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Oreochromis mossambicus accumulates lead without showing growth inhibition

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ABSTRACT

Oreochromis mossambicus (Tilapia fish) has been found in many high heavy metal content-waters. Tilapia fish is also known to be one of the consumable fish in Indonesia. As many water bodies have been contaminated by Pb due to industrial and household pollution, we investigated the Pb accumulation in the flesh of tilapia fish and its effect in the fish growth at different sub lethal concentrations. Our experiments by exposing tilapia fish for 30 days at different sub lethal concentrations showed that tilapia fish accumulated relatively high concentration at day 30 at 10% LC_{50} but neither at the earlier incubation times nor at other lower concentrations. We also show that tilapia fish did not exhibited any significant growth inhibition at different sub lethal concentrations of $PbCl_2$.

Key words: *Accumulation, Growth, Lead, Oreochromis mossambicus, Pb*

Introduction

Lead (Pb) is a toxic and dangerous heavy metal, unfortunately commonly found as a pollutant in the water body and tends to disrupt the survival of aquatic organisms (Palar, 2002). Pb enters the ecosystem and can be a source of pollutants that affects aquatic biota. This can lead to mortality of fish, particularly in the juvenile phase due to its high toxicity (Darmono, 2006). Accumulation of heavy metals in fish can occur due to contact between heavy metal-containing media and fish and followed by the transfer of the heavy metal from environment to fish by food, gills, and diffusion through the skin surface (Sahetapy, 2011; Ruaeny *et al.,* 2015).

As fish is an important part of human diet, high accumulation of heavy metal in human body via food chains can endanger human health (Nakayama *et al.,* 2010; Sembiring, 2009). Many researchers were conducted to investigate the heavy metal contamination in fish. The content of heavy metals in fish varies in each part. The concentration of heavy metals in fish is high in particular organs such as gonads, bones, and heads. The heavy metal content in the flesh of fish is usually lower than the mentioned organs but since it is an important part that is most consumed part by humans, it has higher impact to heavy metal accumulation in the human body (Usero *et al.,* 2003).

The accumulation of Pb can influence growth rate of aquatic organisms. The longer exposure and the higher Pb concentration will decrease the growth rate. This is due to the enzyme activity inhibition by Pb through the formation of compounds between heavy metals and sulfhydryl (S-H) groups (Sahetapy, 2011). Enzymes that have S-H groups are the group of enzymes that are most easily blocked. The S-H group is easily bound to heavy metal ions that enter the body, resulting the bonds formed between the S-H group and heavy metals and followed by enzyme actvity inhibition or even inactivation (Palar, 2002). These conditions will interfere the metabolic system of the body which later also cause growth inhibition.

Since *Oreochromis mossambicus* or tilapia fish is a bio-indicator for monitoring pollution in fresh water due to its the potential to accumulate heavy metals (Arain *et al.,* 2008) and its broad tolerance to salinity (Suseno *et al.*, 2010), here we investigated the accumulation of Pb in and the effect on its growth.

Materials and Methods

Fish acclimation

We used *O. mossambicus* at the juvenile phase for all following experiments. We chose fish with certain weight between 2 - 2.5 gram. The fish have been acclimatized for 2 weeks prior the following experiments. The fish condition was as followed: the volume of media used was 1 L for 0.8 g of fish, fish have been fed twice per day and the water used to acclimatized has been changed every four days.

Lethal concentration 50 (LC_{50}) determination

The acclimatized fish were used for this experiment. We chose fish with the weight between 2.8 – 3.5 gram and used 20 fish for each concentration of Pb. We added $PbCl_2$ in the media with different final concentration (0 mg/L, 20 mg/L, 40 mg/L, 80 mg/L, 160 mg/L, 320 mg/L, and 640 mg/L). The fish were incubated for 4 days and the number of the living fish was counted every 24 h. The results were then calculated to obtain LC_{50} concentration using probit analysis with SPSS.

Pb accumulation measurement

We used similar fish criteria as in LC50 determination experiment for this experiment. We incubated the fish for 30 days with different concentration of $PbCl_2$. The concentration of $PbCl_2$ we used were 0%, 2.5%, 5% and 10% of LC_{50} obtained from prior experiment. We harvested the flesh from 6 fish every 10 days to measure the Pb content using Atomic Absorption Spectrophotometer (AAS) (Dai *et al.*, 2010). The experiments were done in 3 independent replications.

Growth measurement experiment

We used the same condition and fish criteria as in Pb accumulation measurement experiment. We observed the specific growth rate (SGR) and the daily length increasing every 10 days for 30 days using the following formula.

$$SGR = \{\ln(Wt) - \ln(W1)\} \times \frac{100}{t}$$

We measured the weight of the fish before the experiment (W1) and measured the weight of the fish at the measurement day (Wt; t = day of measurement). Then we calculated the the SGR using the formula as explained in (Schram *et al.,* 2009).

We calculated the daily length increasing using formula as shown below.

$$dL = \frac{Lend - Lstart}{t} \times 10$$

We measured the initial length of the fish before starting the experiments (L_{start}) and the measure the length of the fish every 10 days (L_{end}), with t as day of measurement (Fonds *et al.,* 1992).

Statistical analysis

We analysed the data statistically using unpaired Student's t test to analyse the significance of concentration on the Pb content in tilapia fish and using paired Student's t test to analyse the significance of Pb content in media on SGR and the daily increase of length of fish.

Results and Discussion

LC_{50} determination

We determined the LC_{50} concentration to obtain the right concentration variables where there will be no mortality until the end of the experiment. We put 20 tilapia fish in the media with different concentration of $PbCl_2$. We counted the survived tilapia fish daily until day 4 (Fig. 1). As shown in the Fig.1, at concentration 320 mg/L of $PbCl_2$, there were still 80% of the population were survived. Higher survival rate was observed for lower concentration of $PbCl_2$. At concentration of 640 mg/L, there were no fish survived at day 4. We then analysed the results and obtained that the LC_{50} concentration at day 4 was 426.7 mg/L. We then used this concentration as a basis to perform further experiments.

As shown in the Fig. 1, higher concentration of heavy metal, in this case $PbCl_2$, tends to have higher mortality rate but there is threshold concentration where fish can survive from the Pb exposure. As

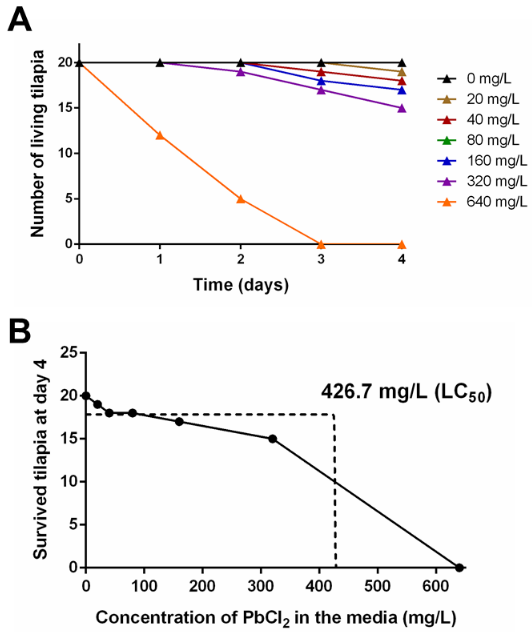

Fig. 1. Number of living tilapias (A) and survived tilapias (B) after exposed to different concentration of Pb during 4 days.

mentioned above that the heavy metal can enter the body of fish through gills, skin surface and by food ingestion. The xenobiotic compounds in the body induce fish to physiologically minimize the toxic effects. Fish has defensive mechanisms against the heavy metal exposure by performing the detoxification and excretion processes, so the toxic effect of the heavy metal can be minimized, until the threshold is reached then later it can cause mortality. The mortality indicates that fish can no longer cope the toxic effects by physiological adaptation. Pb can cause mortality by inhibiting the acetylcholinesterase which leads to the accumulation of acetylcholine in the central nervous system and induces tremors, convulsions, and death (Sahetapy, 2011).

We then performed the Pb bio-accumulation experiment by letting the tilapia fish in the Pb-containing media for 30 days with different $PbCl_2$ concentrations. The concentration variations we calculated from the LC_{50} at 4 days from previous experiment. We took samples every 10 days starting from day 0. We harvested the flesh of the fish and analysed the Pb content using AAS. As shown in Fig. 2, there were no significant Pb-content detected in the fish for all concentrations except 10% LC_{50} until day 30, which showed more than 30 mg/kg.

We did not observe the significant Pb accumulation until day 30 for concentration 2.5% and 5% LC_{50} was probably due to the detoxification and excretion process performed by fish can still cope the accumulated Pb. Whereas at day 30 for concentration 10% $LC_{50,}$ the Pb content in the flesh was detected in relatively high concentration, which probably due to the capability of the tilapia fish to do detoxification process of accumulated Pb could no longer cope with the accumulated Pb in the body. This later then caused more and more Pb accumulated in the body.

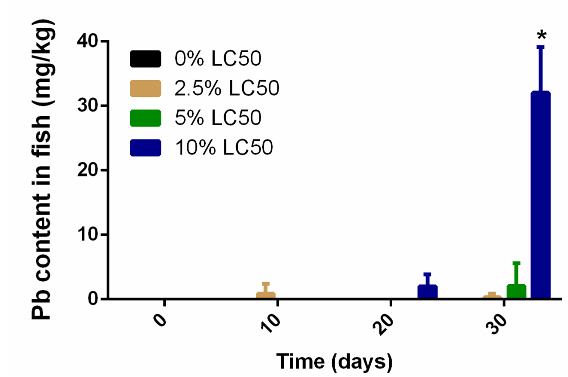

Fig. 2. Pb content in fish after exposed to different concentration of Pb at different exposure time.

The Pb exposure to the fish caused Pb accumulation in the body. The Pb accumulation in the body is due to the tendency of heavy metal to form a complex compound with the organic compounds in the body. The heavy metal accumulation in the particular part of the body is due to the presence of metallothionein groups, which is the sulfhydryl group, and the amine group that can bind Pb covalently. The heavy metals that enter the fish body will be distributed through the blood system to the whole tissues in the body, including the muscle tissues (Harteman, 2011; Usero *et al.*, 2003).

We then measured the weight and the length of the fish every 10 days to calculate the specific growth rate (SGR) and the daily length increase. These parameters represent the growth of the fish. We would like to investigate whether $PbCl_2$ exposure can affect the growth of the fish. Growth can be interfered by the exposure of the heavy metals. The interference is due to the disturbance of the metabolism activities which are regulated by enzymatic activities. The exposure or presence of Pb in the body can bind to the sulfhydryl groups of the enzymes and inhibits their activity or even inactivate them (Landis *et al.*, 2017).

Our results exhibited that the exposure of $PbCl_2$ showed no significant effect both on SGR and daily length increase. Even at the highest concentration of $PbCl_2$ (10% LC_{50}), the growth of tilapia fish was not significantly affected (Fig 3). This is consistent with the Pb content data in the fish which we did not detect any significant amount of the $PbCl_2$ in the flesh sample of the fish, except the highest concentration at day 30. This might show that tilapia fish is, if we may say, highly tolerance with the Pb exposure. Tilapia fish detoxification and excretion process to discard any Pb in the body might be quite efficient, so the Pb that enters the body can be excreted back to the environment, until certain concentration. The efficient excretion process and heavy metal tolerance of tilapia fish make the growth of the fish stay unaffected until certain concentration.

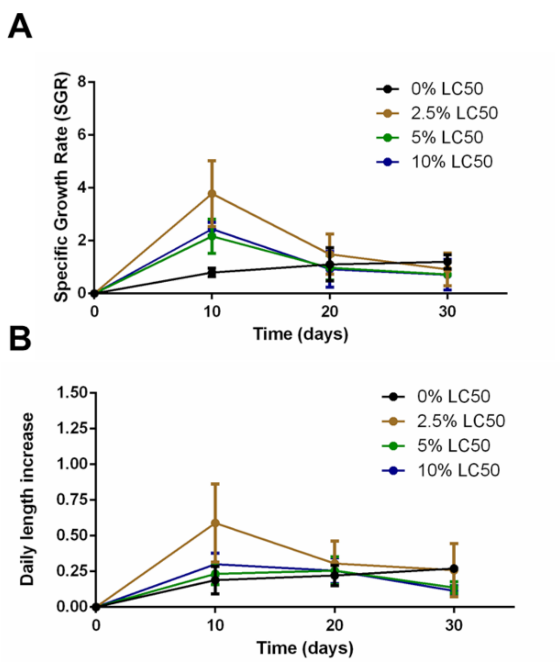

Fig. 3. Specific growth rate (A) and daily length increase (B) of tilapia after exposed to different concentration of Pb at different exposure time.

Conclusion

O. mossambicus showed low Pb accumulation from 0 – 20 days but at day 30 at concentration 10% LC_{50}, we detected relatively high Pb content in the flesh. *O. mossambicus* also showed no growth inhibition until day 30 even at the highest Pb concentration in media used in the experiments.

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