

## Effects of Concentration, Body Size and Food Type on the Bioaccumulation of Hg in Farmed Tilapia *Oreochromis Mossambicus*

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**Abstract:** Kinetics of bioaccumulation of Hg by *Oreochromis mossambicus* were investigated in this study. We experimentally determined the assimilation efficiency (AE) of ingested prey, the uptake-rate constant from the aqueous phase, and elimination-rate constant of mercury. By the steady state of the exposure period, the Concentration Factor (CF) of Hg was 130.66 to 150.58. The biological half-lives at slow components between small and large fishes did not change significant, and were found to be 46.8–46.5 and 72.2–75.3 days. Predictions of Bio Concentration Factor (BCF) in *O. mossambicus* tissues using a biokinetic model and the measured AE and efflux rates ranged from 743.31 to 4825.95.

**Key words:** Bioaccumulation, mercury, kinetic, *Oreochromis mossambicus*

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### INTRODUCTION

Since some of the most heavily industrialized areas of the Jakarta bay coastal are sited on the banks of estuaries, these waters and those of other confined areas are particularly risk from mercury contamination. Adequate management of our environment requires the correct tools which will allow us to accurately predict the fate and effects of contaminants within the environment. Considering that mercury is the only element capable to biomagnificate along the food chain, carnivorous fish tend to accumulate high concentrations of this element (Tsui and Wang, 2004). For this reason apex predator fishes may become the main pathway for human contamination through the consumption of contaminated specimens (Ferreira *et al*, 2004). The consumption of contaminated fish is a well-documented pathway leading to Hg exposure in humans. The cases of Minamata disease in Japan provide extreme examples of the severe and persistent toxicity caused by chronic Hg pollution in rivers and bays (Harada, 1995). The Mozambique tilapia, *Oreochromis mossambicus* is widely used as food and has been introduced in various localities for aquaculture in Jakarta bay coastal. The Mozambique are omnivores that consume detrital material,vegetation with various ranges from diatoms to macroalgae to rooted plants, invertebrates, and small fry. They are however highly tolerant of brackish water. Because of human health concerns, mercury accumulation in commercial predatory fish has been studied thoroughly and concentrations have been found to increase with species trophic level and longevity, as well as fish age (Monteiro *et al*, 1996).

Bioaccumulation can be defined as the net accumulation of a metal in a tissue of interest or a whole organism that results from exposure. The bioaccumulation of trace elements in aquatic organisms can be described with a kinetic model that includes linear expressions for uptake and elimination from dissolved and dietary sources (Reinfelder *et al*, 1998 and Chen and Liao, 2004) Predictive trace element bioaccumulation models therefore need to account for accumulation from both water and food. The biokinetics of Hg in fishes are less well studied Most previous studies measured the concentration of different Hg species in different tissues of fishes (Wang and Wong, 2003). The routes of Hg accumulation, including the relative importance of different Hg species (inorganic and organic) and exposure pathways (aqueous vs dietary), are not yet well understood. Most field studies examined the trophic transfer of Hg by collecting various abiotic (water and sediment) and biotic (phytoplankton, zooplankton, and fish) compartments and then analyzing the respective Hg concentrations However, these studies did not provide information regarding the uptake and removal kinetics of the Hg compounds, which are important parameters in interpreting and predicting the food-

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chain transfer of Hg. (Tsui and Wang, 2004). On other hand, in estuarine and coastal environments, various environmental factors including pollutant input, salinity, temperature, and food availability can vary widely. Those external factors were known to influence on the metal bioaccumulation in organisms by changing either the bioavailability of dissolved and particulate metals in water or physiological attributes of organisms (Casas and Bacher, 2006; Lee and Lee 2005)

Kinetic modeling has recently been successfully employed in predicting the exposure pathways and concentrations of metal contaminants in marine invertebrates (Ferreira *et al*, 2004). There are, however, only a few reports addressing the application of kinetic modeling to trace element accumulation in fish, although such a model has been developed for fish. Mechanistic understanding of Hg bioaccumulation may be possible through measurements of several physiological parameters described in the kinetic model, including the contaminant assimilation efficiency (AE) from the ingested food source, the uptake rate constant from the aqueous source, and the efflux rate constant.

Previous research has recognized the *O.mossambicus*'s potential as a bioindicator organism to assess spatial and temporal trends in concentrations of bioavailable contaminants (Noegrohati. 2006). However, there have been limitations to using this research for quantitatively describing bioaccumulation in these fishes and their influence on geochemical cycling. For example, the relative bioavailability of different trace element sources (food and water) for *O.mossambicus* has never been evaluated. In this study, we quantified Hg bioaccumulation from both the aqueous and dietary phases uptake by mozambicus tilapia under varying concentration of Hg and food types.

## MATERIAL AND METHODS

### ***Fish and Radioisotopes:***

farmed tilapia *Oreochromis mossambicus* (3.2 to 5.8 cm) were taken from a fish farm in Panimbang, Banten Province Indonesia, and were maintained in aerated brakish water and fed commercial feeding twice a day. The radiotracers:  $^{203}\text{Hg}^{2+}$  ( $t_{1/2} = 46.9$  d, in 0.1 N HCl purchased from Center for Radioisotope Production-National Nuclear Energy Agency of Indonesia)

### ***Hg Uptake at Different Ambient Concentrations:***

The uptake of Hg was determined at 4 different ambient Hg concentrations: 0.421, 2.105, 10.90  $\mu\text{g l}^{-1}$  (added as stable Hg,  $\text{HgCl}_2$ ). Radioactivity addition was 1,28 kBq  $\text{l}^{-1}$ . Three individual fish were exposed in 6 l of 0.22  $\mu\text{m}$  filtered brakish water at each concentration. At time intervals the fish were removed from the radioactive medium, rinsed twice (transferred from one beaker to another) with filtered non-radioactive water and their radioactivity counted non-destructively by a NaI gamma detector at 279 keV, and was corrected for counting efficiency and geometry. Following the radioactivity measurements, the fish were returned to the radioactive medium. The radioactivity in the water was measured at the beginning of exposure and during the measurements of radioactivity in fish at each time point.

### ***Measurements of Hg Assimilation Efficiency(AE):***

The AE of  $^{203}\text{Hg}^{2+}$  were measured using technique, described in Wang &Wong (2003) with some modification. Three diets were used: brine shrimp *Artemia* sp. The brine shrimp were radiolabeled with  $^{203}\text{Hg}^{2+}$  in 200 ml 0.22  $\mu\text{m}$  filtered brakish water. Radioactivity additions were 3.7 kBq for  $^{203}\text{Hg}^{2+}$ . The macro algae were radiolabeled in 2 l filtered seawater spiked with 3.7 of  $^{203}\text{Hg}^{2+}$ . After 36 h exposure to radiotracers, the prey (macroalage and brine shrimp) were removed from their exposure medium, rinsed thoroughly with seawater, and fed to the fish naturally. After the radioactive feeding, the fish were placed in nonradioactive water (20 l) and depurated of their ingested metals for 48 h. The radioactivity remaining in the fish was quantified non-destructively at 3, 6, 12, 18, 24, 36, and 48 h. Water was renewed every 12 h to ensure that the amount of radioactivity in the water was negligible within the 48 h depuration period. The fish were fed with nonradioactive shrimp meat twice a day during the depuration period. The AE was calculated as the percentage of metal retained in the fish at 24 h.

### ***Data Analyses:***

Uptake of the radiotracers from water was expressed as change in concentration factors (CF). The concentration factor (CF) of  $^{203}\text{Hg}^{2+}$  was calculated as the ratio of the radioactivity in the fish ( $\text{Bq.g}^{-1}$ ) to the radioactivity in the water ( $\text{Bq.ml}^{-1}$ ), calculated as the mean before and after exposure for each time point). The uptake-rate was calculated as the slope of the linear regression between th CF and the time of exposure multiplied by the dissolved Hg concentrations. Uptake kinetics in were described using a single-component first-order kinetic model:

$$CF_t = CF_{equil}(1 - e^{-kt}) \quad (1)$$

where  $CF_t$  and  $CF_{equil}$  are concentration factors at time  $t$  (d) and steady state, respectively, and  $k$  is the rate constant ( $d^{-1}$ ) [4]. Radiotracer elimination was expressed in terms of percentage of remaining radioactivity, i.e. radioactivity at time  $t$  divided by initial radioactivity measured in the organisms at the beginning of the depuration period. When radiotracer loss plotted against time displayed an exponential shape, the kinetics were described by single-component exponential model:

$$At = A_0 e^{-k_d t} \quad (2)$$

where  $A_t$  and  $A_0$  are remaining activities (%) at time  $t$  (d) and 0, respectively, and  $k_d$  is the depuration rate constant ( $d^{-1}$ ) which allows the calculation of the radiotracer biological half-life ( $t_{1/2b}$ ).

$$t_{1/2b} = \frac{\ln 2}{k_d} \quad (3)$$

#### Modeling Exposure and Food-chain Transfer:

Under steady-state conditions, Hg accumulation in fishes can be calculated by the following equation (Wang and Wong 2003):

$$BCF = \frac{k_u}{k_e} + \frac{AE \cdot IR \cdot BCF}{k_d} \quad (4)$$

where  $BF$  is the Hg Bioaccumulation Factor in the fish ( $ml \cdot g^{-1}$ ),  $k_u$  is the metal net-uptake rate-constant from the aqueous phase ( $ml \cdot g^{-1} \cdot d^{-1}$ ),  $k_e$  is the elimination rate constant following uptake from the dissolved phase ( $d^{-1}$ ),  $AE$  is the metal-assimilation efficiency,  $IR$  is the fish feeding rate (in fraction of body weight  $d^{-1}$ ) and  $k_d$  is the elimination-rate constant following uptake from food ( $d^{-1}$ ). In this experiment we used an  $IR$  value of 0.073/d dry-wt basis (Pickhardt *et al*) The growth rate constant was ignored in the calculation. Assuming that  $Cf$  can be predicted based on the bioconcentration factor of metals ( $BCF$ , under assumption of equilibrium) in the prey and on  $Cw$  ( $Cf = BCF \times Cw$ ).

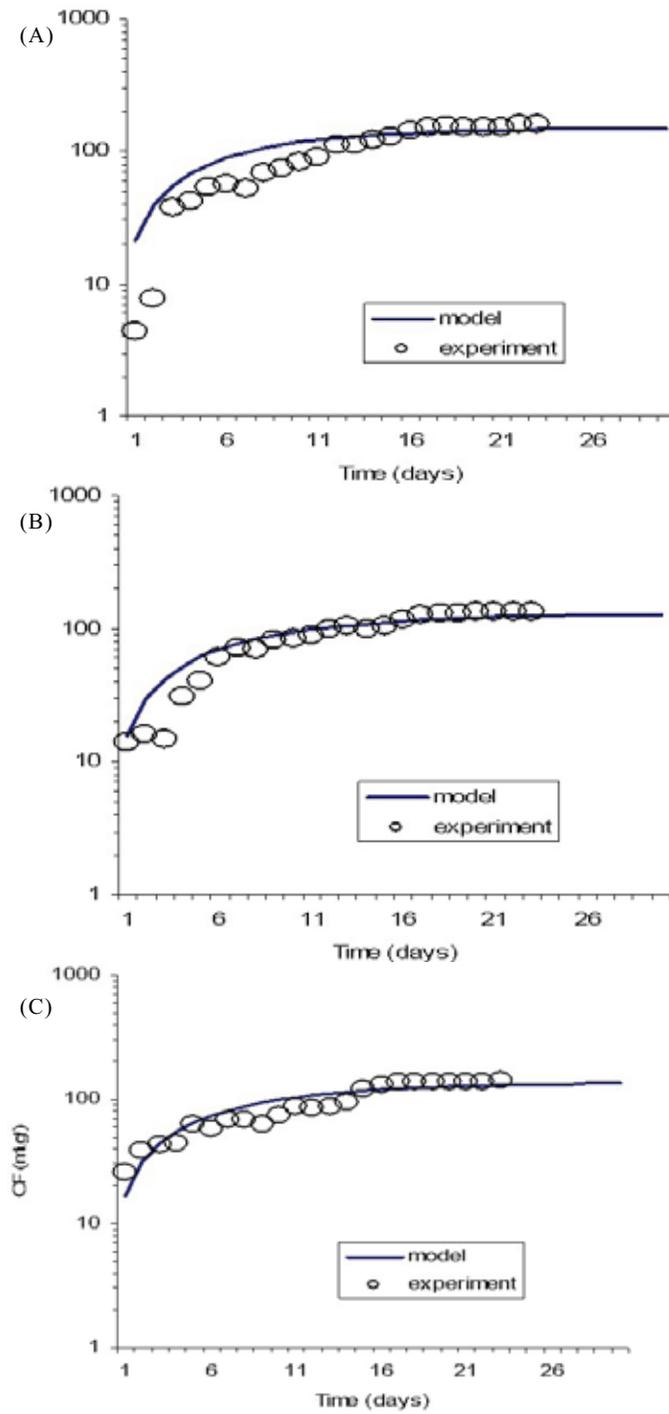
## RESULT AND DISCUSSION

#### Bioaccumulation $Hg^{2+}$ from the Aqueous Phase:

Bioaccumulation studies of Hg in fish tend to minimize the importance of direct aqueous uptake of Hg by fish, because the main pathway for Hg accumulation in food webs is trophic transfer from invertebrates and fish prey to planktivorous and piscivorous predators (Luoma and Rainbow, 2005; Zhao *et al*, 2001). However, subtle differences in uptake rates for Hg to fish, when combined with the temporal variability in dissolved Hg in most water ecosystems, can alter fish Hg burdens (Wang and Wong, 2003). In this experiment, the accumulation of Hg was determined by exposing the organisms to Hg in difference exposure concentrations. The results of the uptake Hg at difference concentration are shown in Figure 1.

Although the experimental contamination via brackish water was only carried out for a short period of time, the activities recorded in the whole *oreochromis mossambicus* suggest that they would efficiently accumulate this element directly from water. *Oreochromis mossambicus* showed a rapid accumulation of Hg during the first 16d followed by a slower accumulation phase. Between 1 and 16d of exposure, the quantified concentration factor ( $CF$ , radioactivity in fish divided by radioactivity in the water) exhibited an approximately linear uptake pattern. There was however a significant difference in the calculated  $CF$  among the 3 experimental concentrations.

By the steady state of the exposure period, the  $CF$  of Hg was 130.66 to 150.58. Because Hg accumulation in tilapia was linear over 0 to 16 d exposure, it was possible to calculate uptake rate of  $Hg^{2+}$  from kinetic measurements. We regressed the calculated  $Hg^{2+}$  Concentration Factor of tilapia againsts time of exposure (0 to 15d) at each  $Hg^{2+}$  concentration. The slope of linear regression represented the uptake rate. At the highest concentration ( $10.90 \mu g \cdot l^{-1}$ ) was 1.14 times lower than that at  $0.421 \mu g \cdot l^{-1}$ . Lacoue- Labarthe *et al* (2009) found that the  $CF$  of Hg by the fish *Sepia officinalis* was about 260 at the end of the uptake 10d. The uptake rate constant was 6.19 to  $8.73 \text{ ml} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$  Metal uptake rates in this experiments decrease with concentration, up to dissolved concentrations. It must be recognized that that metals cross cell membranes in a process that is essentially passive, although endocytosis may occur. It is thought that cell membranes possess aqueous channels that are lined with hydrophobic portions of protein and lipid molecules. The diameter of these channels could impede solute transport due to steric hindrances at the site of entrance. Type B metals such as Hg can form complexes that cross membranes based on their lipid solubility (Carvalho *et al* 1999).



**Fig. 1:** Measured internal concentrations factor (CF) of Hg as function of time for the different exposure concentrations (a:  $0.421 \mu\text{g l}^{-1}$  of Hg ambient water concentration; b:  $2.105 \mu\text{g l}^{-1}$  of Hg ambient water concentration; c:  $10.90 \mu\text{g l}^{-1}$  of Hg ambient water concentration) and the model fits for the accumulation of Hg

Depuration rate from the most important physiological compartments in the fish were usually similar whether food or the dissolved phase was the source exposure. Retention of the Hg following 8 d is shown in Fig. 2. Depuration patterns could be described by 1-compartmental first-order exponential loss patterns.

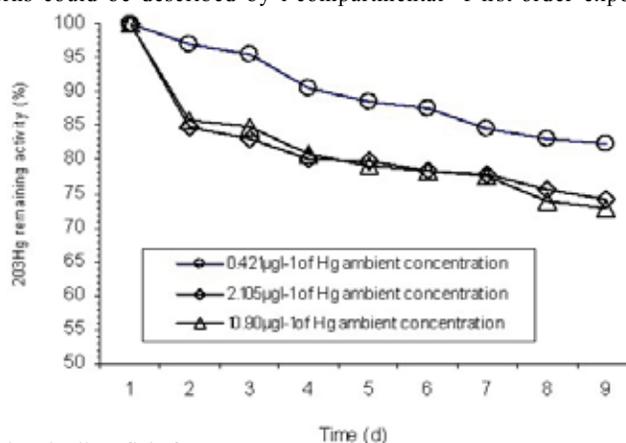


Fig. 2: Mercury depuration in live fish from aqueous exposures

The depuration of Hg from *O. mossambicus* was fairly slow. The Hg efflux rate constant was calculated slope of the linear regression between percent retained in the fish and the time of depuration. The calculated efflux rate constants were display in Tabel 1. Depuration half-life ( $t_{1/2b}$ ) was calculated to be 25.70 to 30.41 d, indicating that it will take a long time to eliminate Hg from the whole body of *Oreochromis mossambicus*.

Table 1: Whole-body uptake and loss kinetic parameters of <sup>203</sup>Hg in whole tilapia following different exposure experiments

Condition	Uptake		Depuration	
	$CF_{ss}$ (ml.g <sup>-1</sup> )	$k_u$ (ml.g <sup>-1</sup> .d <sup>-1</sup> )	$k_e$ (d <sup>-1</sup> )	$T_{1/2}$ (d)
Uptake brackist water	130.66 to 150.58	6.19 to 8.73	-	-
Loss brackist water	-	-	0.022 to 0.027	25.7 to 30.41

Body size also influences contaminant accumulation in fish. Size-dependent accumulation of trace metals has been reported for both freshwater and marine bivalves (Strong, C. R., and Luoma 1991). Smaller individuals generally have faster rates of uptake and accumulate greater contaminant concentrations than larger individuals. Physiological differences in metabolic demand are the primary cause for the differential contaminant accumulation, although size-dependent feeding behavior, surface to volume ratios and concentrations of enzymes that influence uptake may also play a role (Bruner *et al* 1994). Because of the possible influence of body size  $s$  on accumulation of Hg<sup>2+</sup>, the effects of these variables on tilapia concentration factor were examined.

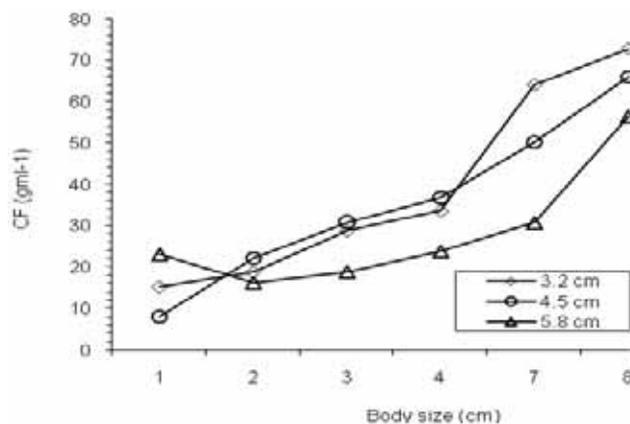
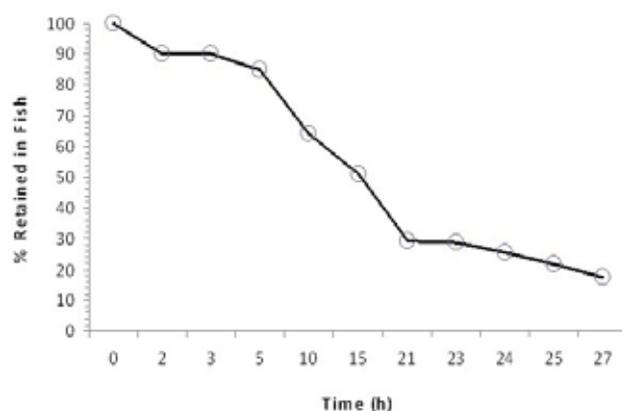


Fig. 3: Measured internal concentrations factor (CF) of Hg as function of time for the different body size in *O Mossambicus* determined after 8 d of exposure and the dissolved Hg

A linear pattern of  $\text{Hg}^{2+}$  uptake over also observed at difference body size. The calculated Concentration Factor in tilapia was increased with a decrease in body size (Fig 3). Comparison of biokinetic parameters between the 3 deference of fish size classes, indicated that bioconcentration of Hg was greater for the smaller fish. Smaller fish (3.2 cm length) had significantly greater uptake for all four Hg than the 4,5 and 5,8 cm sized tilapia. The uptake rate constant was 6.19 to 8.73  $\text{ml.g}^{-1}.\text{d}^{-1}$  At the smallest body size (3,5 cm) was 1.14 times lower than that at 0.421  $\mu\text{g l}^{-1}$ .

#### **Bioaccumulation $\text{Hg}^{2+}$ from the Foods phase:**

It is well established that dietary exposure to metals can result in accumulation of metals in aquatic organisms. After ingestion, some of the dietary metal can be released from the ingested particle into the gastrointestinal fluids of the animal and become available for assimilation into the tissues of the animal and the tissues of its consumer (i.e., trophic transfer). Assimilation efficiency (i.e., the net amount of metal retained in tissues relative to the amount ingested from food) is a common measure of the bioavailability of a chemical from food (Wang and Fisher, 1999). In our experiment, retention of ingested  $\text{Hg}^{2+}$  by the fish following short-term pulse radioactive feeding is shown in Fig. 3.



**Fig. 4:** Mercury depuration in live fish from dietary exposures as a percentage of the initial burden after consumption of labeled artemia prey for  $\text{Hg}^{2+}$

After 1 h of radioactive feeding, the ingested Hg was well retained by *O. mossambicus*, whereas the ingested  $\text{Hg(II)}$  was rapidly released within the first 10 h of depuration. Because there was little further loss of  $\text{Hg}^{2+}$  after 24 h depuration, the assimilation efficiency (*AE*) was calculated as the percentage of metal retained in the fish after 24 h. *AE* value were 25.34% for Hg. In this study, the efflux rate constants for  $\text{Hg}^{2+}$  from radiolabel artemia was 0.09337 $\text{d}^{-1}$ . In another study (Wang and Wong, 2003) the *AE* of  $\text{Hg}^{2+}$  was 20%. On the other hand, the assimilation efficiencies can vary widely depending on the metal, its form and distribution in prey, species digestive physiology (e.g., gut residence time), environmental conditions (e.g., temperature), food quality, food ingestion rate, and metal concentration in the diet.

Applications of one-compartment biokinetic models using laboratory-based measurements of key model parameters (assimilation efficiency, metal uptake rates from water and food elimination rates) have been extended to field situations for populations of a diverse array of aquatic species, including freshwater and marine bivalves, various crustaceans such as copepods, amphipods, and crab, aquatic insects, and fish (e.g., Luoma and Rainbow, 2005). Site specific model predictions for metal concentrations in animal tissues are strikingly close to independent field measurements for diverse water bodies, suggesting that it is possible for risk assessors to account for the major processes governing contaminant concentrations in aquatic animals and that laboratory-derived kinetic parameters are applicable to natural conditions (Luoma and Rainbow, 2005).

In our experiment, modeling analysis of the exposure and trophic transfer factor of Hg in tilapia requires measurements of several of the parameters in Eq. (4), including *AE*, *ku*, *ke*, *kef*, *IR* and *BF* Values of *AE*, *k<sub>w</sub>*, and *k<sub>ef</sub>* have been taken from this study and *BCF* (2000) was taken from literature (Wang and Wong, 2003). Because the *ku*, and *ke* values for *O. mossambicus* were variable depending on environmental conditions (ie. Hg ambient concentration), range of  $\text{Hg}^{2+}$  concentration was used for the model calculation. The growth rate was 0.0207  $\text{d}^{-1}$  (Pathiatne, 1999) and we assume the *IR* was 0.1 to 1%. Figure 5 shows the predicted percentage of  $\text{Hg}^{2+}$  from the dietary phase.

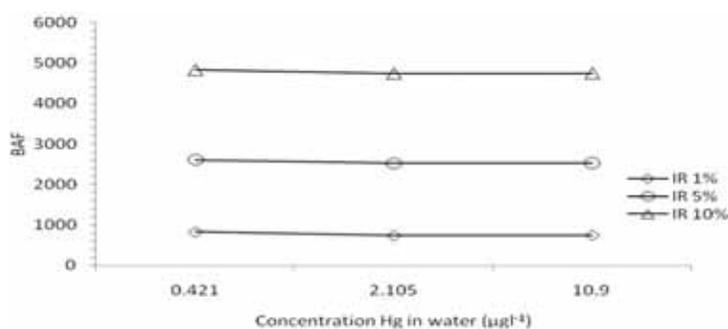


Fig. 5: Predict model of Hg bioaccumulation for *O. mossambicus*

The model predicted Hg Bioaccumulation Factor for *O. mossambicus* that were well within the range observed for concentration Hg in water were 743.31 to 4825.95. The uptake of  $Hg^{2+}$  by *O. mossambicus* was predominantly due to the food exposure (87.7- 93.5%) at intermediate to high BCFs in *artemia* at different IR values.

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