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Whole Effluent Toxicity (WET) Testing of Tin Mining Effluent and Receiving Water on Zebrafish, *Danio rerio* (Hamilton, 1822)

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ABSTRACT

Mining is one of the anthropogenic activities that can negatively affect the environment especially the waterways. Discharges from mining activities are usually in acidic state and containing elevated concentrations of metals. The exposure to these contaminants may cause several harmful effects not only to aquatic organisms but also to human health. The Whole Effluent Toxicity (WET) test was applied to evaluate the toxic effects of tin mining effluents to aquatic organisms. An acute toxicity test with zebrafish (*Danio rerio*) was conducted where fish was exposed to 3.13%, 6.25%, 12.5%, 25% and 50% effluent for 96 hours under static renewal test system. Effects of effluent exposure were determined using endpoints with mortality of median lethal concentration (LC_{50}) value. Results indicated that the LC_{50} value of zebrafish when exposed, was 14.21% effluent. The physicochemical properties of the effluent were also evaluated in order to assess the cause-effect relationships of the effluent. The low pH values of the mine effluent might be the main reason contributing to the fish mortality. This approach provides additional information of tin mining effect on freshwater fishes as well as to human health.

Keywords: Acute toxicity, LC_{50}, tin mining effluent, whole effluent toxicity (WET), zebrafish

INTRODUCTION

Active or abandoned mining sites can contribute to complex environmental situations. Environmental alterations from mining have caused serious problems such as decrease in river water quality and accessibility, habitat and biodiversity loss, and species extinction. Discharges from mining activities are responsible

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for chemical contamination of the river ecosystems (Ahmad & Sarah, 2014). Elevated concentrations of toxic chemicals can adversely affect fish and finally risk human health through consumption.

Mixed composition and interaction of the effluent within itself and surrounding have made the effluent complicated. To stand alone on only one or two known toxicants of the effluent, and to be tested for the toxicity test may cause this toxicant to be either less or more toxic to aquatic organisms. Whole Effluent Toxicity (WET) testing has been applied to assess the potential effects of these complicated effluents on aquatic life. WET test makes it possible to account for the uncharacterized sources of toxicity together with the physical and the chemical interaction of the effluents.

WET test can be performed by various tests on common species. Fish has been widely used in ecotoxicology to test chemicals and effluents toxicity (Lammer et al., 2009). In this study, zebrafish *Danio rerio* was used to assess the acute toxicity of the effluent from the tin mining industry. Zebrafish is among the most used fish species in ecotoxicology study because this fish is easily bred and has short generation times (Lourenço et al., 2017) which makes it suitable for test throughout the whole life cycle. Zebrafish are able to withstand higher level of chemicals in the water and also able to introduce any kind of genetic changes (Howe et al., 2013). Besides, zebrafish can create better model system which the genetic is similar to human (Howe et al., 2013). The objective of this study was to determine the sensitivity of zebrafish to tin mining effluent. This will allow detailed assessment on the potential risk of discharges from mining industry.

MATERIALS AND METHODS

Test Organism

Zebrafish (*Danio rerio*) was obtained from a commercial fish supplier. The fish was acclimatized for two weeks in non-chlorinated tap water (Temperature 28±1°C; pH 7.6±0.2; DO 6.6±0.3 mg/L) in four separate 60-L aerated aquaria (50 fish per aquarium) before the start of the effluent exposure. Fish was fed with commercial feed (Tetra Bits Complete, Germany) once daily at a ratio of 2% of the body weight (approximately 0.01 g).

Table 1
Physicochemical parameters of mining effluent and receiving water

	Unit	**Effluent**		**Receiving water**	
		Mean	SD	Mean	SD
pH	-	2.653	0.006	6.593	0.076
Temperature	°C	27.300	0.173	24.333	0.058
DO	mg/L	6.297	0.117	7.633	0.040
TDS	mg/L	1202.000	93.552	90.467	0.058

Table 1 *(continue)*

	Unit	Effluent		Receiving water	
Conductivity	µS/cm	1997.333	16.503	137.500	0.000
Salinity	ppt	0.967	0.058	0.067	0.058
Turbidity	NTU	212.667	8.021	45.567	0.153
BOD	mg/L	4.117	0.153	0.990	0.166
COD	mg/L	51.800	1.997	1.800	0.794
AN	mg/L	0.210	0.060	0.077	0.021
TSS	mg/L	85.333	3.786	35.333	1.155

Effluent and Receiving Water Samples

Effluent samples were collected at the treatment/settling pond of the mining company (5° 36′ 17.0″ N, 101° 2′ 10.3″ E) in Kepayang River. Meanwhile for receiving water, samples were collected at Kampung Alai in Rui River (5° 37’ 13.7″ N, 101° 5’ 7.4″ E). Samples were collected in 10-L acid-washed polyethylene bottle and transported to the Aquatic Research Laboratory, Universiti Putra Malaysia at 4 °C until use. Physicochemical properties of both waters were tested *in situ* and *ex situ*, and presented in Table 1.

Test Procedure

Preliminary test was done using 100% of mining effluent that showed 100% fish mortality (10 fish per aquarium; triplicate) within only 2 hours of exposure. Therefore, the test was conducted starting from 50% of mining effluent. The fish were exposed over 96 hours to 50%, 25%, 12.5%, 6.25%, and 3.13% of mining effluents, and also to 100% of receiving water following the US EPA method (United States Environmental Protection Agency [US EPA], 2002). Clean non-chlorinated tap water was used as control (0%) and for dilution water. Each treatment group consisted of 4 replicates with 10 fish per aquarium. The fish were placed in 2L aquarium tank. Water temperature of all treatment groups were maintained at 28±1 °C with a 14-h:10-h of light: dark photoperiod, and were constantly aerated with DO maintained at 6.6±0.3 mg/L. Based on Table 1, only pH, TDS, conductivity and turbidity of all treatment groups were monitored throughout the 96-h test since their values were of concern and had exceeded the Malaysia’s National Water Quality Standard (NWQS) for natural environment for aquatic organisms (Department of Environmental Malaysia [DOE], 2015). Water was changed 100% daily for all treatment groups. The fish was transferred to another aquarium filled with new treatment water by using a scoop net. The fish was not fed during the 96-h test. Observations were made every 4-h for 24-h and every 24-h thereafter until 4 days (96-h). Any changes on the behavioral of the fish were monitored and recorded. Behavioral parameters that were observed were swimming movement, mouth and operculum movements, and hyperactivity. The dead fish were counted and immediately removed.

Data Analysis

The acute toxicity of effluent was estimated as the median lethal concentrations (LC_{50}). The 96-h LC_{50} value was calculated by Probit Analysis with 95% confidence limits using IBM SPSS Statistics 22.

RESULTS AND DISCUSSIONS

The percentage mortalities of zebrafish to different concentration of the tin mining effluent, and at different exposure time is presented in Table 2. Results showed 100% mortality of fish was when exposed to 50% and 25% effluents in less than 24-h. Meanwhile, about 30% mortality of fish was recorded for 12.5% of effluent and only 5% mortality recorded for the receiving water. No mortality observed for the control. The values of pH, TDS, conductivity and turbidity were also presented in Table 2. The pH value at 25% effluent was still very low (pH 4.91) that could cause death to most aquatic organisms, although the TDS, conductivity and turbidity values have dropped to the safe level of NWQS (Table 2). Besides, zebrafish can tolerate a wide range of salinities and conductivities of up to 14 ppt (Lawrence, 2007) and 1500 µS/cm (Avdesh et al., 2012), respectively. Moreover, Wojtas et al. (2015) found that this fish could also tolerate high water turbidity above 300 NTU. Previous studies on the effect of low pH water have shown an increase in fish mortality due to gills damage (Sharma, 2003; Slaninova et al., 2014). In fact, zebrafish have been reported to be typically found in alkaline water condition with an average pH of 8.0 (Lawrence, 2007). Therefore, these present results indicated that the fish died due to the low pH of the mining effluent.

The behavioral changes of the fish towards the toxicant were also investigated. When exposed to higher concentrations of effluent (50% and 25%), fish immediately showed

Table 2
Percent mortality of fish after exposure and physicochemical properties of different concentration of the treatment waters

Concentration (%)	No. of fish	Mortality (%)				pH	Conductivity (µS/cm)	TDS (mg/L)	Turbidity (NTU)
		24-h	48-h	72-h	96-h				
50	40	100	100	100	100	3.26	1174.9	706.7	106.67
25	40	100	100	100	100	4.91	734.3	441.9	53.33
12.5	40	15	27.5	30	30	6.19	469.0	282.5	26.80
6.25	40	2.5	7.5	7.5	7.5	6.67	320.4	193.2	13.33
3.13	40	0	0	0	2.5	7.04	240.1	144.6	6.73
0 (control)	40	0	0	0	0	7.56	164.0	97.0	0.37
100 (receiving water)	40	2.5	2.5	2.5	5	6.56	136.8	90.2	45.50

hyper-excitability by erratic swimming and increased operculum movement. Few minutes after that, the swimming behavior started to slow down and the fish tried to remain in vertical position perpendicular to the surface water with the mouth facing upward. Finally, the fish lost its balance, settled to the bottom or turned upside down at the surface water and died. Similar behavioral changes were observed in zebrafish due to metal pollution (Anandhan & Hemalatha, 2009).

On the other hand, the 96-h acute test of LC_{50} for the mining effluent on zebrafish *Danio rerio* was calculated to be at 14.21 % (Table 3). The results showed significant differences in toxicity responses between different effluent concentrations. The results also demonstrated the LC_{50} values calculated for 24-h, 48-h and 72-h of exposure were at 15.85%, 14.46%, and 14.23%, respectively (Table 3). These LC_{50} values are important in setting the benchmark for the safe level of mine-water to be discharged into streams or rivers, in order to protect the aquatic organism, as well as the ecosystem.

Table 3
LC_{50} values for mining effluent at different time of exposure

Test duration (h)	LC_{50} values (%)	95% lower confidence limit	95% upper confidence limit
24	15.85	14.23	18.24
48	14.46	12.91	16.61
72	14.23	12.70	16.37
96	14.21	12.64	16.31

CONCLUSION

This study shows that tin mining wastes are very toxic to the fish. Lethality of fish in the present study was mainly affected by the low pH of the effluent. Further research is needed on ecotoxicological risks of tin mining waste and improved mine drainage treatment in order to support the development of suitable regulations of mine-water discharges.

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