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Effects of Temperature and Different Feed Uptake on Mercury (Hg) and Cadmium (Cd) Accumulation in Nile Tilapia

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ABSTRACT

Climate warming due to increases in atmospheric temperature and heavy metal contamination constitutes a major issue for the aquatic ecosystem. Heavy metal toxicity hinges on an organism's tolerable limit of exposure to these elements, and in the aquatic environment, higher temperatures enhances this toxicity in fish. The purpose of this study was to investigate the combined effects of higher temperature and different feed uptake on Hg and Cd accumulation in Nile tilapia (*Oreochromis niloticus*). Fish were exposed to a single concentration of 10 µg/l mercury (Hg) or 100 µg/l cadmium (Cd) for 30 days under 24 °C (room temperature) and 32 °C (elevated temperature) conditions in a 7-L aquaria. The feed used in this experiment comprised pellets, water and pellets, and water and silkworms, in combination with temperature and heavy metal exposure. Results demonstrated Hg and Cd accumulation in flesh, kidney and liver increased with increasing temperature. The influence of different uptake mediahas an effect on Hg and Cd concentration levels in fish organs. The order of Hg and Cd accumulation in organs, from the highest level to the lowest, was kidney, liver and flesh.

***Key words**: Mercury, Cadmium, Feed uptake, Accumulation, Nile tilapia*

Introduction

Rising temperatures in the environment, as a consequence of global warming, may contribute to the increased susceptibility of aquatic ecosystems and living organisms to various deleterious conditions (Dietrich *et al.*, 2014; Sunardi and Wiegleb, 2016; Kumar *et al.*, 2018). Aquatic organisms demonstrate a sensitive response to shifting temperatures that can hamper their physiological functions, such as thermal tolerance, growth, metabolism, food consumption, reproductive success and the ability to maintain internal homeostasis (Bake *et al.*, 2009; Carroll and Warwick, 2017). The normal metabolic activities of aquatic organisms depend on a certain range of ambient temperatures in the environment, and increasing temperatures that exceed these limits will result in physiological disturbances

(Lapointe *et al.,* 2011).

Not only are aquatic ecosystems negatively impacted by rising temperatures, they often suffer from a high level of contamination from heavy metals. Toxic heavy metals, such as mercury (Hg) and cadmium (Cd), are harmful to the environment; of particular concern is the wide distribution of Hg and Cdvia natural sources, industrial effluents, anthropogenic activities and, on occasion, domestic sewage. Their presence in the aquatic environment often poses adverse effects on aquatic organisms, including fish (Perera *et al.,* 2016).

Fish represent a type of aquatic organism that absorbs heavy metals from its environment, and because of this capability, they are commonly used as biomonitoring agentsfor aquatic environmental pollution. At present, Nile tilapia (*Oreochromis niloticus*) is one of the most commonly cultivated freshwater fish due to high consumer demand (Ayyat *et al.,* 2020).

Numerous studies report that metal toxicity in fish is often increased with increasing temperature, and that it is linked to higher metal accumulation. For example, increasing temperature was found to increase cadmiumtoxicity in yellow perch (Baykan *et al.,* 2007; Grasset *et al.,* 2016), lead toxicity in*Pangasius hypophthalmus* (Kumar *et al.,* 2018), copper susceptibility in fathead minnow (Lapointe *et al.,* 2011), and mercury toxicity in Nile tilapia (Sunardi *et al.,* 2017).

In metal-contaminated environments, fish can accumulate metals through water, sediment and feeds.In particular, heavy metals are considered a high contaminant of fish feeds (Adamse *et al.,* 2017). It is a widely usedmethod in fish farming practice to feed fish different types of food, such as worms (as natural prey) and artificial pellets.However, some studies have found Hg and Cd in commercial fish pellets (Choi and Cech, 1998; Dang and Wang, 2009). Because feed uptake significantly affectsthe accumulation of heavy metals in fish, it is recognised as a predominant route for metal bioaccumulation in fish (Wang, 2002). For example, an assessment of the metal contamination in commercial fish diets and natural prey yielded different accumulated concentration levels of Hg, Cd, C, Zn and Fe in farmed fish (Wang *et al.,* 2012).

Therefore, fish are at potential risk for exposure to dietary metals and could consequently pose a hazard to consumers. For vulnerable populations – such as the elderly, children and pregnant women – limitations on daily metal uptake should be more stringent than for the general population in order to prevent them from experiencing metal-induced adverse effects (Olmedo *et al.,* 2013; Ju *et al.,* 2017).

While studies on temperature effects on heavy metal accumulation in fish are available (Kuz'Mina *et al.,* 2002; Vergauwen *et al.,* 2013), research pertaining to the role of feed media exposure, or its combination with temperature, on such accumulationis still limited. Therefore, this study aimed to investigate Hg and Cd accumulation with increasing temperature and in combination with different uptake routes in Nile tilapia.

Materials and Methods

Nile tilapia of ± 100g were purchased from a local hatchery and brought to the Laboratory of Toxicology, Institute of Ecology, Universitas Padjadjaran. During the acclimation period (about 2 weeks), Nile tilapia wereaerated and kept in a 100-L aquarium at room temperature. Fish were fed with living silkworms every other day during the acclimation period prior to the experiment.

In the experiment, fish were exposed to Hg-or Cd-contaminated water, and different types of feed. Hg and Cd concentrations used in this study were 10 µg/l and 100 µ/l, respectively. Hgwas added as a 0.7-ml stock solution of mercury chloride ($HgCl_2$) into an aquarium containing 7L of water, while Cd was added as 7 ml of cadmium chloride ($CdCl_2$), dissolved in the same volume of water.

The feeds used in this experiment were living worms collected from a heavy metal-contaminated area as the natural feed, and pellets as the artificial feed, which is widely used in aquaculture. Heavy metal content of the artificial feed was measured first in order to be used as test feed. Hg content in pellets was 2.996 mg/kg dw, and Cd content in pellets was 0.04 mg/kg dw.

The experiment was carried out through an exposure process of 30 days with 7L of aerated water, with a single fish in each aquarium. The experiment consisted of different treatments containing different heavy metal levels in the following media: (i) pellets (M1), (ii) water and pellets (M2) and (iii) water and silkworms (M3). One group of experiments was subjected to 24 °C room temperature (T1), while another was maintained in a water temperature of 32 °C (T2), maintained using a thermostat. Each experimental unit was designed with 5 replications. A third group of fish was maintained in metal-free water at 24 °C to serve as the control. Therefore, the final combinations of the treatment were: (a) pellets with a temperature of 24 °C (M1T1), (b) pellets with a temperature of 32 °C (M1T2), (c) pellets and contaminated water with a temperature of 24°C (M2T1), (d) pellets and contaminated water with a temperature of 32°C (M2T2), (e) silkworm and contaminated water with a temperature of 24°C (M3T1), and (f) silkworm and contaminated water with a temperature of 32°C (M3T2). The medium was renewed with fresh metal solution every other day to maintainmetal concentration. The fish were fed ±2 g twice a day (i.e. morning and evening). During the experiment, water quality was also measured periodically, including DO, pH and salinity.

Hg and Cd concentration in fish organs were measured on days 0 and 30. Sample preparation and analysis were carried out according to FAO methods (Pauly, 1983). Fish were dissected to collect flesh, kidney and liver. Prior to analysis, Nile tilapia were thawed at room temperature and washed with deionized water. Each organ was dried at 105 °C until they reached a constant weight. The dried samples were homogenised into a ne powder using a ceramic mortar and pestle. One gram of each of the ground sht issues were transferred to a porcelain basin and placed in a muffle furnace at a temperature of 550°C for 5 h. Samples were digested with 10 ml tri-acid mixture (HNO_3:$HClO_4$:H_2SO_4) in a ratio of 6.5:6:2. The samples were heated at 105 °C until a clear, colourless solution was obtained. The digested samples were allowed to cool, and were then diluted to 100 ml with deionized water and ltered through Whatman filter paper No. 42. The filtrate was diluted to 100 ml with deionized water for determination of heavy metals concentration by atomic absorption spectrophotometer (AAS). Heavy metal concentrations were expressed as mg/kg dry weight.

Data on Hg and Cd concentrations in the different fish organs were then analysed using two-way ANOVA statistical tests with R software. When the results showed significant differences, they were followed by Duncan's new multiple range test (MRT) with a 95% confidence level. These statistical tests were conducted to determine whether the individual treatments or a combination thereof would affect the accumulation of heavy metals in the studied fish organs.

Results

Exposure to Hg at higher temperatures tended to increase Hg accumulation in flesh across all treatments. However, the effect of the elevated temperature was not significant (df=1, p=0.126). Similarly, feed uptake clearly did not increase Hg accumulation in flesh (df =2, p=0.241). The highest Hg accumulation was 3.84 mg/kg in the water and silkworm treatment, while the lowest was 1.52 mg/kg in the pellet treatment. Levels of Hg accumulationin the flesh across all treatment groups, in decreasing order, are: M3T2>M1T2>M3T1>M2T2>M2T1>M1T1.

Likewise, Cd accumulation in the flesh increased at all higher temperature treatments. Based on the type of feed, however, there was no specific pattern with regard to its effect on Cd accumulation in flesh. The highest Cd accumulation was 0.35 mg/kg in the

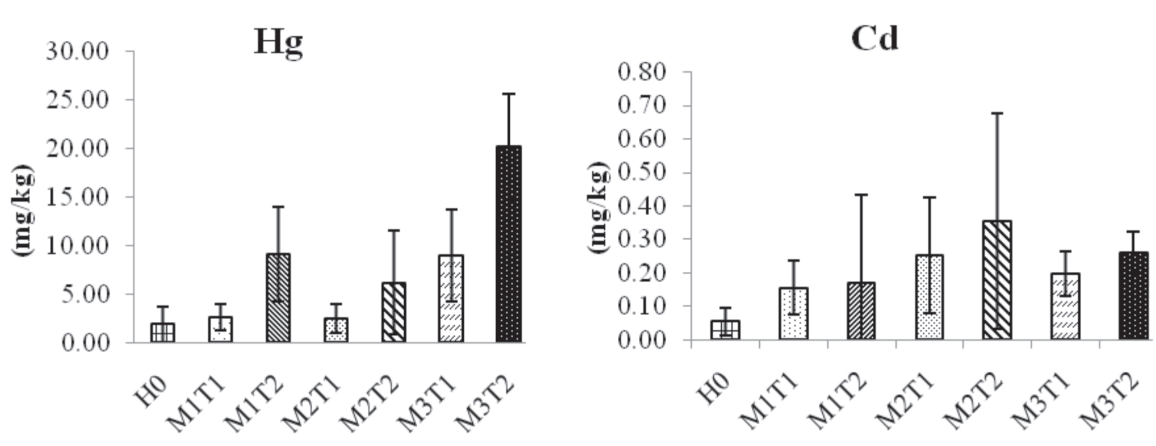

Fig. 1 Hg and Cd accumulation in flesh of Nile tilapia

water and pellet treatment, and the lowest was 0.15 mg/kg in the pellet-only treatment. The order of Cd levels in flesh could thus be presented as follows: M2T2>M3T2>M2T1>M3T1>M1T2>M1T1. Similar to the result of Hg accumulation, there was no significant effect of temperature (df=1, p=0.294), feed type (df=2, p=0.274) and their interactions (df=2, p=0.814) on the accumulation of Cd in flesh.

Remarkably, there was a higher level of Hg accumulation in the liver at 32 °C compared to that at 24 °C in all treatments. The increase in Hg accumulation was significant in each of the units as an effect of higher temperature (df=1, p=0.000). Referring to the effects of feed types, the combination of contaminated water and silkworms led to the greatest amount of Hg accumulation, followed by those treated with contaminated water and pellets, then by pellets only. The highest Hg level in the liver was 20.17 mg/kg in the water and silkworm treatment (M3T2), while the lowest was 2.51 mg/kg in the water and pellet (M2T1) treatment. The order of Hg accumulation in the liver was as follows: M3T2>M1T2>M3T1>M2T2>M1T1>M2T1. The effect of feed types on Hg accumulationin the liver was significant (df=2, p=0.000); in contrast, the effect of interaction between temperature and feed type was not significant (df= 2, p=0.182).

Similarly, higher temperature significantly increased Cd accumulation in the liver (df=1, p= 0.012) in all treatments. The effects of feed type on Cd accumulation was the same as in the Hg treatments; the highest level of Cd accumulation in the liver occurred in the water and silkworm treatment, followed by the water and pellet treatment and then the pellet-only treatment. However, the difference in Cd accumulation in the liver caused by the type of feed was not significant (df=2, p=0.061). The order of Cd accumulation, in decreasing order, was as follows: M3T2>M2T2>M1T2>M3T1>M2T1>M1T1. Notably, the interaction of the type of feed with temperature had no significant effect on Cd accumulation in the liver (df=2, p=0.198).

In general, Hg accumulations in the kidney in the higher temperature treatments are higher than those in the lower temperature treatments. The highest Hg concentration was 13.85 mg/kg in the water and pellet treatment (M3T1), while the lowest was 3.29 mg/kg in M1T1. The order of Hg concentration, from highest to lowest,was as follows: M3T1>M3T2> M2T2>M2T1>M1T2>M1T1. There was a significant effect of feed type on Hg accumulation (df=1, p=0.003), which differed significantly, while temperature and its interaction with feed type had no significant effecton Hg level in the kidney (df= 2, p=0.567).

A higher temperature showed significantly increased Cd level in the kidney (df=1, p=0.000). Similarly, the type of feed had also clearly increased Cd accumulation in the kidney (df=2, p=0.001), as indicated in the interaction of feed type and temperature (df=2, p=0.029). The highest Cd content was 150.63 mg/kg in M2T2, and the lowest was 10.54 mg/kg in M1T1. In decreasing order, the Cd levels in the kidney were as follows: M2T2>M3T2> M2T1>M3T1> M1T2>M1T1.

Discussion

Increased water temperatures affect the toxicity and distribution of chemical compounds in the water,

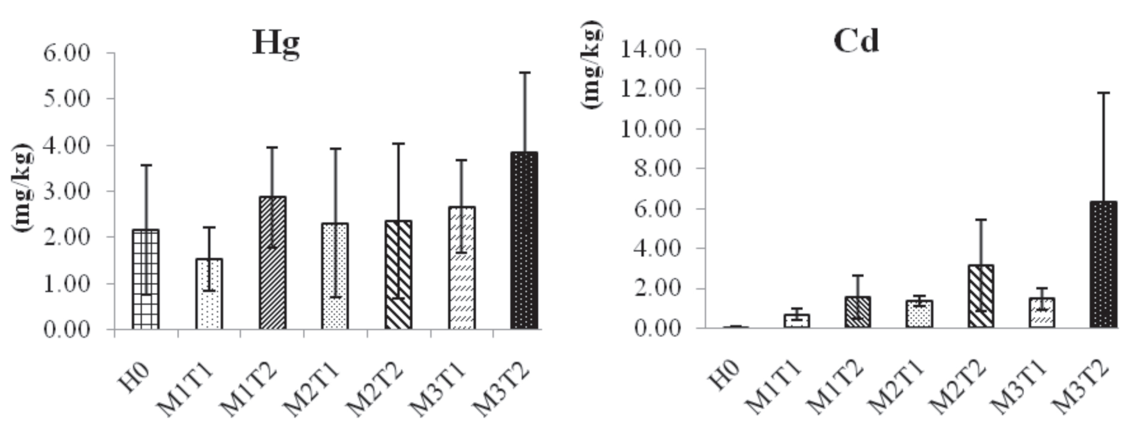

Fig. 2. Hg and Cd accumulation in liver of Nile tilapia

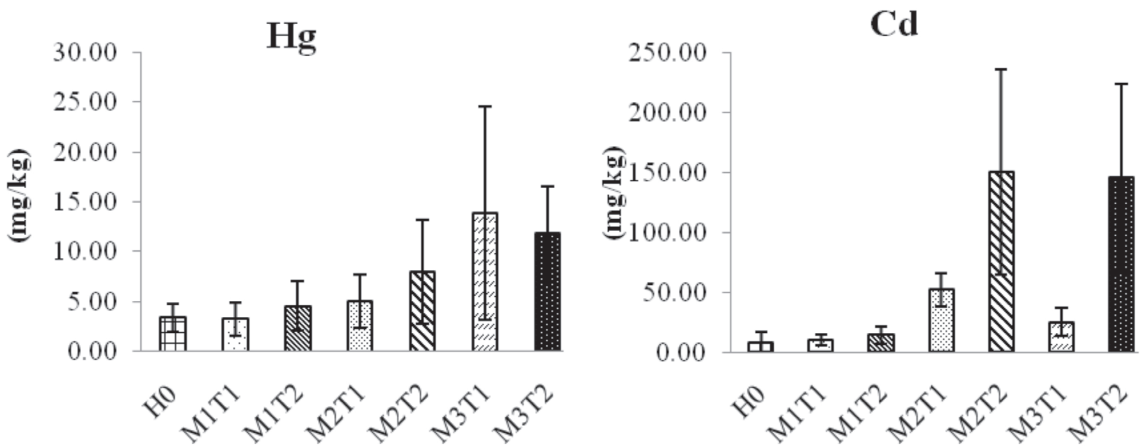

Fig. 3. Hg and Cd accumulation in kidney of Nile tilapia

subsequently impactingthe lives of aquatic organisms in the short, medium and long term (Manciocco *et al.,* 2014). Aquatic organisms become vulnerable to the threat of water temperature increases, causing changes in their metabolism and physiology. In the natural ecosystem, elevated temperature is not a single, standalone stressor, but one which may occur simultaneously with heavy metals and contaminated media.

The results of this study indicated that the accumulation of Hg and Cd in the flesh, liver and kidney of Nile tilapia increased with increasing temperature. The effect of elevated temperature combined with Hg and Cd exposure might have increased fish respiration rate and the uptake of water across the gills (Sunardi *et al.,* 2017). In the same way, high temperature results in a decrease of dissolved oxygen, a situation that fish might be able to tolerate (Kumar *et al.,* 2018). Furthermore, the presence of heavy metals may cause physiological stress in Nile tilapia and disturb their ability to maintain homeostasis. In optimal water temperatures, fish can successfully maintain internal metabolism. Qiang *et al.* (2012) found 29.9 °C to be favourable for fish growth and feed utilisation; however, according to Abdel-Tawwab and Wafeek (2014), 28 °C is better for the growth performance of Nile tilapia.

In addition, the higher accumulation of Hg and Cd in fish may occur with the consumption of contaminated food. Elevated temperature affected fish consumption as a consequence of rapid metabolism. Heavy metal compounds that aretaken up with the food will be ingested into the digestive system and absorbed in the intestine (Kondera *et al.,* 2014). The process of transporting heavy metals into the fish body occurs when the heavy metals are transferred to the digestive tract and to organs such as the liver and kidneys via the circulatory system or enterohepatic circulation (Al-asgah, 2015).

In this examination, pellets and silkworms were used as the contaminated feed for studying Hg and Cd uptake. Water and silkworms became the most influential feed uptake contributing to Hg accumulation in the flesh, kidney and liver. In terms of Hg accumulation, feed uptake had a significant effect on increased Hg levels in the kidney and liver. Meanwhile, the water and pellet combination was the main contributor of Cd accumulation in the flesh and kidney, while it was the water and silkworm combination in the liver.

Differences in the types of feed presented differences in palatability. According to Kolkovski *et al.* (2000), feed palatability is influenced by several physical and chemical factors, such as feed conditions that include shape, size, movement and taste, as well as stimulation given by the sense of smell (olfactory). Natural feed such as *Tubifex* sp. has some advantages; among others, its movement is relatively slow and thereby provides stimulation for fish to eat, its size is in accordance with the mouth of fish, it has a high protein content and it is highly palatable and easily digested (Kicklighter *et al.,* 2003).

The kidney and the liver are the organs that accumulate the highest levels of Hg and Cd. These organs are active in the metabolism of fish with regard to excretion and detoxification. In the blood stream, Hg and Cd are bound to complex protein forms and are then transported first to the liver. In the liver, heavy metals are divided into two parts, some of which will accumulate in the liver, while others will be transferred to bile and converted into inorganic Hg and Cd compounds and then re-detoxified (Evans *et al.,* 1993; Seco *et al.,* 2020). The compound will then be delivered through the blood to the kidney, some of which will accumulate in the kidney, and somewill be removed with urine (Ghedira *et al.,* 2010; Squadrone *et al.,* 2013). Whereas in the flesh, our results showed that the rate of heavy metals accumulation is lower. Although flesh is the organ that accumulates Hg and Cd to a lesser degree, it illustrates the dangers of biomagnification in humans, a process that causes adverse health effects in people who consume large quantities of fish (Jovanovic *et al.,* 2017).

This study demonstrated that higher temperatures increased Hg and Cd accumulation in fish organs. The condition could be worse if the feed of fish is also contaminated with heavy metals. Our research revealed that different feed uptake would contribute to Hg and Cd accumulation levels in fish organs. More specifically, the kidney and liver became the organs most affected by Hg and Cd exposure, as they accumulate higher levels of the metals due to their vital role in metabolism and feed digestion.

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