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Accumulation and elimination of mercury in Nile Tilapia (*Oreochromis niloticus*) under an elevated temperature and its ambient concentrations

SUNARDI[1,2,♥], ANNISA JOVIANI ASTARI[1], TRI DEWI KUSUMA PRIBADI[2], KEUKEU KANIAWATI ROSADA[2]

[1]Master Program of Environmental Studies, School of Graduate Studies and the Institute of Ecology (IoE), Universitas Padjadjaran. Jl. Sekeloa Selatan 1, Bandung 40132, West Java, Indonesia. Tel./Fax.: +62-22-2502176, ♥email: sunardi@unpad.ac.id, sunardi.ioe@gmail.com
[2]Department of Biology, Universitas Padjadjaran. Jl. Raya Bandung-Sumedang Km. 21 Jatinangor, Sumedang 45363, West Java, Indonesia

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Abstract. *Sunardi, Astari AJ, Pribadi TDK, Rosada KK. 2017. Accumulation and elimination of mercury in Nile Tilapia (*Oreochromis niloticus*) under an elevated temperature and its ambient concentrations. Nusantara Bioscience 9: 18-22.* The problems of heavy metal pollution combined with the issue of climate warming has attracted a growing international concern, particularly to those exert very toxic effects to organisms and human, e.g. mercury. There have been evidences for temperature effect on metal uptake, accumulation and toxicity; but only few of those on metal elimination. An experimental work was set up to investigate the effect of higher water temperature and ambient concentration on both accumulation and elimination of mercury in Nile Tilapia (*Oreochromis niloticus*). Using 50-L aquarium, fish were exposed to room and 32^{o}C temperature combined with 10 and 20 µg/kg Hg concentration. Test fish were treated for 28 days for accumulation phase, and then transferred to Hg-free water for 7 days for elimination. Data of the Hg accumulation and elimination from the flesh, liver and kidney were analyzed using one-way Anova. The results indicated that higher water temperature and ambient mercury have increased accumulation in the liver and the kidney, but not in the flesh. Higher rate of Hg elimination occurred in higher water temperature resulting Hg deposits did not differ among treatments. However, the Hg deposits remained higher compared to those in the original state representing a potential risk to either fish or human. The kidney and the liver of Nile Tilapia seemed to be the preferable depository organs for mercury.

Keywords: Ambient concentration, mercury contamination, Nile Tilapia, water temperature

INTRODUCTION

Post Minamata incident, there was a great awareness on the effects of mercury pollution on human and environmental health. However, the environmental problems caused by the mercury contamination seems to be a continual bout since mercury-pollution has been occurring for years, and most probably will continue in the upcoming years. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and heavy metals (ECDG 2002). Reasonably, contamination of the aquatic environment by mercury, and heavy metals in general, has been considered a major threat to the aquatic organisms including fishes.

Fish living in polluted waters tend to accumulate heavy metals in their tissues (Jezierska and Witeska 2006), and studies of metal accumulation in fish living in polluted waters show that considerable amounts of various metals may be deposited in fish tissues without causing mortality (Akan et al. 2012). Heavy metal, such mercury, can be incorporated into food chains and absorbed by aquatic organisms to a level that might affects their physiological state.

However, there is almost no single contaminant works independently in the environment; a contaminant together with a variety of biological and environmental factors may jointly pose adverse effects to organisms. It is regarded that global warming will also amplify the already existing toxicity of many contaminants. IPCC (2001) has employed several models and found an increased likelihood of a 1-7^{o}C increase in mean global temperature within the next hundred years. In addition, Ficke et al. (2007) suggested that the general effects of climate change on freshwater systems will likely be increased water temperatures, decreased dissolved oxygen levels, and the increased toxicity of pollutants.

Like most ectoterms, fish cannot regulate their body temperature in accordance with surrounding environment; as a result most physiological and biochemical processes are temperature dependent (Dame 1996; Heugens et al. 2003). Fish physiology is inextricably linked to temperature, thereby their physiology and life histories will be affected by alterations induced by climate warming. Differences in the ambient temperature, for instance, may affect uptake, elimination, and detoxication rates because of changes in metabolic, locomotory, and feeding activity of organisms (Donker et al. 1998; Fisher et al. 1999; Smit and Van Gestel 1997). Increased global warming may result in higher mercury concentrations in fish through increased water temperatures (Evans et al. 2005).

There is a bulk of literature showing evidence for temperature effects on metal uptake, accumulation and toxicity, but unfortunately we lack data on heavy metal elimination from fish body (see Mubiana and Blust, 2007).

Therefore, understanding the possible roles of temperature and other environmental factors on metal accumulation and elimination is critical in order to correctly relate tissue concentrations to those in the surrounding environment.

A number of wide-ranging monitoring studies have been performed in order to estimate the degree of mercury (Hg) contamination in freshwater ecosystems (e.g. Sliggers and Jager 1993; Yamaguchi et al. 2003). A study of the kinetics of contamination and decontamination, at the organism level and at main organ level, gives a better understanding of the overall bioaccumulation mechanisms involved during acute or chronic exposure. Knowledge regarding contamination of different levels of ambient mercury in fish under climate warming is necessary particularly for ecological and human health risks assessment purposes. This research questions how the elevated temperature influences the accumulation and elimination of Hg in Nile Tilapia (*Oreochromis niloticus* L.), a fish species which is cultured worldwide. The hypothesis of this research is that higher ambient temperature and concentration increase Hg accumulation and depuration as well. Research on relevant topics hold a key position in the estimation of the Hg dose consumed by the human population as it is highly dependent on fish consumption (Dusěk et al. 2005).

MATERIALS AND METHODS

Test animals

Nile tilapia, *O. niloticus,* about 100 grams of body weight were purchased from local hatchery. The fish were transported to the laboratory and acclimated for 14 days prior to the experiment. During the acclimation period, the fish were fed regularly with living silkworms obtained from commercial aquarium shop.

Exposure medium

Mercury stock solution of 10 µg/L was prepared by dissolving 10 µg $HgCl_2$ in 1 litre of distilled water, while stock solution of 20 µg/L was prepared by dissolving 20 µg $HgCl_2$ in 1 litre of distilled water. Ground water was used as dilution medium. Our historical record on the ground water showed no detected Hg.

Experiment procedures

The experiment contained two stages: (i) Hg uptake and accumulation, or exposure phase, and (ii) depuration phase. First stage lasted for 28 days, while elimination stage lasted for 7 days. The experiment employed completely randomized design with two factors: (i) ambient Hg concentrations, and (ii) water temperature levels. The ambient Hg concentrations were set at 10 and 20 µg/L, while temperature levels were set at room temperature (23.5-27.5^{o}C) and 32^{o}C. The purpose of setting Hg in such levels is to enable instrument to detect Hg in the flesh more easily, and in some occasions, such levels may occur in the environment.

A number of 50 L aquarium were used to carry out the Hg exposure in which 4 Tilapia were placed (each was sacrificed in day 10, 28, 35 respectively, and one for back up). Each experimental unit was made triplicates. The expected level of water temperature in the aquarium was adjusted using thermostats, while dissolved oxygen levels were maintained using aerators. To reach the expected level of temperature (32^{o}C), the water temperature in the aquarium was increased gradually with the rate of warming of 1^{o}C per hour. The medium was partially changed with fresh medium every other day (except day 28 to 35, the elimination stage) to keep the concentration more or less stable. During the exposure phase, fish were fed with living silkworms one hour before water siphoning time. At the end of the first stage, the fish were transfered into Hg-free medium to run the elimination process.

In day 10, 28, and 35, an individual test fish was taken out from the aquarium and sacrificed. Samples of edible flesh, kidney, and liver of the fish were collected for measurement of Hg concentrations. Hg in fish tissues were determined using AAS Shimadzu type AA-6300 referring to protocols described by APHA (1995) part SMEWW 3500-Hg. As a reference, a group of untreated fish were killed and analyzed its Hg contamination in the flesh, kidney and liver. In addition to that, the water quality parameters of the exposure medium such pH, DO, and conductivity were periodically measured.

Data analysis

To analyze the the effects of ambient Hg levels and temperature regime on Hg accumulation, *one-way* ANOVA was employed. The analysis was carried out using Minitab ver 17.0.

RESULTS AND DISCUSSION

Results

Mercury in flesh

Exposure of Nile Tilapia with higher ambient Hg concentrations and temperature have increased Hg accumulation in the flesh (Figure 1), however, the magnitude of the accumulations were not significant (*one-way Anova, df=3, p=0.275*). The Hg levels in the flesh have increased up to 3.06, 3.92, 5.56, and 6.08 µg/kg, respectively forC1T1, C1T2, C2T1, and C2T2 after 28 d. Interestingly, when all fish transferred into Hg-free water, the temperature variation affected significantly the Hg deposits (*one-way Anova, df=3, p=0.036*). The Hg level in treatment C2T1 remained remarkably higher (*Tukey pairwise comparison, p=0.048*) than those of other treatments showing that the elimination rate has gone slower. The Hg elimination rate seemed depend on the original concentration in the flesh mainly when temperature was low, and hence determined Hg deposits. But nonetheless, Hg deposition all of the treatments remained higher compared to those of the original levels.

Mercury in kidney

In contrast to the flesh, the kidney responded differently to higher ambient Hg concentration and temperature; they accumulated higher Hg under higher ambient concentration

and temperature (*one-way Anova, df=3, p=0,052*) (Figure 2). All combinations of higher Hg and temperature tended to increase accumulation in the kidney; Mecury accumulated up to 206.79, 466.91, 485.56, and 748.81 µg/kg, respectively for C1T1, C1T2, C2T1, and C2T2. However, Hg levels in the kidney differed significantly only in between C1T1 abd C2T2 treatment (*Tukey pairwise comparison, p=0,035*). In the end of depuration, the Hg deposits in the kidney were not significantly different among the treatments (*one-way Anova, df=3, p=0,678*) meaning the higher water temperature has increased the elimination rate of mercury.

Mercury in liver

Similarly to the kidney's responses, the liver have accumulated higher Hg when treated in a higher level of Hg concentration and temperature. Mercury in the liver elevated up to 85.00, 93.15, 116.76, and 357.06 µg/kg, respectively for C1T1, C1T2, C2T1, and C2T2 (Figure 3). Mercury in liver differed only between C1T1 and C2T2 exposure (*Tukey pairwise comparison, p=0,050*). The depuration process has decreased the Hg levels in the kidneys up to 69.68, 50.53, 64.84, and 57.80 µg/kg, respectively for C1T1, C1T2, C2T1, and C2T2. Mercury deposits were not remarkably different after depuration (*Tukey pairwise comparison, p=0,827*) showing that higher temperatures have sped up the elimination process of Hg.

Mercury deposits among organs

In general, the kidney of Tilapia could accumulate highest level of Hg compared to the liver and the flesh; and the liver accumulated higher Hg compared to flesh (*Hg kidney > Hg liver > Hg flesh*) (Figure 4a). When treated in higher Hg concentration and water temperature, the magnitude of accumulations tended to be bigger. Referring to Hg in the flesh, the magnitudes ranged from 21 to 123 times in kidney, and from 23 to 87 times in liver. The relative ability of the three organs accumulating Hg has been consistently shown in final Hg deposits in each organ after depuration (Figure 4.B). The kidney accumulated 17 to 109 times than flesh did, while the liver accumulated 27 to 69 times Hg in the flesh.

Discussion

People in many developing countries are highly dependent on both capture fisheries and aquaculture to fulfill the need of animal protein. Therefore, the health of freshwater ecosystems securing healthy natural aquatic bioresources, as well as, sufficient water quality for aquaculture activities are inevitable. In Asia and the Pacific, mainly the developing countries such Indonesia, Bangladesh, Sri Lanka, Cambodia, etc have been relying on rivers and lakes for fish production (FAO 2012). As a matter of fact, in these countries pollution of the aquatic environment by inorganic chemicals has been considered a major threat to the aquatic organisms including fishes. The agricultural drainage water containing pesticides and fertilizers and effluents of industrial activities and runoffs in addition to sewage effluents supply the water bodies and sediment with huge quantities of inorganic anions and

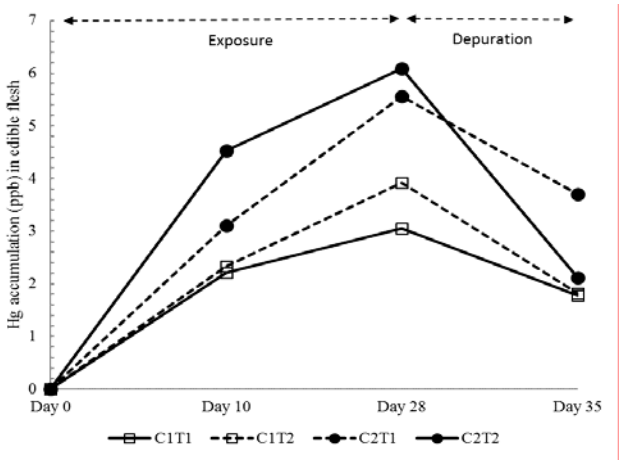

Figure 1. Hg accumulation and elimination in flesh of test Tilapia (Note: C1T1= 10 µg/kg Hg and Room temperature, C1T2= 10 µg/kg Hg and 32°C; C2T1=20 µg/kg Hg and room temperature, C2T2=20 µg/kg Hg and 32°C)

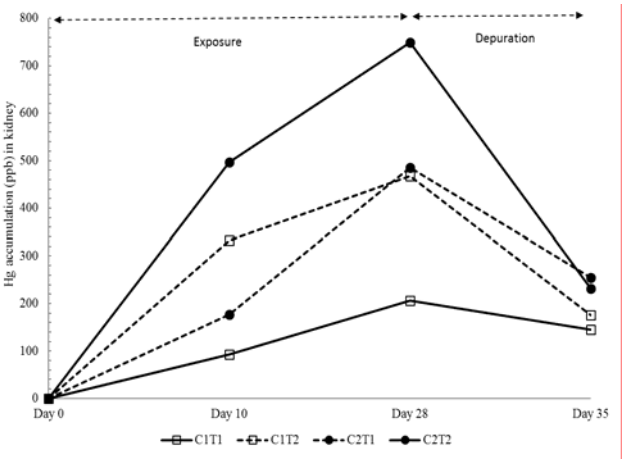

Figure 2. Hg accumulation and elimination in kidney of test Tilapia (Note: C1T1= 10 µg/kg Hg and Room temperature, C1T2= 10 µg/kg Hg and 32°C; C2T1=20 µg/kg Hg and room temperature, C2T2=20 µg/kg Hg and 32°C)

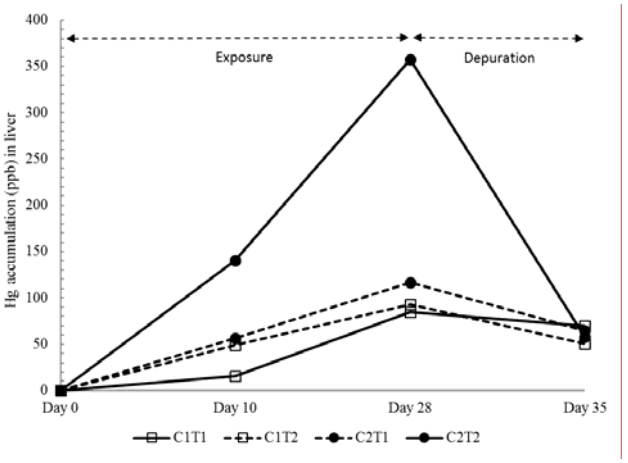

Figure 3. Hg accumulation and elimination in liver of test Tilapia (Note: C1T1= 10 µg/kg Hg and Room temperature, C1T2= 10 µg/kg Hg and 32°C; C2T1=20 µg/kg Hg and room temperature, C2T2=20 µg/kg Hg and 32°C)

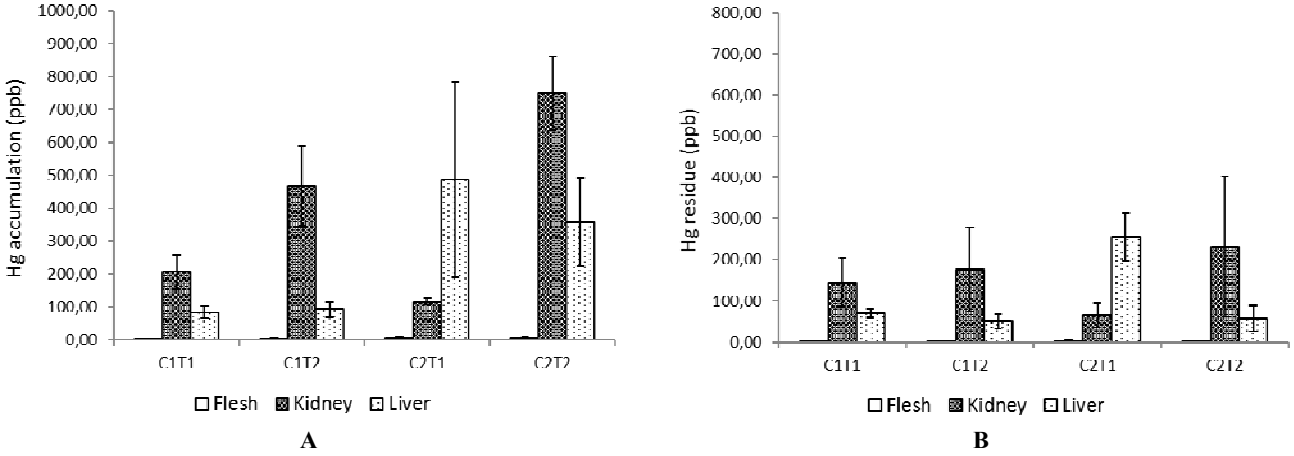

Figure 4. Comparison of Hg deposits among organs of Tilapia. A. After accumulation and, B. After elimination phase

heavy metals (ECDG 2002; Parikesit et al. 2005). Among the toxic elements, mercury is widely distributed in the aquatic environment which is likely to accumulate in fish and represent a potential risk, not only to the fish themselves but also to piscivorous birds and mammals including humans (Adams et al. 1992).

Mercury and other heavy metals can be incorporated into food chain and absorbed by aquatic organisms to a level that might affect their physiological state. Studies on accumulation of Hg in fish living in polluted water show that considerable amounts of Hg may be deposited in fish tissue without causing mortality (Akan et al. 2012). Mercury is accumulated in fish body in different amounts depending on its affinity to various tissues, uptake, deposition and excretion rates. Fish absorb heavy metals from the surrounding environment depending on a variety of factors such as the characteristics of the species under consideration, the exposure period, the concentration of the element, as well as abiotic factors such as temperature, salinity, pH and seasonal changes (Jezierska and Witeska 2006; Ginsberg and Toal 2009; Copat et al. 2013).

Our study revealed that higher ambient concentrations and water temperatures have risen up mercury accumulation in the kidney and the liver of Tilapia, but not in muscle. These studies are consistent with results involving many different organisms, which tend to show a positive correlation between temperature and metal uptake, accumulation or toxicity (See Mubiana and Blust 2007). Heugens et al. (2003) have regarded that temperature is an important factor having a high impact on the rate of most physiological processes mainly on ectotherms, such as fish. Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits) (Dorea et al. (2007).

Unlike accumulation, depuration rate in higher water temperature was remarkably higher leading to levels where mercury residues were not significantly different among treatments. Higher mercury accumulations in higher temperatures were followed by higher rate of detoxication. However, the mercury deposits in all fish organs remained higher compared to their original states; these levels of Hg deposits represent their potential risk to fish itself or human health.

In addition, our study showed that Hg was deposited preferentially in kidney and liver than in flesh; Studies have revealed that liver plays as a depository organ for organic and inorganic forms of Hg (Havelkova et al. 2008; Jezierska and Witeska 2006). Meanwhile, fish flesh, comparing to the other tissues, usually contains the lowest levels of metals (Jezierska and Witeska 2006). The main pathway for inorganic Hg intake into fish is the digestive tract, but other pathways are the skin and gills. Mercury is transported within the organism bound to blood plasma proteins. The liver, as the organ that participates in redistribution, detoxification and transformation of pollutants, is the target for inorganic Hg (Evans et al. 1993; Yamashita et al. 2005; Marsalek et al. 2007).Evan et al. (1993) stated that the liver has the ability to accumulate large quantities of pollutants from the external environment. However, our data indicated that the kidney accumulated higher Hg compared to the liver did. Our data were consistent with the previous study showing that Hg was deposited in higher level in fish kidney, while in liver only in moderate level (Menon and Mahajan 2012).

In context to our global environmental issues, recent study has revealed that climate change will pose negative impacts to freshwater ecosystems (IPCC 2014); the general effects of climate change on freshwater systems will likely increased water temperature (see Sunardi and Wiegleb 2016), decreased dissolved oxygen levels, and increased toxicity of pollutants (Ficke et al. 2007). Evans et al. (2005) have explained that increased air temperature may result in higher mercury concentration in fish through increased water temperatures. Our study indicates that depuration in higher temperature was possible to reduce Hg contamination in any organs; but it will not likely happen in the flesh. It means that, in future, the potential risk of Hg

contamination on freshwater fish and human health will be higher under climate warming.

In conclusion, higher environmental temperature and Hg concentration have promoted accumulation of mercury in fish organs. The Hg depositions differ among fish organs; Kidney and liver seem to be the preferable depository sites for mercury. In higher water temperature, the decontamination rate of Hg becomes higher too leading to a lower level that will not make any differences among temperature variation. However, the fish flesh seems to have a slower of elimination rate in lower temperature making the final Hg deposits stay high. Over all, the final deposits of Hg in higher ambient temperature and Hg concentrations remain higher compared to the original levels representing the potential risks of Hg contaminations.

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