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Capability of Catfish (*Clarias gariepinus)* to Accumulate Hg^{2+} From Water

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Abstract – Mercury is hazardous contaminant that can be accumulated by aquatic organisms such as fishes, mussels etc. Catfish is one of source of animal protein but it also can accumulate Hg^{2+} from water that used in aquaculture. Due to less information about capability of catfish to accumulate Hg^{2+}, therefore we studied bioaccumulation of Hg^{2+} that used biokinetic approach (aqueous uptake-rate, and elimination-rate). Nuclear application technique was applied in this study by using radiotracer of ^{203}Hg. A simple kinetic model was then constructed to predict the bioaccumulation capability of by catfish. The result of experiments were shown that the uptake rate of difference Hg^{2+} concentration were 79.90 to 101.22 $ml.g^{-1}.d^{-1}$. Strong correlation between uptake rates with increasing Hg^{2+}concentration. In addition, the elimination rates were range 0.080 – 0.081 day^{-1}. The biology half time ($t_{1/2b}$) of Hg^{2+} in whole body catfish were 8.50 – 8.63 days. However, no clear correlation between elimination rate with increasing concentration of Hg^{2+}. The calculation of Bio Concentration Factor (BCF) shown catfish have capability to accumulated Hg maximum 1242.69 time than its concentration in water.
Keywords: Radiotracer; Kinetic; Uptake; Elimination; Bioaccumulation

Introduction

Catfish is one of aquaculture species in Indonesia. Catfishes of the genus *Clarias* (Siluroidei, Claridae) are widespread in tropical Africa and Asia (Sudarto, 2007). Because many catfish aquacultures in low quality water, it has possibility contaminated by pollutants such as heavy metals. One of the most hazardous contaminants is mercury (Hg) that input from natural and anthropogenic activities and then will enter In environment waters, mostly of Hg present as in organic (Hg^{2+}) and organic (methyl mercury) (Wang, 2012; Leermakers *et al*., 2005). These mercury species have potential to be accumulated in catfish through water and the food web. Consumption of contaminated cat fish by mercury can effect to human health. WHO (2008) explain that some organs such as: the nervous system, the cardiovascular system and the kidneys are the primary targets for toxicity of mercury and mercury compounds. Moreover, the most of sensitive to toxic effect of mercury are developing organ systems (such as the fetal nervous system). Therefore, it is important to find the information of accumulation of Hg^{2+} in catfish.

A large number bioaccumulation studies of Hg^{2+} and other heavy metals in fresh water fishes have examined but most previous studies measured the concentration of Hg in fish and compare it's concentration in water (Baker *et al.,* 2009; Choy *et al.,* 2009; Casas and Bacher, 2006; Passos *et al.,* 2007; Kasper *et al.,* 2009; Limbong *et al.,* 2004; Eng *et al*., 1989, Prasetyo, 2009; Riani, 2010; Mustaruddin, 2013). However, these previously studies were not provided information regarding the uptake and elimination kinetics of the Hg^{2+}, which are important parameters in interpreting and predicting the Bioaccumulation Factor. On other hand biokinetics of Hg^{2+} in fishes are less well studied. In this study we quantified the biokinetics (uptake and elimination) of Hg^{2+} from water by catfish (*Clarias gariepinus*) to predict its capability to accumulate the Hg^{2} that used radiotracer. A radiotracer technique was used during the present study because it is a very sensitive method and the biokinetics of Hg can be followed non invasively over time (Tsui and Wang, 2004). African catfish (*C. gariepinus*) is one of the popular freshwater fish widely cultured in Indonesia, and is used for human consumption. Herein, we measured a few kinetic parameters (aqueous uptake-rate, and elimination-rate) of Hg^{2+} species in the fish. A simple kinetic model

was then constructed to predict the bioaccumulation capability of Hg^{2+} by catfish from water. This information is important due to both of the environmental assessment in ecosystems and human risk assessment regarding to fish consumption.

Materials and Methods

Radiotracer experiments

The bioaccumulation experiment used methods similar to those of Wang and Wong, 2013; Tsui and Wang 2004 with some modifications. The catfish (*C. gariepinus*) (7.0 to 7.2 cm length) were purchased from a fish farm in Serpong area, South Tangerang, Indonesia. Catfishes were acclimated by maintaining in aerated fresh water and fed with commercial fish feed twice a day. After 14 days acclimatization process, four catfishes were placed in aquarium and exposed to different concentrations of Hg^{2+} (0.001, 0.005 and 0.01 ppm) in the dissolved (0.22-μm-filtered fresh water) for a total of 12 days. Every Hg^{2+} concentration also spiked $^{203}Hg^{2+}$radioisotope into aquarium until each activity concentration was 1 $Bq.ml^{-1}$. Radiotracer of $^{203}Hg^{2+}$($t_{1/2}$ = 46.9 d, in 0.1 N HCl, specific activity = 3$\mu Ci.g^{-1}$) was produced in Center for Radioisotope and Radiopharmaceutical, BATAN Indonesia. The uptake of radioisotopes was followed non destructively over time. The activity concentration of $^{203}Hg^{2+}$ was measured by a gamma spectrometer (NaI detector) at 279 keV, and was corrected for counting efficiency and geometry. At the end of the uptake experiment (8 days), all catfishes were transferred to the uncontaminated running freshwater. The flow rate of the uncontaminated water was set at 1 $l.h^{-1}$ to avoid recontamination. The elimination of the $^{203}Hg^{2+}$ in each catfish is expressed as the percent of the initial activity at the beginning of the elimination experiment.

Model

The uptake kinetics were modelled with a single-component first order kinetic model (Whicker and Schultz, 1982, Metian *et al.*, 2011; Wang, 2012, Cardoso *et al.*, 2013)

$$CF_t = C_{ss}\,(1 - e^{-k_e t}) \quad (1)$$

where CF_t and CF_{ss} represent activity concentration factor at time t (d) and at steady state ($ml.g^{-1}$) respectively, and k_e represents biological uptake rate constant (d^{-1}). If there was no indication of reaching a steady state during the time of exposure (non-significant fit to model 1), a simple linear regression model was applied. Concentration factor, CF is ratio of activity concentration of ^{203}Hg in fish tissue to its activity concentration at water.

$$CF_t = k_u t \quad (2)$$

where k_u is the slope of regression (uptake in ml. $g^{-1}.d^{-1}$). Elimination after return to fresh water was modelled using either a single-component exponential model

$$A_t = A_o e^{k_e t} \quad (3)$$

where A_o and A_t are percent of the initial activity at the beginning of the loss experiment and percent at time t of loss experiment. On other had k_e is elimination rate constant. The Bio Concentration Factor (BCF) is ratio of the steady-state chemical concentrations in an aquatic water-respiring organism and the water determined in a controlled laboratory experiment in which the test organisms are exposed to a chemical in the water (UK-EPA, 2011)

$$BCF = \frac{k_u}{k_e} \quad (4)$$

Result and Discussion

According to Wang (2012), speciation of Hg^{2+} is complicated by their binding to various ligands (e.g., chloride and dissolved organic carbon). Furthermore, Wang (2012) explained that differences in Hg speciation may considerably affect its bioavailability and bioaccumulation in aquatic organisms. In this experiment we calculated all biokinetic parameter from dissolved Hg^{2+}. On other hand interaction between Hg and dissolved organic matter (DOM) significantly influences the Hg speciation, solubility, mobility and toxicity in aquatic ecosystems (Ci *et al.*, 2011). Using the pore-size filter (0.22 µm) we removed DOM to ensure the mercury speciation was dissolved Hg^{2+}.

After 7d experiment, uptake of Hg^{2+}in whole-body catfish displayed linear kinetics and the steady state wasn't reached (Figure 1). The values of Concentration Factor (CF) at the end of experiment were 517.88 to 650.58 $ml.g^{-1}$. This result indicated that catfish have capability to accumulated Hg^{2+} 517.88 to 650.58 time than it's concentration on water. However these are not representing as value of Bio Concentration Factor (BCF) due to this value have to be determined at steady state condition.

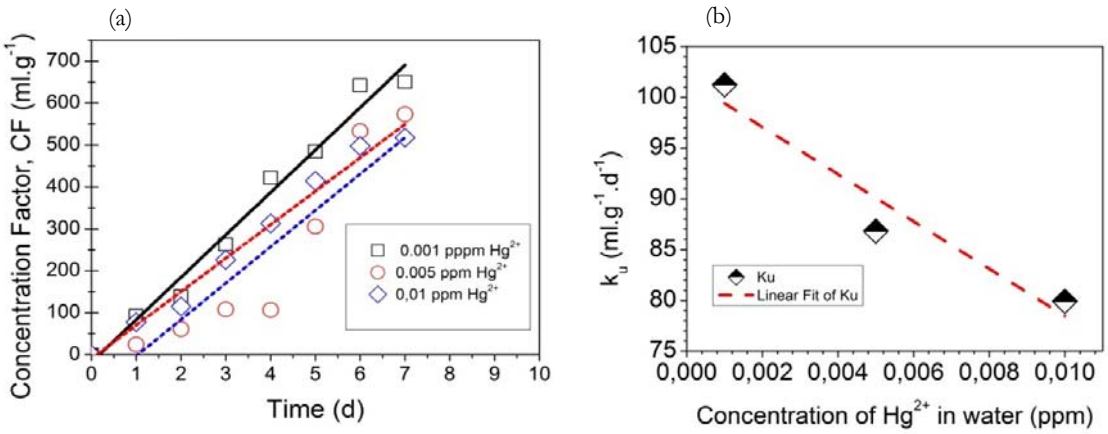

Figure. 1 Bioaccumulation Hg^{2+} (a) Uptake Hg^{2+} from difference concentration (b) Influence of Hg^{2+} concentration in water to uptake rate by catfish

Uptake rate of Hg^{2+} from difference it's concentration in water were 79.90 to 101.22 $ml.g^{-1}.d^{-1}$. Uptake of dissolved metals (Hg^{2+}) from solution through permeable surfaces into the bodies of fish is generally considered to be a passive process (Carvalho *et al*., 1999). Elevated Hg^{2+}concentration in water induce decreasing of Concentration Factor and uptake rate. This result can be explained based on mechanism bioaccumulation Hg^{2+} through water. Mercury enter to fish via gill that follow the mechanism of respiration or water drinking. According to Morgan *et al* (2004), it is generally accepted that the key toxic site of action of heavy metal (such as Hg^{2+}) is the Na^+K^+-ATPase located on the basolateral membrane of gill cells. Furthermore, Morgan *et al.* (2004) explained that this enzyme is responsible for extruding Na^+in exchange for K^+ across the basolateral membrane and into the extracellular fluid, thereby providing much of the energy for active Na^+ and Cl^- uptake. In freshwater fish, this transport is essential to counter act the diffusive loss of Na+ and Cl^- to the hypo osmotic fresh water environment. Water borne Hg^{2+} exposure inhibits the activity of this enzyme causing an inhibition of Na+ and Cl− uptake via the gills (Morgan *et al*., 2004). Furthermore Stohs and Bagchi (1989) explained that specific differences in the toxicities of metal ions may be related to differences in solubility, absorbability, transport, chemical reactivity, and the complexes that are formed within spite of these factors, the basic mechanisms involving production of reactive oxygen species are the same for these transition metal ions in the body. The toxicity of mercury and its ability to react with and deplete free sulffiydryl groups are well known. Elemental, inorganic, and organic forms of mercury exhibit toxicological characteristics including neurotoxicity, nephrotoxicity, and gastrointestinal toxicity with ulceration and hemorrhage. The decrease in free sulfhydryl groups may lead to the formation of an oxidative stress, resulting in tissue-damaging. (Stohs and Bagchi, 1989)

Regarding the elimination step, this study find that the catfish demonstrated a slow decrease of Hg^{2+} concentration in the first day until the end of the experiment. Probably, the catfish needed more time to progressively detoxify and a continuation to this study would be to assign until achieve equilibrium. When non-contaminating conditions were restored, the whole body elimination kinetics of both Hg^{2+} were best described by a one -component exponential model (Figure 2a). The elimination rate were range 0.080 – $0.081 day^{-1}$. The biology half time ($t_{1/2b}$) Hg^{2+} in whole body catfish were 8.50 – 8.63 days. However, no clear correlation between elimination rate and increasing concentration of Hg^{2+} because the linear regression coefficient (Adj.R-Sq) bellow 50% (Figure 2b).

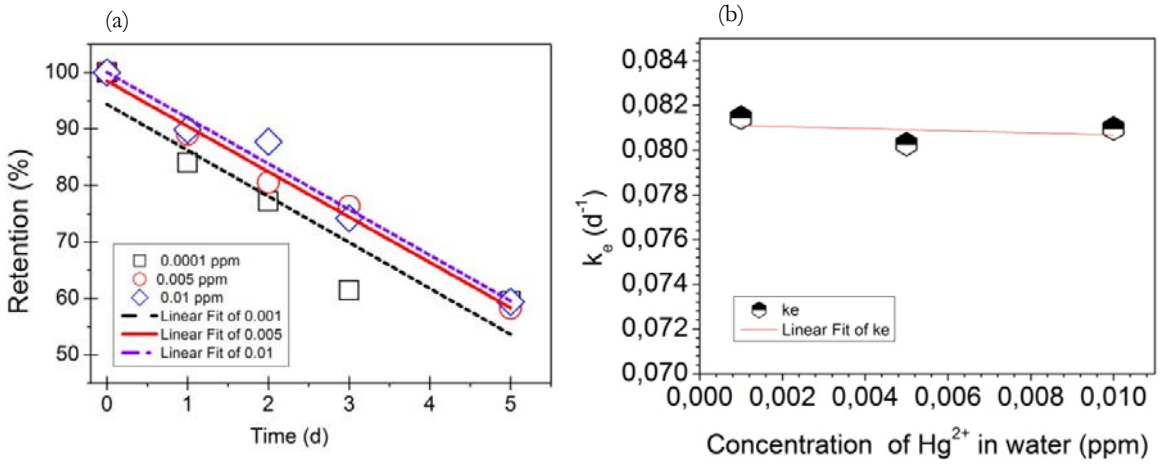

Figure. 2 Elimination of Hg^{2+} (b) Loss of Hg^{2+} in difference concentration, (b) K_e of catfish after accumulated Hg^{2+} in difference concentrations

The uptake experiment only performed for 7 days so that the condition of equilibrium has not been reached. According to Reinardy *et al.* (2011), dissolved contaminants are primarily taken up across gill membranes or epithelia of the gastrointestinal tract depending on exposure (aqueous or dietary); and, if exposure is of sufficient duration, equilibrium will be established between contaminants in tissues and in the abiotic environment. Another way to define the ability of bioaccumulation is the value of BCF. Van der Oost *et al.* (2003) explained the bio Concentration Factor (BCF) of a chemical is the ratio of its concentrations in the organism and in water during steady state or equilibrium. The biokinetic parameter that results from this experiment was display on Table 1. The elevation of Hg^{2+} concentration will decrease the uptake rate, elimination rate, Bio Concentration Factor (Table 1). The model bioaccumulation was displayed at Figure 3.

Table 1. Bioakinetic parameter and BCF calculation

Concentration of Hg^{2+} in water (ppm)	k_u ($ml.g^{-1}.day^{-1}$)	Ke (day^{-1})	BCF ($ml.g^{-1}$)	$t_{1/2}$ (day)
0.001	101.22	0.081	1242.69	8.51
0.005	86.81	0.080	1081.41	8.63
0.01	79.90	0.080	986.78	8.56

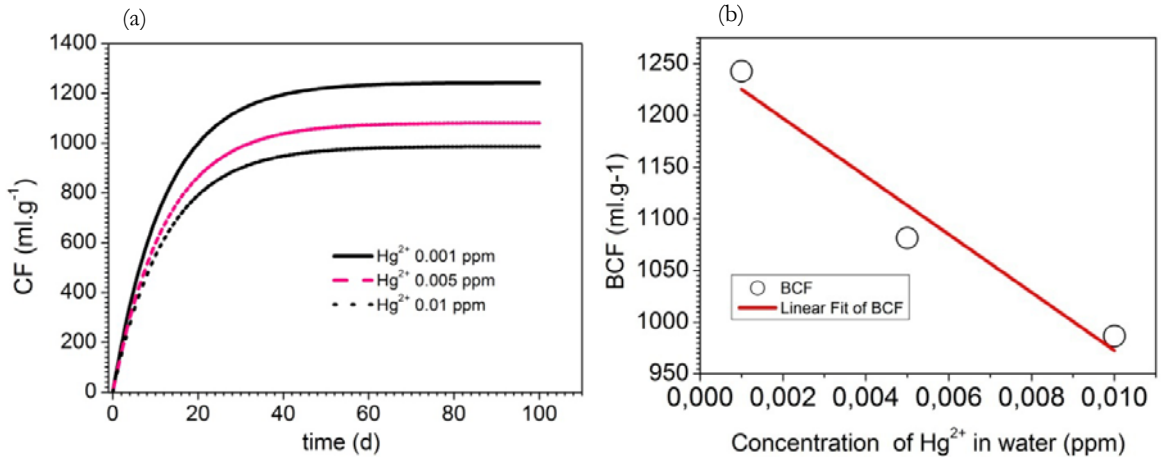

Figure 3. Bioaccumulation model. (a) Prediction steady state condition, (b) Influence concentration Hg^{2+} in water to BCF

Regarding to model, steady state condition was reached after 31 days accumulated Hg^{2+}. The BCF have strong correlated with Hg^{2+} concentration in water because increasing concentration will inhibit the metabolism enzyme of catfish. Comparing with another result was shown at Table 2.

Table 2. Biokinetic for fishes using radiotracer techniques

Biota	k_u ($ml.g^{-1}.day^{-1}$)	k_e (day^{-1})	BCF, calculation ($ml.g^{-1}$)	Refference
Tilapia (*Oreochormis niloticus*)	86	0.039	2205.128	Wang *et al.* (2010)
Mosquito fish (*Gambusia affanis*)	52 – 78	0.021 – 0.042	1857.143 - 2476.19	Pickhardt *et al.* (2006)
Sunfish (*Lepomis microlophus*)	38 – 51	0.003 - 0.0035	1033.30 – 1457.14	Pickhardt *et al.* (2006)
Catfish (*Clarias gariepinus*)	79.90 – 101.22	0.080 – 0.081	986.78 – 1242.69	in this study

The result of this experiment was comparable with another Hg^{2+} bioaccumulation experiment that use radiotracer techniques. Furthermore, protection of human health depends directly on the accuracy of estimates of BCF because its variability such as (1) ecological variability (signal) due to ecosystem-specific differences in Hg uptake and accumulation and (2) methodological variability (noise) due to, for example, differences in species, sex, weight, length, age, trophic position, tissue type, collection season, and Hg analysis (Scudder-Eikenberry *et al*., 2015). Thus, minimizing methodological variability in experiment is critical to BAF-based Hg-risk management. The Hg concentrations in some fishes (including catfish) may be contribute to negative effect to human health, thus Hg exposure to human mainly occurs through dietary intake of contaminated fish (Taylor *et al.,* 2014). Base to this experiment, catfish have capability to accumulated Hg maximal 1242.69 time than its concentration in water. On other hand the threshold levels of Hg^{2+} is 1.0 $mg.Kg^{-1}$ thus concentration of Hg^{2+} in aquaculture water approximately 0.00081 ppm can give maximum concentration level of Hg in catfish.

Conclusions

Uptake rate of Hg^{2+} from difference it's concentration in water were 79.90 to 101.22 $ml.g^{-1}.d^{-1}$. Strong correlation between uptake rates with increasing concentration of Hg^{2+}. The elimination rate were range 0.080 – 0.081day^{-1}. The biology half time ($t_{1/2b}$) Hg^{2+} in whole body catfish were 8.50 – 8.63 days. However, no clear correlation between elimination rate and increasing concentration of Hg^{2+}. Catfish have capability to accumulated Hg (BCF) maximal 1242.69 time than its concentration in water. Due to the threshold levels of Hg in fish products were 1.0 $mg.Kg^{-1}$, therefore concentration of Hg^{2+} in aquaculture should be maximum approximately 0.00081 ppm

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