Study of Bioaccumulation Hg by *Oreochromis mossambicus* Using Radiotracer Techniques - 9189

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ABSTRACT

Industrial wastes are considered critical factors for disturbing the natural environment. Composite effluents tainted with different heavy metals are major environmental pollutants of varied wetland ecosystems. Coastal and estuarine areas seem to play a minor role in the global cycle of mercury. However, these areas can exhibit locally high levels of mercury directly resulting from human pollution. To reveal the presence of pollutants over time and to measure their toxic effect, the use of biomonitors or bioindicators can play a prominent role in the monitoring of aquatic ecosystems. *Oreochromis mossambicus* concentrate heavy metals in their tissues as a result of their capabilities to remove dissolved metals from the water column and can be candidate as bioindicator. Uptake and loss kinetics of gamma-emitting radiotracers of ^{203}Hg were determined following exposures to a one order of magnitude-range of environmentally realistic concentrations of Hg, using highly sensitive nuclear detection techniques. Using the simplest model of accumulation and loss, some of these various factors can be demonstrated, e.g., the effect of respiration rate on the uptake process. In this study, we quantified the various physiological parameters characterizing the metal bioaccumulation from dissolved uptake by *Oreochromis mossambicus*.

INTRODUCTION

Mercury cycles in the environment as a result of natural and anthropogenic activities, and can accumulate most efficiently in the aquatic food web. Although the major environmental risks are associated with organic forms of mercury (i.e., methylmercury), environmental releases are almost always in the inorganic form. Inorganic mercury, which is less efficiently absorbed and more readily eliminated from the body than methylmercury. Mercury bioaccumulates in aquatic organisms, i.e. the concentrations in the organisms' tissues increase to a high dynamic equilibrium or even increase throughout the life span.

The traditional concentration factor approach predicts trace element concentrations in animals based on those in the water typically total dissolved concentatrations. This approach may provide general information about how enriched in particular elements organisms are with respect to their environment, but is insensitive to changes in the pathway of accumulation and environmental and physiological conditions. As alternatives to the concentration factor model, models that include parameters for each of the constituent processes of trace element bioaccumulation have been developed [1]

Recently, studies concerning the ecological risks posed by metal pollution in aquatic environments have focused on bioaccumulation modeling. However, few bioaccumulation or biokinetic studies have concerned the influence of varying environmental factors. Therefore, the comparative influences of many of those factors are not fully understood. Quantitative understanding of how the environmental factors affect metal bioaccumulation is critical to accurately assess the dose of metal that animals in the field will

experience. Therefore, it would be a prerequisite for modeling bioaccumulation and also for the use of biomonitors in environmental monitoring programs [2].

The use of radiotracers to obtain such information involves exposing organisms to radiolabelled forms of the contaminant of interest, often trace metals. The amount of tracer incorporated into the organism is followed over time using gamma spectrometry. Bioaccumulation potential is rapidly screened, and assimilation or uptake efficiencies can be calculated. The laboratory studies which have relied on this radiotracer approach have benefitted from major advantages, such as the relative ease and low cost of measurements, as well as the accuracy and precision of such measurements, even when working with low, environmentally realistic contaminant concentrations [3]. These attributes, which are particularly associated with high specific activity gamma-emitting radioisotopes that do not require destructive analysis of samples, make the use of these isotopes for understanding bioaccumulation very attractive, particularly compared to working with non-radioactive chemicals. However, it is also easy to produce data that can be misleading with this experimental approach, particularly relating to determinations of assimilation of ingested contaminants from sediments in benthic animals, and to the applicability of laboratory experiments using relatively short-term assessments of efflux rates to many natural situations.

Concern about heavy-metal contamination of fish has been motivated largely by adverse effects on humans and wildlife, given that consumption of fish is the primary route of heavy-metal exposure. The Mozambique tilapia, *Oreochromis mossambicus* is widely used as food and has been introduced in various localities for aquaculture, and may be (erroneously) called "Java tilapia" in trade. 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 considered filter feeders because they can literally filter plankton out of the water. They are however highly tolerant of brackish water therefore, this fish could be regarded as an indicator species in the context of ecotoxicology.

In this study, we quantified the various physiological parameters characterizing the metal bioaccumulation from dissolved uptake by *Oreochromis mossambicus* in varying salinity conditions.

METHODS

Fish and radioisotopes. Common coastal fish Mozambique tilapia, *Oreochromis mossambicus* (3.0 to 3.5 cm) were taken from a fish farm in Panimbang Banten Indonesia, and were maintained in aerated brackish water and fed commercial food twice a day. All experiments were performed at ambient water temperature and variated salinity. The uptake and elimination by the fish were studied using radiotracers ^{203}Hg(II) ($t_{1/2}$ = 46.9 d) from National Nuclear Energy Agency's radioisotope production facility. Radioactivity of ^{203}Hg was determined using NaI(Tl) detectors connected to a multichannel analyser (Canberra). Gamma emissions of ^{203}Hg were assayed at 279 keV, and counting times of 10 min were used to reduce stress on live fish but still yield propagation errors of 5% or less. Counts were corrected for decay and background radioactivity

Dissolved Uptake of Hg^{2+}. The Hg and radiotracer of ^{203}Hg were spiked in 0.22-µm-filtered pond water and equilibrated for 3-4 h. The Hg^{2+} concentrations was chosen at 0.0025 µg ml^{-1} of Hg. These concentrations were achieved by adding amounts of ^{203}Hg radioisotopes and stable Hg. The low concentration range used in this study represented the environmentally realistic concentration of Hg in natural waters. *Oreochromis mossambicus* were exposed to different salinity for a more than 14 d, and the uptake of radioisotopes was followed nondestructively over time.

Data analyses. Uptake of the radiotracers from water was expressed as change in concentration factors (CF). The concentration factor (CF) of Hg(II) was calculated as the ratio of the radioactivity in the fish ($Bq.g^{-1}$) to the radioactivity in the water ($Bq.ml^{-1}$), 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:

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

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:

$$A_t = A \quad (2)$$

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

$$T_{1/2b} \quad (3)$$

RESULT

In estuarine and coastal environments, various environmental factors including salinity, temperature, and food availability can vary widely. Those external factors were known to influence the metal bioaccumulation in organisms by changing either the bioavailability of dissolved and particulate metals in water or physiological attributes of organisms. However, few bioaccumulation or biokinetic studies have concerned the influence of varying environmental factors. Therefore, the comparative influences of many of those factors are not fully understood. Quantitative understanding of how the environmental factors affect metal bioaccumulation is critical to accurately assess the dose of metal that animals in the field will experience. Therefore, it would be a prerequisite for modeling bioaccumulation and also for the use of biomonitors in environmental monitoring programs[5]. Physiological parameters related to the metal bioaccumulation varied widely among elements and by external factors, such as salinity and temperature, which could vary the metal bioaccumulation in *Oreochromis mossambicus* by altering uptake and the lost kinetics associated with metals as well as through the changing chemical speciation of metals. Using kinetic modeling in this study, we can evaluate how the variation in salinity can influence the mercury bioaccumulation in *Oreochromis mossambicus*

Our previous experimental investigations of Hg bioaccumulation in *Oreochromis mossambicus* we considered exposure concentrations. The concentrations tested in the previous study (0.001 to 0.005 $\mu g.ml^{-1}$ added Hg^{2+}) were selected in order to include the entire concentration range which can be measured in the coastal waters. Results showed that the bioconcentration of Hg in the Mozambique tilapia was directly proportional to the Hg concentration in brakis water. The Concentration Factor ranging from 719 to 1583 ml g^{-1} [6]. In this study we chosen the variaty of salinity because in an estuary usually exhibits a gradual change in salinity throughout its length, as fresh water entering the estuary from tributaries mixes with seawater moving in from the ocean. Environmental conditions vary with the

seasons, and salinity levels in fish farms can reflect those variations. The result of this experiment was shown that *Oreochromis mossambicus* accumulated Hg^{2+} in all salinity treatments (Fig 1).

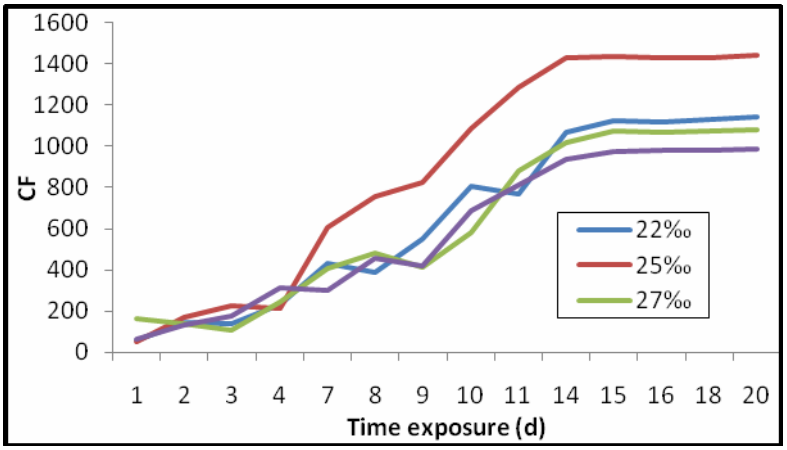

Fig. 1. Uptake ^{203}Hg by *Oreochromis mossambicus* at difference salinity

Uptake of ^{203}Hg in whole body of *Oreochromis mossambicus* displayed linear kinetics at all of the four salinity condition. The steady state or equilibrium was reached within the 15d exposure period. At low salinity (22 and 25$^{o}/_{oo}$) Hg appeared to be accumulated at higher levels than the higher salinity (27 and 30$^{o}/_{oo}$). The calculated CF at steady state condition were 973 to 1439. Uptake rate constants were: 0.107; 0.1604; 0.101 and 0.096 $l.g^{-1}.d^{-1}$ respectively for 22; 25; 27 and 30$^{o}/_{oo}$ of salinity. According to *Lee et al* (2005), salinity can change the dissolved metal speciation, most importantly, the ratio of free ion metals, which are known as the most bioavailable[5]. However, only the free ion model cannot account for the increase in metal uptake at lowered salinity levels. The major ion (*e.g.* Ca^{2+} and Mg^{2+}) concentration in the solution can also influence the heavy metal uptake by changing the permeability of the epithelial structures, competing for binding sites with the apical membrane surfaces, and decreasing metal transfer from the epithelium to the blood with increasing intracellular levels of calcium[5]. On other hand, according to Gungor *et al* (2001), the influence of salinity on the rate of accumulation of radionuclides in marine organisms is highly variable where in previous bioaccumulation study of ^{137}Cs has shown isopods of low salinity regime was increased significantly but the same study also indicated that the bioaccumulation rate in fish species was decreased at low salinity. This explanation may apply for the comparison of the accumulation rates of marine organisms living under different salinity regimes [7]. In our study, we showed that at narrow range of salinity condition at (22 to 30$^{o}/_{oo}$) the uptake rate of Hg by *Oreochromis mossambicus* was not influence . At the end of the exposure time, non-contaminating conditions were restored and loss kinetics of ^{203}Hg were followed in the field for 4d. Loss of kinetics from whole soft parts were best described by a a single-component exponential. The elimination of ^{203}Hg from the fish following 4 d exposure is shown in Fig. 2. There was little difference in the loss of accumulated Hg between salinity condition. Over the 4 d elimination period, only 19,15% to 23,57 % of the accumulated Hg was lost by the fish.

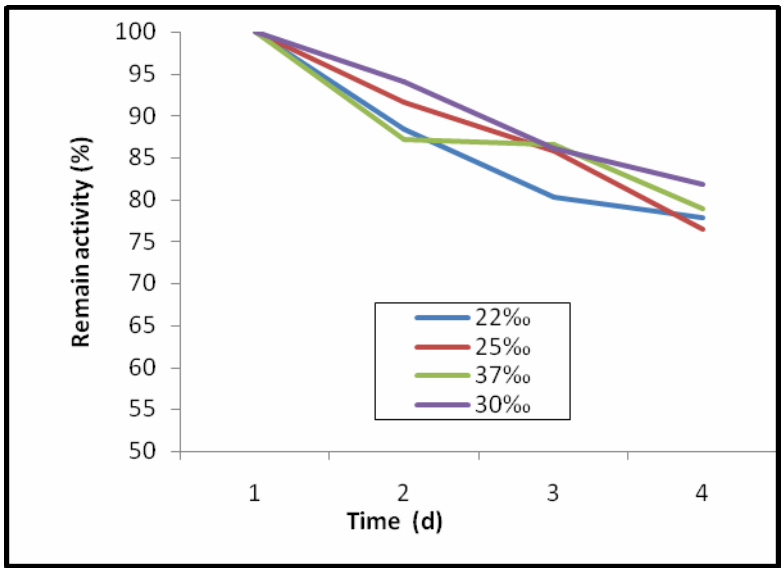

Fig 2. Depuration ^{203}Hg by *Oreochromis mossambicus* at difference salinity

The biokinetic parameters of the depuration constant were estimated to 0.075; 0.076; 0.064 and 0.062 d^{-1}. Whole body loss was relatively slow for 5 days, with mean biological half-lives of 9.3; 9.067; 10.84 and 11.1 d.

To illustrate the relationship between time exposure and Concentration Factor under difference salinity condition we use kinetic modeling. According to Fisher (2003), a mechanistic understanding of the processes involved in the uptake and accumulation of metals by aquatic organisms requires coupling of the different processes in a dynamic manner[3]. To do this, accumulation models can be constructed that link exposure to uptake, compartmentalization and excretion. These models are powerful tools to analyze the separate events and predict the combined result of the processes in a space- and time- resolved manner. Important model input, usually determined from laboratory experiments under well-defined conditions, includes information concerning the concentrations and speciation of the metal present in the exposure media (e.g., water, sediment, food), and data on the kinetics of uptake and elimination of the metal by the organism. Kinetic modeling as result of experiment is shown in Fig 3.

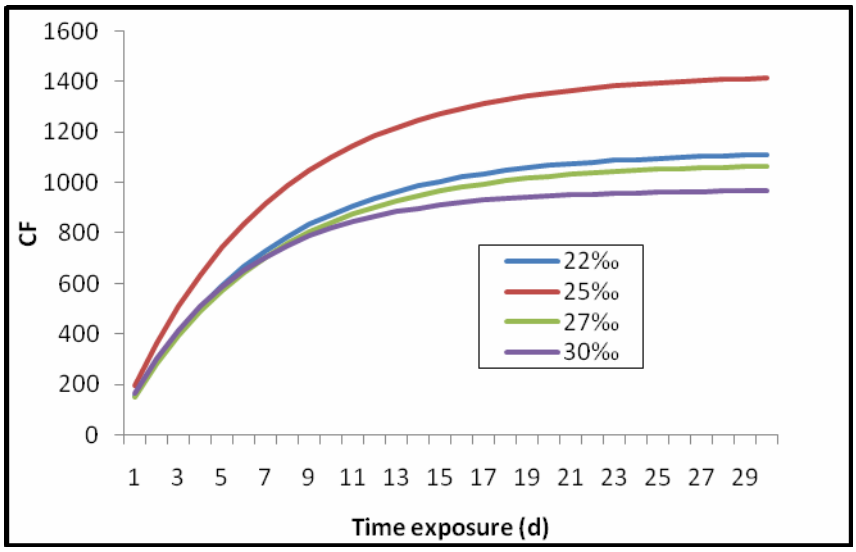

Fig. 3 Modeling between time exposure and Concentration Factor under difference salinity

The equation to exposure time for 22, 25, 27 and 30‰ of salinity respectively were: $CF_t = 1120(1 - e^{-1}$, $CF_t = 1431(1 - e^{-1}$, $CF_t = 1075(1 - e^{-1}$, and $CF_t = 973(1 - e^{-1}$.

In the light of all these observations and model, it was indicated that *Oreochromis mossambicus* can tolerate and bioaccumulate Hg and can serve as effective biomonitors or indicators of water quality at northern of Java costal. Advantages of this fish over most other organisms for biomonitoring studies, are their large size and their limited mobility. They are also abundant in many types of aquatic environments of coastal fish farm and are relatively easy to collect and identify

CONCLUSION

In conclusion, our results show that bioaccumulation concentration factor of Hg by *Oreochromis mossambicus* are 973 to 1439. Uptake rate constants were 0.0913 to 0.1604 $l.g^{-1}.d^{-1}$. The calculated elimination-rate constants were 0.062 to 0.075 d^{-1}.

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