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Original Article

Heavy metals accumulation in gills and muscles of Mozambique Tilapia (*Oreochromis mossambicus*) exposed to crude leachate

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Abstract

Objective of this study was to measure heavy metals accumulation in Mozambique Tilapia (*Oreochromis mossambicus*) exposed to crude leachate. Tilapia was exposed to leachate from municipal landfills at the volume of 2% to 20% v/v for 96 hours. Heavy metals were measured using inductive coupled plasma optical emission spectrometry (ICP-OES) Optima 8300. All heavy metals were higher in the gills compared to the muscles and the concentration was higher in old leachate. The highest element detected in fish gills was Cu (22.72 ± 0.60 mg/kg) followed by Ni (1.66 ± 0.021 mg/kg) and Pb (0.50 ± 0.011 mg/kg). Ni also was highly detected in fish muscle with the mean of 1.32 ± 0.024 mg/kg. Cd was commonly detected in muscle and gills of young and old leachate (0.14 ± 0.01 mg/kg). It was concluded that heavy metals detected pose a risk of metals exposure through Tilapia consumption.

Keywords: Heavy metals, Crude leachate, Tilapia, Bio-accumulation, Municipal waste

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Introduction

Landfill leachate contains various contaminants from the products that being used at home such as metals, ammonia, organic compounds and other toxicants that pollute the environment (Mor et al., 2006; Olivero-Verbel et al., 2008; Pivato and Gaspari, 2006; Slack et al., 2005). The toxic and genotoxic organic and inorganic compounds in landfill leachate can cause toxicity in aquatic life and effect human health (Bortolotto et al., 2005; Fauziah et al., 2013; Sivaperumal et al., 2007).

Aquatic living organisms such as fish, shrimps, snails and aquatic plants can accumulate heavy metals (Saei-dehkordi and Falah, 2011). Fish is an important food source in many natural food chains and a major protein consumed by human (Taweel et al., 2013). It can accumulate organic and inorganic pollutants from water (Eneji et al., 2011; Ismaniza and Idaliza, 2012). Heavy metals in fish were taken up through ion-exchange of dissolved metals across lipophilic membranes and absorption on tissues and membrane surface to the gills, muscles and liver (Sivaperumal et al., 2007).

Mozambique Tilapia (*Oreochromis mossambicus*) is the edible fish with high local demand in Malaysia. It is a species that can adapt to various environmental condition, high resistant to disease and vulnerable to leachate toxicity (Emenike et al., 2011; Umi Raihana et al., 2014). Uncontrolled leachate production and accidental discharge of untreated landfill leachate from the nearest outlet point of waste landfill to the surface water may cause toxicity to tilapia. Consume fish that highly exposed to heavy metals lead to human threatening disorders and may cause fatality if its effect the kidney and liver (Saei-dehkordi and Falah, 2011). For example, Cu can cause toxicity and atherosclerosis to human (Saei-dehkordi and Falah, 2011; Jomova and Valko, 2011). Cd deposited in tissues, kidney, liver, pancreas and human lungs can cause several cancers such as prostate, lung, and gastro-intestinal cancers (Jomova and Valko, 2011). Pb poses serious threat especially to children cognitive skills. Ni is carcinogenic and may cause allergies to high sensitive people.

Owing to this fact, the toxicity effects and metals accumulation in Tilapia from the leachate exposure was explored in this study. Tilapia was exposed to several volume of crude leachate for 96 hours and the level of heavy metals in the gills and muscles was detected. This study provides baseline information of the metals accumulation in the fish that can produce health effects.

Material and Methods

Leachate sampling site

Crude leachate was collected at the inlet point of the leachate pond. Samples were grabbed from a young (5 years operation) and old municipal landfill (17 years operation) located at the central region of Peninsular Malaysia (Selangor).

Fish preparation

Mozambique Tilapia (*Oreochromis Mossambicus*) in uniform size were transported to the laboratory into the 50-L aquariums and acclimatized for 14 days prior to experiment (Emenike et al., 2011; Alkassabeh et al., 2009; Chen and Liao, 2004). Water in the aquarium was aerated for a week to remove residual chlorine, a toxic to the fish. The fish was fed with Aquadene fish food pellet that not interfere the metals accumulation. The feeding was terminated 48 hours before the experiment to reduce metabolic wastes (Alkassasbeh et al., 2009; Emenike et al., 2011).

Fish exposure to leachate

Tilapia was exposed to leachate at 2 to 20% v/v. These concentrations imitate the dilution factor of the river network. For instance, 2% v/v represents the most diluted at the farthest leachate outlet point whereas 20% v/v leachate concentration represents the least diluted at the closest leachate outlet point. The aquarium temperatures were maintained at 25+1◦C as rapid changes may affect the vital function of the fish and a photoperiod of 12 hour of light and 12 hours of dark were maintained as it can influence the physiology and behaviour of fish (Batt et al., 2005; Alkassasbeh et al., 2009). The experiment was conducted for 96 hours. The dead fish were removed immediately to prevent the depletion of oxygen in the aquarium. The fish in control experiment were kept in non-leachate water in the aquarium and was done simultaneously with the test experiment.

Sample preparation and digestion

The fish were cut with a stainless steel knife to prevent heavy metals contamination during the process. Samples were oven dried for 24 hours at 105◦C (Eneji et al., 2011; Obasohan, 2008). The dried samples were grounded and 1g of the samples was put in a crucible (Eneji et al., 2011). 5 ml of concentrated Nitric acid (69%), 1 ml of 70% Perchloric acid and 1 ml of concentrated Sulphuric acid was added to the samples and allowed to stand for two hours (Obasohan, 2008). The mixture of acid and samples in the flask were swirled gently and heated on a hot plate at 100-105◦C. The solution was allowed to cool before made up to 20 ml by the addition of deionised water. Samples were analysed for using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) Optima 8300.

Results and Discussion

Table 1 shows heavy metals concentration in muscles and gills of Tilapia exposed to young and old leachate. All heavy metals were higher in the gills exposed to old leachate (Table 1). The highest element detected in fish gills was Cu (22.72 ± 0.60 mg/kg) followed by Ni (1.66 ± 0.021 mg/kg) and Pb (0.50 ± 0.011 mg/kg). Ni also was highly detected in fish muscle with the mean of 1.32 ± 0.024 mg/kg. Cd was commonly detected in muscle and gills of young and old leachate (0.14 ± 0.01 mg/kg). Statistical analysis indicate Cu ($t = -2.839$, $p = 0.018$) and Ni ($Z = -2.082$, $p = 0.037$) were significantly higher in fish gills exposed to old leachate. Similar trend was observed for Cu in fish muscle (Z = -2.722, p = 0.006). High heavy metals accumulation in fish gills and muscles occur at the moderate leachate volume.

Table-1: Heavy metals concentration (mg/ kg) in muscle and gills of Tilapia exposed in young and old leachate

Landfill	Conc. (v/v)	Muscle (mg/ kg dry weight)				Gills (mg/ kg dry weight)			
		Cu	Cd	Pb	Ni	Cu	Cd	Pb	Ni
Young	2%	5.13±0.202	0.13±0.004	0.06±0.016	0.22±0.007	6.56±0.212	0.08±0.0001	BDL	0.12±0.008
	4%	3.41±0.110	0.11±0.002	BDL	0.08±0.004	1.94±0.048	BDL	BDL	0.07±0.001
	6%	5.11±0.112	0.12±0.001	BDL	0.19±0.002	4.49±0.105	0.07±0.002	0.28±0.031	0.17±0.005
	8%	4.8±0.082	0.14±0.001	BDL	0.27±0.002	14.94±0.086	0.14±0.001	BDL	0.51±0.003
	10%	5.51±0.063	0.12±0.001	BDL	0.17±0.002	3.81±0.011	0.04±0.003	BDL	0.30±0.013
	20%	4.31±0.002	0.10±0.001	0.09±0.032	0.45±0.002	4.14±0.103	0.06±0.001	BDL	0.48±0.020
Average		4.71±0.75	0.12±0.01	0.08±0.02	0.23±0.12	5.98±4.63	0.07±0.05	0.28±0.001	0.27±0.19
Old	2%	6.95±0.018	0.10±0.001	BDL	0.19±0.005	10.93±0.032	0.08±0.001	0.12±0.0001	0.79±0.040
	4%	11.67±0.115	0.11±0.002	0.14±0.004	0.66±0.00	12.84±0.0001	0.07±0.001	BDL	1.34±0.033
	6%	11.72±0.222	0.10±0.001	0.01±0.008	0.93±0.029	22.72±0.600	0.11±0.001	BDL	0.69±0.025
	8%	18.30±0.358	0.13±0.002	0.03±0.011	1.32±0.024	14.55±0.040	0.08±0.001	0.50±0.011	1.66±0.021
	10%	5.40±0.048	0.08±0.0001	BDL	0.11±0.008	10.53±0.062	0.07±0.001	0.04±0.001	0.25±0.002
	20%	8.19±0.088	0.08±0.0001	0.11±0.002	0.24±0.003	10.34±0.098	0.04±0.000	0.06±0.001	0.42±0.024
Average		10.37±4.64	0.10±0.02	0.07±0.06	0.58±0.48	13.65±4.73	0.08±0.02	0.18±0.22	0.86±0.54
t-value/ *Z*-score [c]		-2.722[a]	2.070[b]	-1.026[a]	-1.203[a]	-2.839[b]	-0.475[b]	-1.428[a]	-2.082[a]
p-value		0.006*	0.599	0.305	0.229	0.018*	0.645	0.153	0.037*

* Concentration is significant at the 0.05 level (two-tailed) between fish exposed in young and old leachate,
[a] Z- score, [b] t-value, [c] Significant difference of heavy metals by landfill leachate,
BDL= below detection limit

Findings in this study detected higher metals concentration in Tilapia gills compared to muscles. Gills, liver and kidney are the active metabolite organs in fish that accumulate higher heavy metals compared to the intestine and muscle (Uysal et al., 2008; Eneji et al., 2011). Heavy metals are bound to metal binding proteins such as metallothioneins in gills and accumulated in the organ. Furthermore, gills are known to absorb high contaminants from water and being used in the fish toxicity bioassay experiment (Taweel et al., 2013).

Gills generally are exposed to high metals at the beginning of exposure and the metals are rapidly removed from the gills at the end of the exposure (Jezierska and Witeska, 2006). According to Obasohan, (2008), the absorption of bio-available metals from the aquatic environment is mainly occurs through gills, skin and mouth and these metals are mainly stored in fish liver (Taweel et al., 2013). Results in this study are consistent with most literatures. For example, Shukla et al., (2007) also determined higher Cu (69.78 mg/ kg) and Cd (48.16 mg/ kg) in fish gills than in the muscles (3.38 mg/kg for Cu and 2.37 mg/ kg for Cd).

This study found Cu as the highest element detected in Tilapia followed by Ni, Pb and Cd. These are among the common inorganic metals found in landfill leachate together with Mg, Fe, Zn, Mn, Mo, and Cr where they are highly toxic for biota (Yi et al., 2011). Cu cause structural changes and increased ion permeability of the gill epithelia and lead to the impairment of the respiratory and destruct the ion regulatory functions in Tilapia (Obasohan, 2008). Ni can accumulate in different tissues of fish when they exposed to high contaminants (Obasohan, 2008). Strong bonding of metals to volatile fatty acids (VFAs) in Tilapia may cause high metals accumulation. Cd in Tilapia was possibly related to complexion of the element with the mucus in the gill lamellae where it is impossible to be removed before the analysis (Eneji et al., 2011; Karadede and Unlu, 2000). The affinity between metals also influence the

metal uptakes in Tilapia organ varies (Yilmaz et al., 2007). Besides, other factors such as the availability of the metals in the water, the intrinsic fish processes and the trophic structure also influence the metals uptake (Eneji et al., 2011).
A relatively high metal concentration in the Tilapia exposed to old leachate in this study is possibly due to high bioaccumulation of investigated metals which preferentially stored in the fish liver or muscle (Obasohan et al., 2008). Difference feeding habits and fish behaviour also determined difference heavy metals and level detected (Eneji et al., 2011). Low pH in the leachate may produce organic acids that cause high metal solubility (Umar et al., 2010) and increased the bioaccumulation in Tilapia. High level of ammonia, moderate concentration of BOD5 and COD and low ratio of BOD5/ COD in old leachate (Zainol et al., 2012; Rivas et al., 2004) possibly contribute to this.
High metals accumulation was assumed to be in concentrated leachate, resembled the closest leachate outlet point (20% v/v). However, results in this study shows high metals accumulation in Tilapia occurs in diluted leachate (between 4% to 8% v/v), resembled the farther distance. This is possibly due to the saturation of metal binding proteins (hepatic metallothionein) (Kusemiju et al., 2012) in Tilapia, not being able to bind more metals in the concentrated leachate.
There are few limitations encountered while conducting this research. Due to restriction to enter the landfill site, this experiment only used raw leachate from two landfills. Thus, results in this study cannot be generalized to represent the whole municipal landfill areas in the country. Despite of this limitation, this study provides baseline information on the level of heavy metals accumulation in Tilapia exposed directly from the raw leachate of landfills in 92 hours monitoring which rarely done before. Future study may include different types and location of landfill to see the differences in the accumulation level. The experiment also was restricted to only four common elements of heavy metals; Cu, Cd, Pb and Ni. Future study is suggested to consider other compound in the experiment such as ammonia, Chromium (Cr) and Zinc (Zn). This will help to study the affinity effects between metals and its connection with physicochemical properties.

Conclusion

In conclusion, all heavy metals tested were higher in the gills compared to the muscle of the Tilapia and the level was higher in old leachate. The highest element detected was Cu followed by Ni > Pb and > Cd. High metals accumulated in Tilapia occurs at diluted leachate of 4% to 8% v/v. Heavy metals detected in this study indicate a probable risk of metals exposure to human through Tilapia consumption.

Contribution of Authors

Rahman URA: Conceived idea, conducted experiment and write up of article
Ismail SNS: Helped in the experiment and article write up
Abidin EZ: Article write up
Praveena SM: Article write up

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