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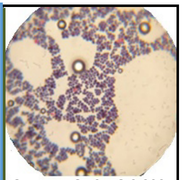

JOBIMB VOL 9 NO 2 2021
S. aureus in sachet water

Evaluation of Toxicity Effect of Palm Oil Mill Effluent Final Discharge by using *Daphnia magna*

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ABSTRACT

Palm Oil Mill Effluent (POME) final discharge has a risk to the ecosystem due to various harmful contaminants including organic and inorganic materials. In this study, biological monitoring method was used to evaluate the toxicity effects of POME final discharge using *Daphnia magna*. The physical and chemical nature of toxicants present in the effluent were characterized through acute Whole Effluent Toxicity (WET), and Toxicity Identification Evaluation (TIE) tests. The Toxicity Unit (TU) and median lethal concentration (LC_{50}) of the POME sample were 11.09 and 9.02% (v/v) respectively. From TIE test, the toxicants present in the effluent can be characterized as filterable and oxidisable through filtration and aeration treatment. The presence of cationic metals, chlorine and disinfection by-products were also determined by the toxicity reduction of the effluent after treatment using ethylenediaminetetraacetic acid (EDTA) and sodium thiosulphate. From TIE test, the filtration treatment at pH 10 of the POME final discharge was the most effective method in reducing the toxicity of the effluent with a value of TU, 1.16 and LC_{50}, 86.34% (v/v). It is recommended that biological tests using *Daphnia magna* can be made as potential methods to indicate the effects of POME final discharge to the aquatic ecosystem.

INTRODUCTION

Ecosystem refers to a geographic area that contains biotic factors such as plants, animals and organisms, as well as abiotic factors including rocks, temperature and humidity [1]. For aquatic and terrestrial ecosystem testing, the species selected should be the keystone species for the trophic level being evaluated [2]. Freshwater crustaceans, *Daphnia magna* are widely used in ecotoxicological studies as they are sensitive to toxicants, easy to culture and multiply. In addition, they are also the crucial members of the zooplankton in numerous lakes [3].

Since Malaysia is experiencing vast progress in the development of new oil palm plantations and palm oil mills, the generation of huge types of oil palm waste has become a critical issue as it creates waste disposal and poses challenges in achieving environmental sustainability vision [4]. The poor quality of POME final discharge and chemical compounds present in the effluent will cause harmful effects to aquatic life such as rivers and oceans in a short- and long-term period. Hence, environmental water quality monitoring is required to ensure that POME is safe to be discharged and also achieve the effluent standard limit set by the Department of Environment (DOE) Malaysia. POME refers to the effluent from the last stages in palm oil processing which is made up of various components such as water, total solids, suspended solids and palm oil [5]. POME final discharge can be one of the threatening water pollutants that can cause adverse impacts to the environment such as reduction of oxygen level in the water, highly toxic to aquatic life and eventually cause unwanted changes in the ecosystem balance. These negative impacts can lead to deterioration of the surrounding environment as well as dangerous consequences for the intended use of water [6].

A biological-based monitoring method that usually utilizes invertebrates and fish as the bioindicator of water quality should be a great complementary approach, along with physical and chemical monitoring in evaluating the toxicity of POME final discharge. Biological methods can be used to evaluate the biological responses towards the toxicants that may present in the POME to indicate comprehensive effects of contaminants in a water body [7]. In this analysis, a Toxicity Identification

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Evaluation (TIE) test was performed to characterize the physical and chemical features of components in POME final discharge that contribute to its toxicity using *Daphnia magna*. Since *Daphnia magna* species are sensitive to contaminants, they are recommended to be used as a bioindicator or a model organism for toxicity testing [8]. Integration of biological monitoring as one of the additional approaches in palm oil mills to evaluate the toxicity of POME will bring a lot of benefits for the ecosystem and human health in the future.

MATERIALS AND METHODS

Collection of Palm Oil Mill Effluent (POME) final discharge

The sample of POME final discharge was collected from Pasoh Palm Oil Mill located in Negeri Sembilan, Malaysia (Google Coordinate: 3.0506, 102.2864). The distance between the laboratory and the palm oil mill is 117 kilometres (Google, 2021). Hence, for convenience, the tests and analysis of the sample were conducted off-site and additional measures were given to preserve and minimise the error.

The POME final discharge sample (3L) was filled into three glass laboratory bottles with screw caps that were wrapped with aluminium foil. The bottles were filled to ensure the absence of air spaces between the content and lid and, stored in a chilled box (0-6°C). Tests and analysis of POME were done according to US EPA, 2002 [9].

Colorimetric procedure

The sample COD value was measured colourimetrically at 420 nm using a spectrophotometer (UviLine 9400, EU). The COD value was obtained in mg/L [10].

Preparation of *Daphnia magna* cultures feed

Dry *Saccharomyces cerevisiae* or baker's yeast (10 mg) was transferred into a 100 mL glass screw-cap bottle (100 mL of distilled water) and mixed using a magnetic stirrer or vortex (Velp Scientifica, EU). This stock solution was renewed every week at room temperature [11].

Set-up and maintenance of *Daphnia magna* culture

Fifteen neonates of D. *magna* (24 h age) were cultured in a plastic aquarium (13 L water with 1 mL dechlorinator). The culture (20 ± 1°C) is photoperiod-controlled at 16 h under light illumination and 8 h in dark conditions (US EPA, 2002) and inspected daily. Daphnids were fed once daily (0.5 mL yeast stock solution) with constant feeding schedules. New cultures were prepared every two weeks [12].

Acute Whole Effluent Toxicity (WET) testing of POME

Multi-concentration or definitive test was conducted according to the acute toxicity test comprised of control and five effluent concentrations, with four replicate chambers per concentration. The concentrations (0%, 6%, 12%, 25%, 50% and 100% (v/v)) were used with POME final discharge sample and distilled water. A static non-renewal test was conducted for the acute toxicity test of POME, where D. *magna* were exposed for 48 h. Test solution (20 mL) was transferred in a 50 mL glass beaker including the control (4 replicates) and D. *magna* were added and exposed in each test solution concentration (48 h).

The WET test was conducted in a photoperiod condition. The endpoint was the survival of D. *magna* as the indicator for toxicity of the POME sample. The results were conveyed as median lethal concentration (LC_{50}) values, which refers to the lethal concentration required to kill 50% of the test organisms. The LC_{50} values were determined using Probit analysis and converted into Toxic Unit (TU) [9].

Toxicity Identification Evaluation (TIE) of POME final discharge

The toxicity identification evaluation (TIE) test was conducted according to the Chronic Phase I procedures implemented by the United States Environmental Protection Agency [13].

Baseline whole effluent toxicity (WET)

Five different concentrations including control were used (0%, 6%,12%, 25%, 50%) and 100% (v/v) POME in four replicates. Test solutions (10 mL) were transferred into glass beakers and D. *magna* (10 individuals) were added and exposed to the solutions (48 h) under light illumination and dark condition respectively (20 ± 1°C).

The endpoint used for the WET test was the survival of the test organism. Hence, the death or percentage of mortality of D. *magna* was the result used to determine the toxicity of the POME sample to D. *magna*. The median lethal concentration (LC_{50}) values were calculated and converted into toxic units (TU) values [13].

Ethylenediaminetetraacetic acid (EDTA) test

Ethylenediaminetetraacetic acid (EDTA) stock solution (0.1 g/L) was prepared and added (0.4 mL, 0.2 mL, 0.1 mL, 0.05 mL, 0.025 mL, 0.0125 mL and 0.0 mL) into plastic cups containing POME concentration (100%, v/v) in duplicates. The test solutions were mixed thoroughly and pH was observed to ensure constant pH, then left for 2 h. The WET test was conducted as described previously. The percentages of mortality of D. *magna* were compared to the untreated effluent [13].

Sodium thiosulphate test

Sodium thiosulphate stock (0.1 g/L) was prepared. Six different volume of sodium thiosulphate (1.0 mL, 0.8 mL, 0.6 mL, 0.4 mL, 0.2 mL and 0.0 mL) were added into plastic cups containing 100% (v/v) POME in triplicates and mixed thoroughly with a glass rod. The pH was checked and re-adjusted to the initial pH (pHi). The WET test was carried out and the percentages of mortality of *D. magna* in the 48 h acute WET test were compared with the treatment without sodium thiosulphate [13].

Filtration Treatment

POME sample (150 mL) was centrifuged (10,000 x g, 4°C, 30 min.) using high-performance Beckman Coulter Centrifuge (US) and filtered using 1000 mL laboratory filtration with a vacuum pump (0.45 µm cellulose acetate membrane filter). POME sample test solutions (20 mL) of 0%, 12%, 25%, 50% and 100% (v/v) were prepared for WET tests using *D. magna* in triplicates. Finally, LC_{50} values and toxicity units were calculated using the data [13].

Aeration Treatment

The POME sample and distilled water were aerated for 60 min using air pumps simultaneously. Subsequently, the aerated POME sample (20 mL) in concentration, 0%, 12%, 25%, 50% and 100% (v/v) were prepared with the aerated distilled water. The WET test was conducted in triplicates for each concentration. Lastly, the results obtained were used to determine LC_{50} values and toxicity units of the POME sample [13].

Adjustment of pH of POME

The initial pH (pHi) was measured and pH adjustments of the POME sample (480 mL) were conducted. The first set POME sample (240 mL) was adjusted to pH 3, while the second set

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(240 mL) to pH 10. The POME samples (120 mL) were used for the pH adjustment test. Both sets were readjusted again to pHi and 20 mL of POME sample (0%, 12%, 25%, 50%, and 100%, v/v) were prepared in triplicate. The WET test was conducted and median lethal concentration (LC_{50}) values and toxic units were calculated from the data [9].

Filtration and pH adjustment

Two sets of POME samples (120 mL) that were adjusted to pH 3 and pH 10 were filtered. The pH 3 and pH 10 of POME sample filtrates were readjusted to pHi before the WET test. From the data obtained, LC_{50} values and toxic units were determined [13].

Aeration and pH adjustment

The two sets of 120 mL of POME sample that was adjusted to pH 3 and pH 10 were aerated using an air pump for 60 min. The aerated POME sample (20 mL) in concentration, 0%, 12%, 25%, 50% and 100% (v/v) were prepared with the aerated distilled water. After aeration, both POME samples were readjusted again to pHi as the pH of effluent may deviate due to aeration. Lastly, acute whole effluent toxicity (WET) test was conducted and LC_{50} values and toxic units were calculated based on the data obtained [13].

Calculation of corrected percentage mortality of *Daphnia magna* in acute whole effluent toxicity (WET) tests

The calculation of corrected percentage mortality (%) according to Abbott's formula is given in the following equation [14]:

$$CPM\ (\%) = \frac{\%\ \text{alive control} - \%\ \text{alive treated}}{\%\ \text{alive control}} \times 100\% \quad \text{(Eq. 1)}$$

where:

CPM is Corrected Percentage Mortality
% alive control = Percentage of living test organisms in control
% alive treated = Percentage of living test organisms in the test solution

Average percentages were calculated as the tests for POME solution were conducted in replicates [15].

Determination of median lethal concentration (LC_{50}) using Probit analysis

The mean percentage of corrected mortality was transformed into Probit values according to Finney's table [16]. The log of POME sample concentrations was obtained, and regression analysis was performed by plotting a graph of Probit mortality versus the log of the POME concentrations. The LC_{50} value was obtained by finding the log of POME concentration associated with the Probit value of 5 in the y-axis of the graph, which corresponds to 50% mortality by using the linear interpolation method. Finally, the inverse log of POME concentration that was correlated with the Probit value of 5 was calculated to obtain the actual LC_{50} value [15].

Calculation of Toxic Unit (TU)

From the value of LC_{50}, Toxic Unit (TU) was obtained by using the following formula [9]:

$$\text{Toxic Unit (TU)} = \frac{100}{LC_{50}} \quad \text{(Eq. 2)}$$

Where

LC_{50} = Median Lethal Concentration of POME, % (v/v)

Toxicity Identification Evaluation (TIE)

Toxicity characterization of POME final discharge

In this study, a TIE test was conducted to characterize physical and chemical contaminants exhibiting acute toxicity to *Daphnia magna* that provided details on, chelatability, filterability, and pH sensitivity of causative toxicants in POME [17]. Characterization of toxicants in this process involves manipulations at the initial pH (pHi) of the POME final discharge and compared with the POME sample without pH manipulation.

RESULTS AND DISCUSSION

Filtration treatment

The LC_{50} value of the POME final discharge before giving any treatments such as filtration, aeration and pH adjusted were similar, 9.02%, and the TU value were 11.09, 11.03 and 9.20 respectively. Since many fine particles were present in the POME final discharge sample, centrifugation was carried out to eliminate the particles. LC_{50} value of the POME sample increased from 9.02% to 11.79% (**Fig. 1**).

Simultaneously, TU reduced from 11.09 to 8.48 indicating the toxicity level of the POME sample was reduced after the filtration treatment test. Rezakazemi, Khajeh & Mesbah [18] reported that toxicants bound to suspended solids or present freely in the POME effluent can be filtered out during the filtration treatment.

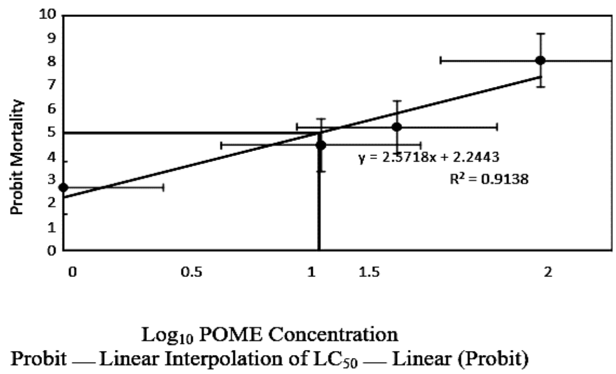

Fig. 1. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of filtered POME final discharge in a 48hour acute WET test. Data are the mean (± SEM) percentage of *D. magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 of *D. magna* individuals per POME concentration treatment.

Aeration treatment

After aeration LC_{50} of POME final discharge increased from 9.02% to 10.87% (**Fig. 2**). Correspondingly, the TU of the aerated POME was lower than the TU of the untreated POME which was from 11.83 to 9.20. The toxicity of POME was reduced after aeration treatment because dissolved gases such as carbon dioxide were removed from the effluent into the surrounding air due to the aeration turbulence. Dissolved materials such as hydrogen sulphide and volatile organic chemicals (VOC) were oxidized and formed precipitate during aeration and removed via filtration or flotation [19].

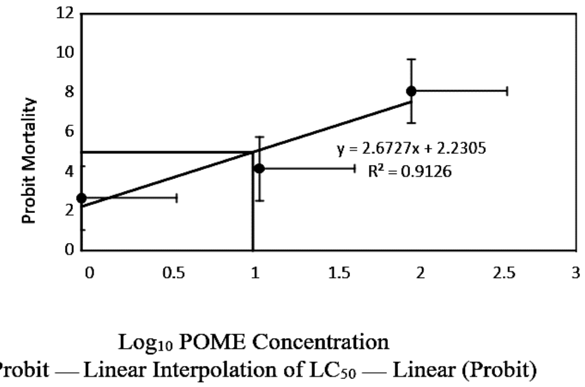

Fig. 2. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of aerated POME final discharge in a 48 h acute WET test. Data are the mean (± SEM) percentage of *D. magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 of *D. magna* individuals per POME concentration treatment.

Sodium thiosulphate treatment

Sodium thiosulphate was added via a gradient concentration of thiosulphate approach to discover the degree of causative toxicants in POME final discharge. **Table 1** shows the mortality percentages of *D. magna* were reduced with the addition of sodium thiosulphate and eventually become zero with 0.8 mL and 1.0 mL of the thiosulphate. The results show the higher the concentration of sodium thiosulphate, the lower the toxicity level of POME final discharge which causes lethality to *D. magna* as compared to treatment without sodium thiosulphate. Without treatment, there was 100% mortality of *D. magna* due to the high toxicity of POME final discharge. The toxicity of the POME sample was reduced as sodium thiosulphate reduced the chlorine and neutralized other chemicals such as bromine and iodine in the original POME sample [20].

Table 1. Comparison of survival readings of *Daphnia magna* in POME final discharge with different concentrations of sodium thiosulphate addition.

Sodium Thiosulphate (mL)	Replicate			Mean	Standard Deviation	Relative Standard Deviation (%)
	1	2	3			
1.00	0.00	0.00	0.00	0.00	0.00	0.00
0.80	0.00	0.00	0.00	0.00	0.00	0.00
0.60	20.00	18.00	20.00	19.30	1.15	5.96
0.40	40.00	38.00	36.00	38.00	2.00	5.26
0.20	80.00	80.00	80.00	80.00	0.00	0.00
0.00	100.00	100.00	100.00	100.00	0.00	0.00

Ethylenediaminetetraacetic acid (EDTA) treatment

The mortality percentage of D. *magna* in POME final discharge started to decrease when 0.025 mL EDTA was added and the mortality percentage reduced with the increasing volumes of EDTA from 0.05 mL to 0.2 mL (**Table 2**). The mortality seems to increase again when 0.4 mL of EDTA was added indicating that during the mid-range of EDTA addition, the cationic metals were chelated and formed metal complexes. Hence, the POME final discharge became less toxic. Nevertheless, higher addition of EDTA caused an increment in toxicity of POME sample due to unreacted EDTA becoming toxic to *D. magna,* while lower EDTA addition was not sufficient to chelate metals in the effluent [13].

Table 2. Survival of *D. magna* in POME final discharge mixed with EDTA.

EDTA stock Solution (mL)	Replicate			Mean	Standard Deviation	Relative Standard Deviation (%)
	1	2	3			
0.4000	80.00	78.00	80.00	79.30	1.15	1.45
0.2000	40.00	40.00	42.00	40.70	1.15	2.83
0.1000	60.00	62.00	58.00	60.00	2.00	3.33
0.0500	70.00	70.00	70.00	70.00	0.00	0.00
0.0250	90.00	88.00	86.00	88.00	2.00	2.27
0.0125	100.00	100.00	100.00	100.00	0.00	0.00
0.0000	100.00	100.00	100.00	100.00	0.00	0.00

pH alteration treatment

The pH of POME final discharge was adjusted to provide more information on the character of toxicants and to discover whether the causative toxicants were pH-dependent [13] (US EPA, 1992). In this study, the POME final discharge was prepared in two sets; pH 3 and pH 10. After the pH alteration, the pH was adjusted again to the initial pH (pHi) to avoid misinterpretation of the toxicity of the POME with the pH-dependent compounds [21] (Melo et al., 2013). **Fig. 3** and **Fig. 4** show the LC_{50} of POME final discharge adjusted to pH 3 and pH 10 was 10.87% (v/v) and 27.06% (v/v) respectively, indicating the increase of LC_{50} values if compared to the original POME final discharge (9.02 % (v/v)).

The TU of POME final discharge altered to pH3 and pH 10 were lower than the TU of the unmanipulated sample with 9.20 and 3.70 scores respectively. This is due to the changes in solubility, polarity, volatility and stability of causative toxicants when pH was altered. This action resulted in the reduction of bioaccessibility toxicants and at the same time reduced their toxicity to *Daphnia magna*. It was mentioned metal toxicities in POME final discharge were reduced by pH alterations through their changes in solubility and speciation [22]).

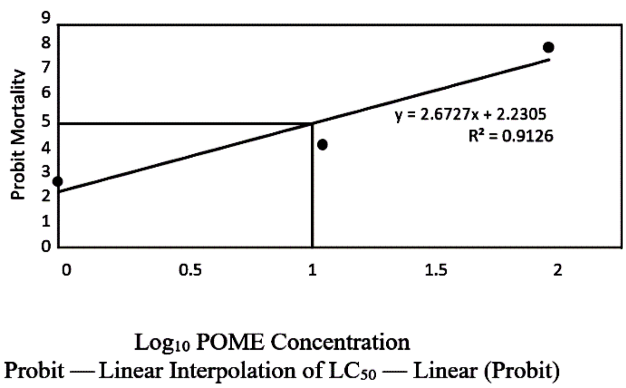

Fig. 3. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of POME final discharge that was adjusted to pH 3 in a 48 h acute WET test. Data are the mean (± SEM) percentage of *D. magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 of D. *magna* individuals per POME concentration treatment

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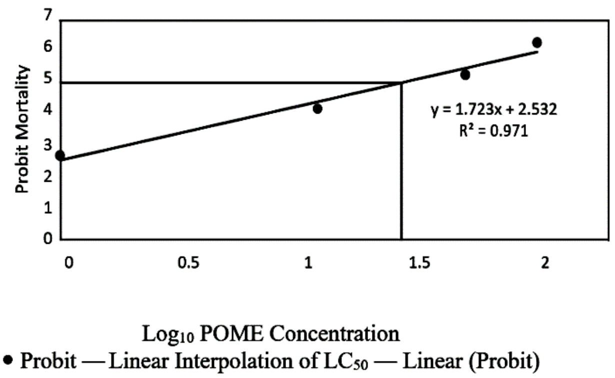

Fig. 4. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of POME final discharge that was adjusted to pH 10 in a 48 h acute WET test. Data are the mean (± SEM) percentage of *D. magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 *D. magna* individuals per POME concentration treatment

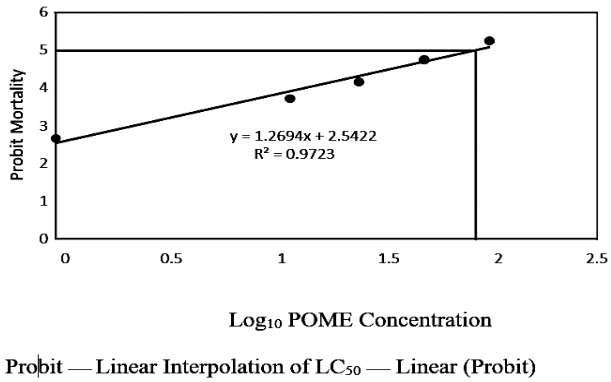

Fig. 6. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of filtered POME final discharge that was adjusted to pH 10 in a 48 h acute WET test. Data are the mean (± SEM) percentage of D. *magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of D. *magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 D. *magna* individuals per POME concentration treatment

pH alteration combined with filtration treatment

Changes in pH can affect the solubility of contaminants in POME final discharge and finally form a precipitate. The pH alteration and filtration tests were conducted to determine whether the precipitated toxicants are filterable [13]. **Fig. 5** and **Fig. 6 show** the LC_{50} of filtered POME final discharge increased to 23.12% (pH3) and 86.34% (v/v) (pH 10) as compared to the sample without pH adjustments (9.02% v/v).

TU of POME final discharge (filtered, pH3 and pH 10) were lower than the TU of the untreated sample, 4.32 and 1.16 respectively. The reduction of POME final discharge toxicity level was facilitated with the filtration of the precipitate which was formed due to the pH alterations [23]. Therefore, the contaminants were separated, and the toxicants absorbed into particles were also removed [18].

pH alteration combined with aeration treatment

Aeration treatment of POME final discharge at pH 3 or pH 10 makes the contaminants in the effluent more oxidisable, spargeable or sublatable [13]. Thus, pH adjustment and aeration treatment tests were conducted to determine the degree of the treatments that can reduce the toxicity of the sample while using D. *magna* as a biological indicator. The results after aeration (**Fig. 7** and **Fig. 8)** show the LC_{50} of the samples at pH 3 and pH 10 were 25.97% and 30.94% (v/v) respectively as compared to the LC_{50} for the sample without pH adjustments (9.02 % v/v). The TU of aerated samples (pH3 and pH 10) were lower than the unaerated sample (3.85 and 3.23). It can be concluded that combination treatment such as pH alteration followed by aeration treatment was more effective to reduce the toxicity of POME final discharge as compared to pH alteration alone or aeration treatment alone. Zhang et al. [24] (2019) reported that the total ammonia (NH_3) level in POME can be air-stripped at pH 10.

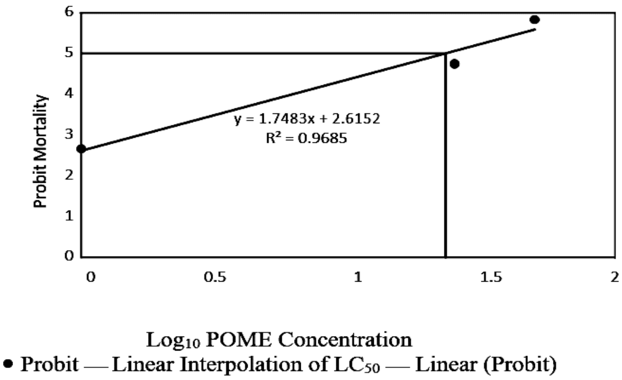

Fig. 5. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of filtered POME final discharge that was adjusted to pH 3 in a 48 h acute WET test. Data are the mean (± SEM) percentage of *D. magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 of *D. magna* individuals per POME concentration treatment.

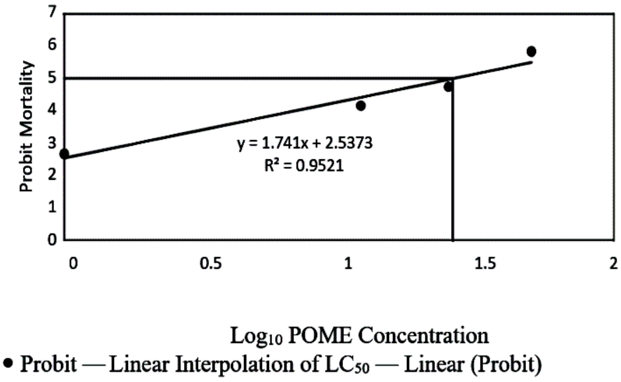

Fig. 7. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of aerated POME final discharge that was adjusted to pH 3 in a 48 h acute WET test. Data are the mean (± SEM) percentage of D. *magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 D. *magna* individuals per POME concentration treatment.

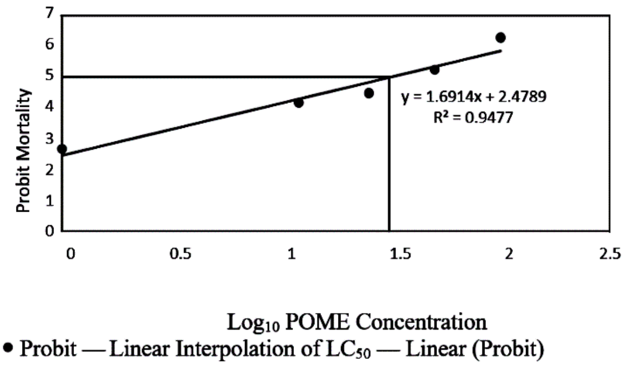

Fig. 8. Probit transformed responses for total death of *Daphnia magna* exposed to different concentrations of aerated POME final discharge that was adjusted to pH 10 in a 48 h acute WET test. Data are the mean (± SEM) percentage of *D. magna* corrected mortality exposed with different concentrations of filtered POME. The dashed horizontal line corresponds to the Probit score associated with a 50% death of *D. magna*, while the vertical dashed line represents true median lethal concentration (LC_{50}) for the concentration-response curve. n=10 *D. magna* individuals per POME concentration treatment.

Table 3. Summary of LC_{50} values and TU of POME final discharge with different pH adjustments and manipulations.

Manipulations	pH	LC_{50} (%, v/v)	Toxic Unit
pH adjustment	pHi	8.45	11.83
	pH3	9.20	10.87
	pH10	27.06	3.70
pH adjustment and filtration	pHi	11.79	8.48
	pH3	23.12	4.32
	pH10	86.34	1.16
pH adjustment and aeration	pHi	10.87	9.20
	pH3	25.97	3.85
	pH10	30.94	3.23

Table 3 shows POME final discharge with pH adjustment (pH 10) provides the highest LC_{50} value (86.34%) and the lowest TU (1.16). Based on this finding, it is highly recommended to adopt the finding as a basic platform to explore a method for reduction of toxicity level in POME final discharge.

CONCLUSION

Based on the initial pH of POME final discharge, the filtration and aeration treatments were able to reduce the toxicity of the POME. After filtration and aeration, the TU of effluent decreased and the LC_{50} increased to imply that the toxicity of the effluent was reduced. Cationic metals present in the effluent as the percentage of mortality of D. *magna* reduced as EDTA chelated the cationic metals in the effluent and rendered them as non-toxic matter. STS results suggested the presence of chlorine and by-products in the effluent was reduced after POME final discharge was added with STS. The combination of pH alteration (pH 10) and filtration treatment of the POME sample was the most effective in reducing the toxicity of the POME as the TU obtained was the lowest, 1.16 and the LC_{50} obtained was the highest, 86.34% (v/v). In conclusion, biological toxicity-based monitoring is an effective complementary approach to chemical-based (conventional) monitoring of POME final discharge in assessing the toxicity level of POME final discharge that will be released into water bodies.

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