7)$$

The input for the bioaccumulation model will be obtained from simulated estuarine ecosystem of fish-water microcosm. If the assumption that heavy metals uptake in gills and gut epithelias are mostly from waterborne exposure holds for other heavy metals, then the obtained parameters from bioaccumulation model from fish-water microcosm can be used to predict the bioaccumulation due to waterborne and particulate heavy metal exposure in fish-water-sediment microcosm. However, the level of waterborne heavy metals in fish-water-sediment microcosm is determined by desorption equilibrium coefficients,  $1/K_D$ , of the sediments, which is influenced by salinity [1].

Further, in light of the transportation of chemical across a biological membrane, the hydrophilic and/or lipophilic fractions of available heavy metal may give significant effects in the uptake and accumulation mechanisms. Finally, for consumer safety, the distribution of heavy metals within specific internal organs, especially heavy metals in carcass, will be evaluated.

## EXPERIMENTAL SECTION

### Material

All chemicals used in the present work are of analytical grade. The estuarine sediment was collected from Gombol, Segara Anakan estuary, Cilacap, Cental Java, kept in wet condition and processed as soon as

possible. The sediment used in this study was characterized as heavy clay, consisting of 60 % clays, 27 % fines, 13 % sands with relatively high content of organic matter, i.e. 3.64 % (Soil Laboratory, Faculty of Agriculture, Gadjah Mada University).

The seawater was collected from Parangtritis, South coast of Special Province of Jogjakarta, mixed and let stand for one week. The water phase used in this study was a mixture of seawater:Aqua® (1:1), with salinity of 12‰, and pH 7. The fish, *Oreochromis nilotycus*, of about 2 months old were collected from Ngaglik, Sleman, Special Province of Jogjakarta. The fish were maintained in sediment-free aquarium with running brackish water (a mixture of seawater:Aqua® 1:1) for 24 hours, and then acclimatized in laboratory condition for 2 weeks period. Heavy metal concentration in the pellet used for fish feeding contains  $Cu^{2+}$  3.14  $\mu\text{g/g}$ ,  $Zn^{2+}$  53.39  $\mu\text{g/g}$ ,  $Cd^{2+}$  0.76  $\mu\text{g/g}$ , and  $Hg^{2+}$  0.34  $\mu\text{g/g}$ .

Analytical instruments for Cu, Zn and Cd determination were Flame/Zeeman Atomic Absorption Spectrophotometer Hitachi Model 180-60/80, with flame of air as oxydant and acetylene as fuel. The absorbance resonance line used for Cu, Zn and Cd determination were 324.8 nm, 213.8 nm, and 228.8 nm, and the limit of detection, LOD, were 0.041 mg/L, 0.023 mg/L, and 0.012 mg/L 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 absorbance resonance line of 253.7 nm, with a limit of detection, LOD of 1.18  $\mu\text{g/L}$ .

### Experimental Design

In the real field condition, exposure, bioavailability, and bioaccumulation are linked, therefore heavy metal bioaccumulation dynamics will be studied in a simulation of Segara Anakan specific condition. The study on physicochemical aspect of bioaccumulation includes study on fractions of the bioavailable heavy metals in sediment, and bioaccumulation in *O. nilotycus*, both due to waterborne and particulate heavy metals exposure, while the study on physiological aspect of bioaccumulation include the distribution of heavy metals within specific organs of the organism.

### Determination of heavy metals fractions in sediment

Into each of 24 polyethylene vessel of 1.5 L, previously washed with 1M HCl, 60 g wet sediment was added. They are divided in 3 groups of 8 vessels and enriched with heavy metal solutions prepared from  $CuSO_4 \cdot 5H_2O$ ,  $ZnSO_4 \cdot 7 H_2O$ ,  $Cd(NO_3)_2 \cdot 6H_2O$  and  $Hg(NO_3)_2 \cdot H_2O$ . First group was enriched with 37.2 mg

Cu(II), 6.1 mg Zn(II), 6.5 mg Cd(II) and 0.86 mg Hg(II), the second group was 74.3 mg Cu(II), 12.3 mg Zn(II), 13.0 mg Cd(II) and 1.73 mg Hg(II), and the third group was the control without enrichment. All of these were mixed and then leave it over night, further they were added with 900 mL mixture of seawater:Aqua® 1:1, and kept at room temperature. Duplicate samples were taken at 1, 7, 14 and 28 day after enrichment. Samples of water and sediment were analyzed for waterborne Cu, Zn, Cd and Hg, and their total soil concentration. Another portion of sediment was analyzed for its lipophilic Cu, Zn, Cd and Hg concentration. The concentration of the residual heavy metal was calculated by the difference of total soil concentration and lipophilic concentration

### **Bioaccumulation of Heavy Metals in *Oreochromis nilotycus***

#### **(a) Bioaccumulation due to Waterborne Heavy Metal Exposure (Fish-Water Microcosms)**

Into each of 4 polyethylene tanks of 38 cm x 29 cm wide x 27.5 cm depth, previously washed with 1M HCl, 30 L of mixture of seawater:Aqua® 1:1 was added. The first 2 tanks were enriched with 2.5 mg Cu(II), 2.3 mg Zn(II), 3.3 mg Cd(II) and 0.08 mg Hg(II). The second 2 tanks were enriched with 5.1 mg Cu(II), 4.5 mg Zn(II), 6.5 mg Cd(II) and 0.16 mg Hg(II). After mixing and let it stand overnight, 15 fishes was added into each tank. The tanks were aerated and kept at room temperature for 28 days. The fish were fed at rate of 0.2 g pellets per fish per day. Samples of water, and internal organs of 5 *O. nilotycus* (gill, liver, carcass, and bones) were collected before the addition of fish into the tanks, denoted as samples of 0 day, and further samples were collected at 7, 14 and 28 days. All heavy metal measurements were performed on duplicate samples.

#### **(b). Bioaccumulation due to Waterborne and Particulate Heavy Metal Exposure (Fish-Water-Sediment microcosms).**

Into each of 6 polyethylene tanks of 38 cm x 29 cm wide x 27.5 cm depth, previously washed with 1M HCl, 30 kg of wet sediment was added. The first 2 tanks was enriched with 1486.8mg Cu(II), 368.5 mg Zn(II), 390 mg Cd(II) and 86.4 mg Hg(II); the second 2 tanks was 2973.6 mg Cu(II), 737 mg Zn(II), 780 mg Cd(II) and 172.7 mg Hg(II), the third 2 tanks was the control without enrichment. All of these were mixed and then leave it over night, further, a mixture of seawater:Aqua® 1:1 of 45 L and 5 fish was added into each tank,. The tanks was aerated and kept at room temperature for 28 days. The fish were fed at rate of 0.2 g pellets per fish per day. Samples of water, sediment and internal organs of 5 *O. nilotycus* (gill, liver, carcass, and bones) were collected before the addition of fish into the tanks, denoted as samples of 0 day, and further collected at 28 days. All

heavy metal measurements were performed on duplicate samples.

### **Analytical Procedures**

**Water samples.** After filtration, the heavy metal in water samples was complexed with ammonium pyrrolidine dithiocarbamate, APDC, (at pH = 4), and extracted into CHCl<sub>3</sub>. The heavy metals in CHCl<sub>3</sub> extract was reextracted into H<sub>2</sub>O with aqueous HNO<sub>3</sub> solution (to pH = 2), and subjected into the appropriate AAS for quantitative determination of the heavy metals. Recoveries of 80 %, 112 %, 139 % and 77 % for Cu, Zn, Cd and Hg were obtained respectively.

**Sediment samples.** Total concentration of heavy metals in sediment samples were determined in the appropriate AAS after wet digested with 3:2 mixture of HNO<sub>3</sub>:HCl at 80°C. The clear solution obtained was filtered and diluted appropriately to obtain the required concentration for quantification in the AAS. The lipophilic heavy metal fractions of the sediment was continuously extracted using 100 mL mixture of hexane:acetone (1:1) in a soxhlet extraction apparatus for 12 circulations. The extract was evaporated and the residue was wet digested as above. Recoveries of 68 %, 69 %, 122 %, and 119 % were obtained for Cu, Zn, Cd and Hg respectively.

**Fish samples.** The gill, liver, carcass, and bones of *O. nilotycus* were dissected and soaked in the digestion mixture of HNO<sub>3</sub>:HCl (3:2) over night, then proceed as in sediment. The respective recoveries for Cu, Zn, Cd and Hg were 133 %, 93 %, 123 % and 74 %.

### **Data Evaluation**

The obtained kinetics parameters for bioaccumulation of heavy metals due to waterborne exposure in simulated estuarine fish-water ecosystem were calculated based on the model developed. The data obtained were then used to predict the concentrations of heavy metals in *Oreochromis nilotycus* exposed to waterborne and particulate heavymetals in simulated estuarine fish-water-sediment ecosystem at the 28<sup>th</sup> day of exposure, and factors significantly influencing their bioavailability, uptake and bioaccumulation processes, were determined.

## **RESULT AND DISCUSSION**

### **Heavy Metal Fractions in Sediments**

The availability of heavy metal either from previous contamination or newly enriched heavy metals

**Table 1.** The fractions (%) of hydrophilic, lipophilic, total available and residual heavy metal in sediments

Heavy metals	Enrichment mg	Fractions			
		Hydrophilic (%)	Lipophilic (%)	Total available (%)	Residual (%)
Cu	0	0	<b>0.31</b>	0.31	99.69
	37.2	0	<b>1.14</b>	1.14	98.86
	74.3	0.21	<b>1.19</b>	1.41	98.59
Zn	0	<b>1.56</b>	0.92	2.48	97.52
	6.1	<b>8.04</b>	0.44	8.48	91.52
	12.3	<b>4.76</b>	0.78	5.54	94.46
Cd	0	<b>50.43</b>	5.36	55.80	44.20
	6.5	<b>36.25</b>	0.03	36.27	63.73
	13	<b>50.51</b>	0.01	50.52	49.48
Hg	0	34.69	<b>11.22</b>	45.92	54.08
	0.86	1.28	<b>11.88</b>	13.16	86.84
	1.73	0.57	<b>13.68</b>	14.25	85.75

in sediment, were studied. The data are presented in Table 1.

Even though during the 28 days of incubation, sediment may undergoes diagenetic changes, no significant differences was observed in heavy metal concentrations of hydrophilic and the lipophilic fractions ( $P < 0.05$ ), indicated that heavy metal species in both fractions are chemically formed.

Most of the added heavy metals are associated with permanently negative charged sites of sediment, and considered to be unavailable, and only small fractions are available. The total available fractions for Cu, Zn, Cd, and Hg are about 1, 7, 43, 14%. Under simulated estuarine ecosystem, which is favourable for chloro-complex formation, the availability of soft cations Cd and Hg were higher than hard cations Cu and Zn, which prefer to form organo-complex.

As expected, the enrichment of Cu into the sediment increases its lipophilic fraction, indicated its preference to associate with organic matter. On the contrary, Zn enrichment increases the hydrophilic fraction, indicate that Zn prefer to associate with the available ligands in water phase, i.e.  $Cl^-$  which has high solubility (432 g/100 mL).

Differ from Cu and Zn, Cd and Hg enrichment increases their residual fractions, indicating their preference to associate with permanently negative charged sites of sediment, possibly sulphides. The sulphur concentration in sediment of Segara Anakan was reported between 0.2-0.4%. A drastic decrease in lipophylic fraction was observed in Cd enrichment, while in Hg enrichment, the decrease was observed in hydrophilic fraction.

The differences of the enriched heavy metal behavior in this estuarine ecosystem may affect the bioavailability and uptake rates. However, the total available fractions of Cu, Zn and Hg was correlated to the desorption coefficient,  $1/K_D$  [1], with degree of fitness,  $R^2$ , 0.9378, which is in accord with the previous study [1, 7].

### Bioaccumulation of Heavy Metals in *Oreochromis niloticus*

Heavy metal concentrations in a given organism are in a state of dynamic equilibrium, and are the net result of both uptake and elimination processes occurring simultaneously, therefore bioaccumulation reflects the balance between uptake and excretion.

### Bioaccumulation model due to waterborne heavy metal exposure

In this simulated estuarine fish-water (F-W) ecosystem, the heavy metals are added into the system in the form of soluble inorganic salts, therefore all of the heavy metals in water phase can be considered as hydrophilic, and the influence of the dynamic nature of metal speciation is limited. This study describes more of the influence of *O. niloticus* physiological aspects.

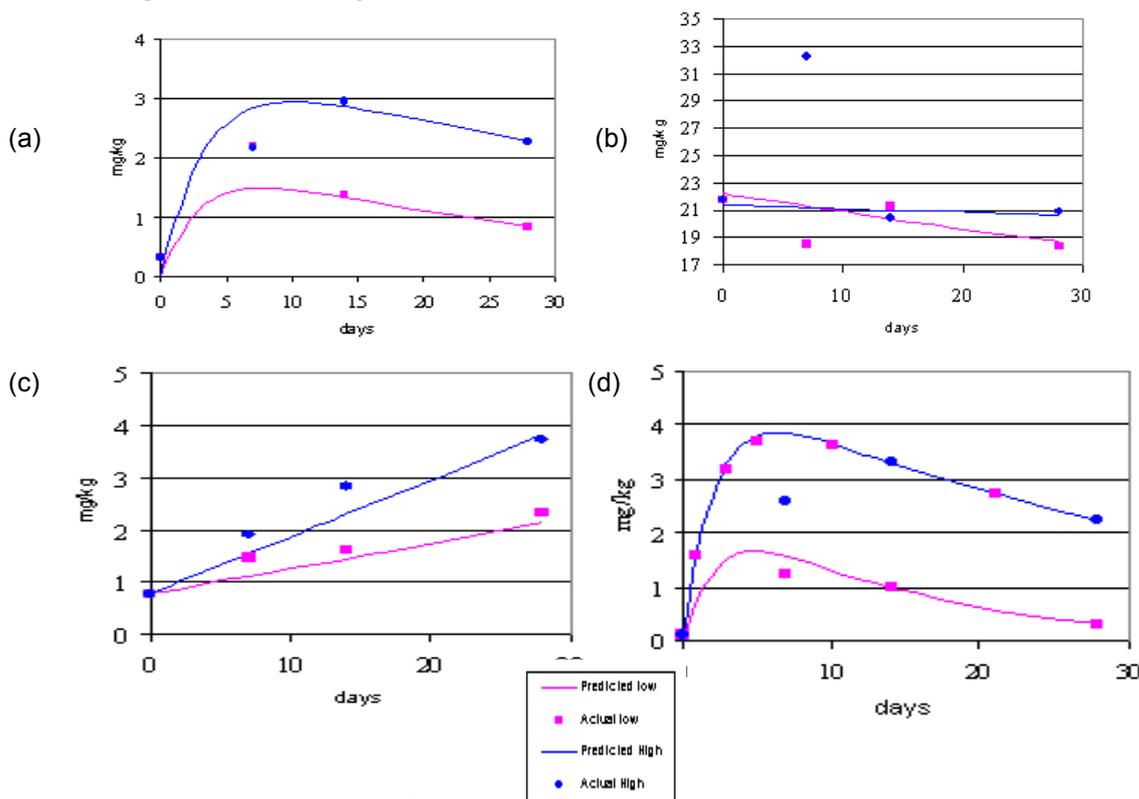
The kinetics parameters of heavy metal bioaccumulation in *O. niloticus* due to waterborne heavy metal exposure are presented in Table 2, and the appropriateness of the model to experimentally determined data are visualized in Fig.1.

The uptake and excretion rate of Cu and Hg can be described mathematically as first order processes; therefore the bioaccumulation of Cu and Hg followed equation (4). Their uptake and excretion rate constants are in the same order, but the uptake efficiency factor ( $E_W$ ) of Cu was much lower than Hg. Cu is an essential heavy metal and known for its tight homeostatic regulation. Kamunde *et al.* [4] observed that Cu elevation or decline in specific tissue of *Oncorhynchus mykiss* resulting from the demand for new Cu uptake. These data suggest that Cu homeostasis in fish entails not only regulated the uptake, but also cellular Cu transport mechanisms and excretion. In this study, the homeostasis regulation of Cu was presented as lower  $E_W$  at higher exposure concentration. On the other hand, higher  $E_W$  was observed at higher exposure concentration of Hg, indicating that there is no homeostasis regulation occurred for non-essential heavy metal Hg. As the consequence, the estimated BCF of Cu decreases with increasing exposure concentration, while the estimated BCF of Hg increases with increasing exposure concentrations. Similar trend was observed in  $C_{fish}/C_{water}$  determined at 28<sup>th</sup> days of heavy metal exposures. Since it was

**Table 2** The kinetic parameters of heavy metal bioaccumulation in *Oreochromis niloticus* due to waterborne exposure

Heavy metals	Enrichment mg	Actual water conc. mg/L	Uptake efficiency factor L/fish,day*	Rate constant		Calculated maximum		Estimated BCF $E_w k_w/k$	$C_{fish}/C_{water}$ at 28 <sup>th</sup> day
				Uptake $k_w$	Excretion $k$	t days	C		
Cu	2.546	0.015	2.273	0.305	0.035	8.0	1.495	1134	56
	5.092	0.104	0.613	0.286	0.018	10.2	2.945	547	38
Zn	2.275	0.092	4.095		0.006				179
	4.549	0.144	2.658		0.001				199
Cd	3.264	0.143	0.109	0.089					21
	6.528	0.270	0.070	0.126					24
Hg	0.078	0.008	5.308	0.414	0.080	4.9	1.656	1582	62
	0.156	0.009	8.843	0.465	0.028	6.4	3.836	8458	362

\* mean weight of fish 0,017 kg

**Fig. 1** The appropriateness of the bioaccumulation model and its experimentally determined concentrations of Cu (a), Zn (b), Cd (c) and Hg (d)

determined before the steady state was attained, its understandable that  $C_{fish}/C_{water}$  ratio was lower than the predicted BCF.

In these calculations, the heavy metals uptake from food pellet was not considered. Compared to waterborne

heavy metals absorption by the fish  $g^{-1}day^{-1}$  ( $= k_w \times E_w$ ), the uptake from food pellet at feeding rate of  $0.2 g fish^{-1} day^{-1}$ , which is equal to Cu  $0.056$ , Zn  $0.963$ , Cd  $0.014$  and Hg  $0.006 \mu g, fish g^{-1}, day^{-1}$ , were negligible.

Zn is an essential heavy metal, therefore it is homeostasis regulated. Glover and Hogstrand [8] reported that intestinal mucus of *Oncorhynchus mykiss* was one important regulatory locus, promoting zinc uptake at low concentrations yet buffering the fish against high luminal zinc loads, therefore, uptake efficiency decreases as the exposure concentration increases. In this simulated estuarine ecosystem, the body burden of Zn in the fish were high due to the previous exposure, as shown by its high  $C_{\text{fish}}/C_{\text{water}}$  ratio, therefore no more nutritional Zn was required, only excretion mechanisms was observed as homeostasis regulation manifestation.

Cd is non-essential heavy metal, however, the uptake efficiency decreases as the exposure concentration increases. The experimental data showed that the exposure time employed in this study was less than the time required to reach maximum concentration,  $T_{\text{max}}$ , and equation 5 was used to predict fish concentration. For that reason, the  $C_{\text{fish}}/C_{\text{water}}$  ratio were similar at different exposure concentrations.

It is clear that every heavy metal has its own bioaccumulation dynamics and it is important to note that the parameter values driving the model are not constant but varied among metals, organisms, and environmental conditions.

#### **Bioaccumulation due to waterborne and particulate heavy metal exposure**

In this simulated estuarine fish-water-sediment (F-W-S) ecosystem, beside the physiological aspects, the influence of the dynamic nature of metal speciation is more pronounced, and external equilibrium of all forms of heavy metals will take place.

Based on the assumption that heavy metals uptake in gills and gut epithelias are mostly from waterborne exposure, the heavy metal bioaccumulation

due waterborne and particulate heavy metal exposure can be predicted using the obtained kinetics parameters of the bioaccumulation model above. The heavy metals concentration in *O. nilotycus* after 28<sup>th</sup> days exposures, were predicted based on hydrophilic fraction and from the total of both hydrophilic and lipophilic sources, and compared to the measured concentration (Table 3).

These data showed the tight homeostasis regulation for Cu and Zn as in the previous waterborne heavy metal bioaccumulation experiment. To accomplish the demand for Cu and Zn at low concentration of hydrophilic fractions, the uptake of Cu and Zn was not only from the hydrophilic fractions, but also supplied by the labile/ exchangeable species of Cu and Zn in the sediment. In fact, they are in equilibrium. Whenever the exposure concentrations are high, the Cu and Zn uptake are accomplished solely by the hydrophilic fraction, as shown in the closeness of prediction calculated from hydrophilic fractions uptake to the measured concentrations.

Cd and Hg are classified as soft cations, therefore Cd prefer to form strong complexes with  $\text{Cl}^-$  ligand either in water phase or at the surface of the particles. For that reason, most of the available Cd present as hydrophilic fraction (Table 1), and the model predicted concentrations due to hydrophilic fraction uptake were close to the measured concentrations.

On the other hand, Hg undergoes many complexation reactions with  $\text{Cl}^-$  and  $\text{OH}^-$  ligands in both water phase and surface particles, and form complexes by coordination with oxygen donor atoms at the surface of particles [9]. Even though in this simulated ecosystem, most of the available Hg was lipophilic fraction (Table 1), the measured concentrations reflected that hydrophilic fractions were the main source for Hg uptake.

**Table 3** The relative importance of heavy metals sources bioaccumulated in *O. nilotycus* After 28 days of waterborne and particulate heavy metals exposure, based on the closeness between predicted and of actual concentration

Heavy metals	Model predicted concentration at 28 <sup>th</sup> days					Remark (relative importance of the sources)
	Source: hydrophilic mg/L	Calculated mg/kg	Source: total hydro + lipo. mg/L	Calculated mg/kg	Measured concentration at 28 <sup>th</sup> days mg/kg	
Cu	0		1.327	75.440	4.400	Homeostasis regulated
	0.259	9.117	2.550	89.676	12.058	Mostly from hydrophilic
Zn	0.031	6.714	0.066	14.169	15.867	Homeostasis regulated.
	0.142	32.599	0.251	57.651	22.084	Mostly from hydrophilic
Cd	0.354	3.502	0.355	3.516	2.245	Solely from hydrophilic
	1.482	26.859	1.484	26.895	13.674	
Hg	0.009	0.395	0.096	4.245	0.518	Solely from hydrophilic
	0.011	4.154	0.120	46.391	2.142	

**Table 4** Distribution of heavy metals in specific internal organs of *O. nilotycus* at the 28<sup>th</sup> day of higher heavy metals exposure in different microcosms, and their  $C_{\text{carcass}}/C_{\text{hydrophilic}}$  ratio

Heavy metals	Microcosms	Sources		Amount of heavy metals (wet basis)				$C_{\text{carcass}}/C_{\text{hydrophilic}}$
		$C_{\text{Hydrophilic}}$ μg/mL	$C_{\text{Lipophilic}}$ μg/mL	Gill μg/fish	Liver μg/fish	Carcass μg/fish	SET μg/fish	
Cu	F-W*)	0.104		3.91	45.47	0	0	0
	F-W-S**)	0.259	2.291	10.13	33.10	72.17	18.81	43
Zn	F-W	0.158		14.82	31.50	106.85	79.86	105
	F-W-S	0.142	0.109	17.75	35.96	293.44	132.52	164
Cd	F-W	0.270		2.30	16.49	16.99	5.67	11
	F-W-S	1.482	0.002	9.73	50.26	203.94	33.07	11
Hg	F-W	0.015		0.98	7.43	7.84	8.70	130
	F-W-S	0.011	0.109	61	7.77	28.39	7.18	210

\*) F-W= Fish-Water Microcosms

\*\*) F-W-S= Fish-Water-Sediment Microcosms

Cd and Hg are non essential heavy metal, therefore the homeostasis regulation were not as tight as in Cu and Zn. As in the previous experiment of bioaccumulation due to waterborne exposure, in this study, homeostasis regulation for both cations appeared only at high exposure concentration.

The closeness between model predicted concentration calculated from hydrophilic fraction to the measured concentration in waterborne and particulate heavy metals, confirm that the internalization of metals into the cells of gills and internal epithelias follows similar mechanisms.

#### Heavy Metal Distribution within Specific Organs in *Oreochromis nilotycus*

Following the uptake, the heavy metals is transported and distributed by circulatory system into specific organs. The physiological flow of <sup>64</sup>Cu following an acute waterborne exposure (48 hours) in rainbow trout (*Oncorhynchus mykiss* [4], was from water across the gill membrane, excreted into plasma, and then distributed via arterial circulation and deposited into specific tissues. The uptakes of heavy metals are strongly related to the presence of MTs in the cytosol of hepatocytes. When the synthesis of these proteins is not enough to sequester all the incoming metal ions, the protein binding sites become saturated and level off. In this case, the remaining free metal-protein complex can be re-absorbed by the circulatory system, and re-distributed to other organ or tissues.

As in the preceding experiments, similar physiological flow seems to be happened in *O. nilotycus*. It is interesting to note that the amount of Cu, Zn and Hg in liver of fish both from F-W microcosms and from F-W-S microcosms, were similar (Table 4), even though their hydrophilic fractions are different. From Cu data, it was clearly shown that before the protein binding sites in liver

were becoming saturated; no Cu was detected in the following organs, i.e. carcass and the Slowly Equilibrated Tissue (SET), but at higher hydrophilic fraction in water phase, then Cu was detected in carcass and SET. For that reason, carcass is a more relevant indicator for heavy metal bioaccumulation than liver; beside it is an important parameter for consumer safety. The closeness of  $C_{\text{carcass}}/C_{\text{hydrophilic}}$  ratio in simulated fish-water microcosm and in fish-water-sediment microcosm indicate a similar heavy metal source accumulated in the carcass of *O. nilotycus*, i.e. the hydrophilic fraction in the water phase.

This study confirmed the bioaccumulation dynamics of non essential heavy metals Cd and Hg in carcass of *Mugil* Sp. and *Geloina* sp. residing in Segara Anakan estuary, Central Java, Indonesia [10]. These aquatic organisms are the protein source for the local residence of Segara Anakan, and concentrations of Cd and Hg in the edible part (carcass) were higher at dry season than at wet season, due to its higher salinity. Despite the organisms differences, the model predicted Cd wet basis concentrations of *Mugil* Sp and *Geloina* sp. at dry season of 1.4 mg/kg were in agreement with the measured concentration, of 2.2 mg/kg, respectively.

#### CONCLUSION

From these bioaccumulation studies, it was confirmed that internalization of metals into the cells of gills and epithelias are similar, and governed mostly by the waterborne heavy metals. The level of waterborne heavy metals is determined by desorption equilibrium coefficients,  $1/K_D$  [1]. In the previous work, due to their preference in complex formation, at higher concentration of  $\text{Cl}^-$  ligands in water phase, only part of the enriched hard cations Cu and Zn are transferred into the overlaying water, resulting in smaller  $1/K_D$ , on

the contrary, more soft cations Cd and Hg were transferred into the overlaying water phase resulting in higher equilibrium coefficient for desorption,  $1/K_D$  [1]. Similar trend were observed in this work (Table 1).

The bioaccumulation model showed that Uptake Efficiency factor,  $E_W = V_W F$  (in  $L\ org^{-1}\ day^{-1}$ ) of essential heavy metals such as Cu and Zn, decreases as exposure concentration increases, due to homeostasis regulation in *O. nilotycus*, while for non essential heavy metal Hg, it is increases as the exposure concentration increases, causing the Estimated BCF and  $C_{fish}/C_{water}$  ratio at 28<sup>th</sup> day for Hg were higher than Cu. No uptake of Zn was observed in this study, demonstrating that due to previous Zn exposure, no more nutritional Zn was required, as pointed out by high  $C_{fish}/C_{water}$  ratio in fish-water microcosms and  $C_{carcass}/C_{hydrophylic}$  ratio in fish-water-sediment microcosm. Therefore only excretion was observed as manifestation of homeostasis regulation. However, in this study, the  $E_W$  factor of Cd indicated that there was a homeostasis regulation. Unfortunately only uptake rate constant could be determined; therefore the  $C_{fish}/C_{water}$  was at the same ratio.

From the study of heavy metal distribution within specific internal organs, it was revealed that due to the capacity of liver in accumulating these heavy metals, the concentration in carcass was more relevant in describing the bioaccumulation condition. The dynamics of heavy metal bioaccumulation in these simulated estuarine ecosystem is applicable to explain the observed Cd and Hg body burden in aquatic organisms of Segara Anakan estuarine ecosystem within the same order and higher at dry season than at wet season.

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#### REFERENCES

1. Noegrohati, S., 2005, *Indo. J. Chem.*, 5, 3, 236-244
2. Campbell, P. G.C., 2002, Predicting metal bioavailability – applicability of the Biotic Ligand Model, in *CIESM, 2002. Metal and radionuclides bioaccumulation in marine organisms*, CIESMWorkshop Monographs n°19, 128 pages, Monaco
3. Phinney J.T., and Bruland K.W., 1994, *Environ. Sci. Technol.*, 28: 1781-1790.
4. Kamunde, C., Clayton, C., and Wood, C.M., 2002, *Am J Physiol Regul Integr Comp Physiol* 283:69-78
5. Soto, M., Marigómez, I. and Cancio, I., 2005, *Biological aspects of metal accumulation and storage*, zoxcauri @lg.ehu.es, downloaded at 6/19/05
6. Shargel, L., and Yu, A.B.C., 1993, *Applied Biopharmaceutics and Pharmacokinetics*, 3<sup>rd</sup> Ed., Appleton & Lange, Norwalk, USA, 169-179
7. Zhang, M.K., He, Z.L., Stoffella, J., Calvert, D.V., Yang, X., and Sime, P.L., 2003, *Envir. Geol.* 44, 1–7
8. Glover, C.N. and Hogstrand, C., 2002, *J. Exp. Biol.* 205, 141–150
9. Schnoor, J.L., 1990, *Environmental Modelling, Fate and Transport of Pollutants in Water, Air and Soil*, John Wiley & Sons, INC, Singapore, 381-431
10. Noegrohati, S., 2005, *Indo.J.Chem.* 5, 2, 135-142