

## Original Article

# Effects of Treated Palm Oil Mill Effluent Application on the Soil Microbial Community Structure and Oil Palm Plantation Productivity

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## ABSTRACT

The palm oil mill effluent (POME) is generally treated using biological anaerobic treatment and then utilized for POME land application. This research aimed to study the effect of treated POME land application on soil microbial community structure in oil palm plantation and their impact to the oil palm productivity. The results showed that ubiquinone (UQ) and menaquinone (MK) contents in soils without treated POME application were 0.045 and 0.204  $\mu\text{mol/kg}$ -dry soils, respectively. These were much lower than the UQ and MK contents in soils with treated POME application which were 0.074 and 0.301  $\mu\text{mol/kg}$ -dry soils, respectively. The diversity quinone (DQ) and bioenergetic divergence quinone (BDq) in soils without treated POME application were 11.21 and 0.86, respectively, while in soils with treated POME application were 11.32 and 0.87, respectively. These results indicate that the treated POME application increased the amount of microorganisms but did not change the diversity of microorganisms. Treated POME application also increased the domination of aerobic bacteria in soils. The total UQ, MK, and UQ/MK ratio of soils with treated POME application are higher than those of soils without treated POME application and the application of POME is generally able to increase oil palm productivity.

**Keywords:** oil palm productivity, quinone profile, microbial community structure, POME application, palm oil mill effluent

## INTRODUCTION

Palm oil industry in Indonesia has increased rapidly. During 2007 – 2012 the plantation area grew 6.96% and the palm oil production increased about 6.02% annually [1]. Palm oil industry does not only produce the main product crude palm oil (CPO), but also generates a huge amount of liquid wastes known as palm oil mill effluent (POME) and solid wastes. Each ton of fresh fruit bunch (FFB) produces about 0.75 – 0.90  $\text{m}^3$  of POME or equivalent to approx. 3.33  $\text{m}^3$  of POME per ton of CPO production. The POME treatment has been done by anaerobic treatment process. Since POME contains some organic materials and nutrients, the effluent of the treatment process is then applied to the plantation area

(treated POME land application) for irrigation of the oil palm plantation and where the treated POME serves as fertilizer.

POME contains nitrogen (N), phosphorus (P), and potassium (K) in a relatively high concentration making it a good potential substitute for inorganic fertilizers [2]. Some palm oil industries utilize the treated POME as liquid fertilizer. The treated POME land application will influence the soil health which is identified by changes of microbial community structure in soils leading to the increase of oil palm plantation productivity. The changing of microbial community structure can be evaluated using quinone profile analysis. Research on the changes of microbial community structure in the natural mixed culture becomes very important to define the mechanism of microorganism process

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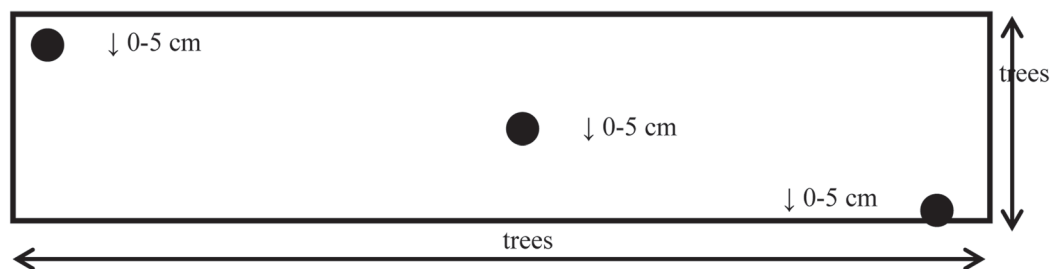


Fig. 1 Schematic diagram of sampling points.

from microorganism community perspective in relation with physico-chemical condition of the environment [3]. It is possible to evaluate the impact of the utilization of the treated POME which is applied to the oil palm plantation. Quinone profile analysis can be used as an index to characterize microorganism community [4–6] and as biomass index [6,7]. Quinone profile analysis has been also applied as an equipment to measure the quantity of microbial community structure changing in tidal flat, land, water, and wastewater. Quinone profile analysis that is used to land ecosystem can explain the effect of fertilizer use and pesticide to the microbial community structure [8]. The purposes of this research were (a) to characterize the soils microbial community structure as result of application POME as liquid fertilizer of the oil palm plantation, and (b) to determine the correlation of microbial community structure with the oil palm plantation productivity.

## MATERIALS AND METHODS

### Equipment and materials

The equipment used in the analysis was a high performance liquid chromatograph (HPLC) with organic carbon detection (OCD) column with octadecyl-silica (ODS) packing (Zorbax-ODS, Agilent Technology, Tokyo, Japan 4.6 I.D. × 250 mm) and multichannel UV (photodiode array detector), model: SPD-M10A, Shimadzu, Kyoto, Japan.

Samples of soils applied by chemical fertilizer (without treated POME) and applied by treated POME were taken from PTPN VII Unit Usaha Bekri in Sinar Banten Village, Central Lampung Regency, Lampung Province, Indonesia. Reagents used for laboratory analysis consisted of methanol, di-isopropyl ether, pure water (distilled water), hexane, diethyl ether, chloroform, and acetone obtained from University of Lampung.

### Methods

The soil samples were taken from three points of each

plantation land blocks (3 blocks) with 0 – 5 cm depth. The soil at a depth of 0 – 5 cm is very sensitive to changes that occur on the surface of the soil. In addition, the materials added to the soil accumulate at the soil depth of 0 – 5 cm. A total of 9 samples were used in this study. The schematic diagram of the sampling points is shown in Fig. 1. The soil samples were freeze-dried for 24 hours at a temperature of –81°C. The quinone was extracted and analysed. The quinone analysis procedure is shown in Fig. 2.

### Extraction using chloroform and methanol

Quinone was extracted from microbial cells contained in the soil samples. The quinone extraction was conducted with organic solvent mixture, (2:1 (v/v) chloroform and methanol mixture). The homogenization was done using a shaker for 30 minutes at room temperature. The sample was then filtrated and the filtrate was placed in an eggplant flask. The purification step was repeated 3 times. The sample in the eggplant flask was evaporated to get the residue. Evaporation process was done in a rotary vacuum evaporator with a heater temperature of 35°C and condensation temperature of 9 – 10°C.

### Quinone purification with hexane

Rough extract quinone was re-extracted using water and 20 mL hexane to remove impurities. The residue was then placed in a 50 mL centrifuge tube and was added with 10 mL of water. The extraction was done 3 times and the homogenization was done in 5 minutes [9,10]. The sample was centrifuged with a 7,000 rpm speed. After centrifugation, the upper layer, consisting of hexane and quinone, was evaporated using rotary vacuum evaporator with a temperature of <40°C [4,9].

### Quinone purification and separation

The resulting quinone extract was then purified and separated based on polarity difference. The column used was Sep-Pak (Waters, Tokyo, Japan) with 10 mm internal diameter, and 600 mg silica gel. Five millilitres of hexane

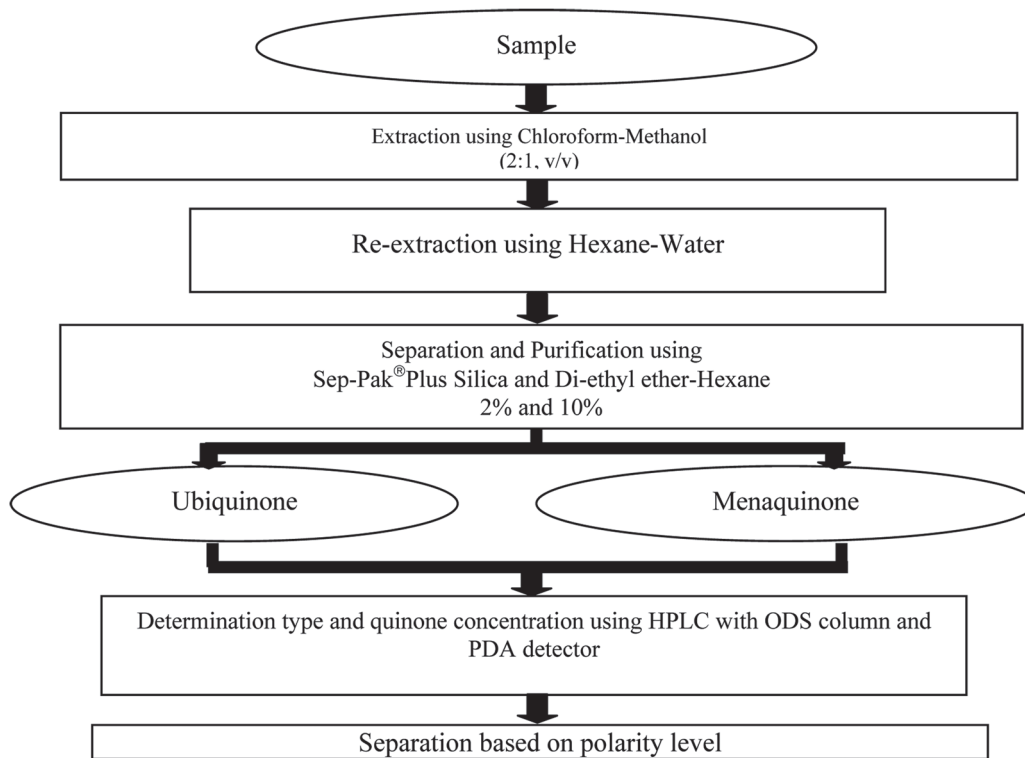


Fig. 2 Quinone analysis flow chart [3,4]

was passed through Sep-Pak cartridge with quinone extract. The menaquinone and ubiquinone were then eluted with 2% and 10% diethyl ether, respectively, in hexane solution. Then, menaquinone and ubiquinone were evaporated and the evaporation residue was re-dissolved in acetone for the subsequent quantitative analysis [10].

#### Species determination and quinone concentration

The quinone type and quinone concentration were determined using HPLC. Methanol and di-isopropyl ether mixture (9:2, v/v) was used as mobile phase at 1.0 mL/min flow rate. The temperature in the oven column was 35°C. Species of quinone was identified-based on HPLC column retention time with quinone standard and spectra absorption. This was done by measuring the UV spectra on each peak inside the multi-channel UV detector. Type of quinone was identified by the linear correlation between the logarithmic retention time of quinone and the number of isoprene unit (ENIU). Quantitative standard for ubiquinone and menaquinone used 10 isoprene unit ubiquinone and vitamin K1 ENIU was predicted with the equation below:

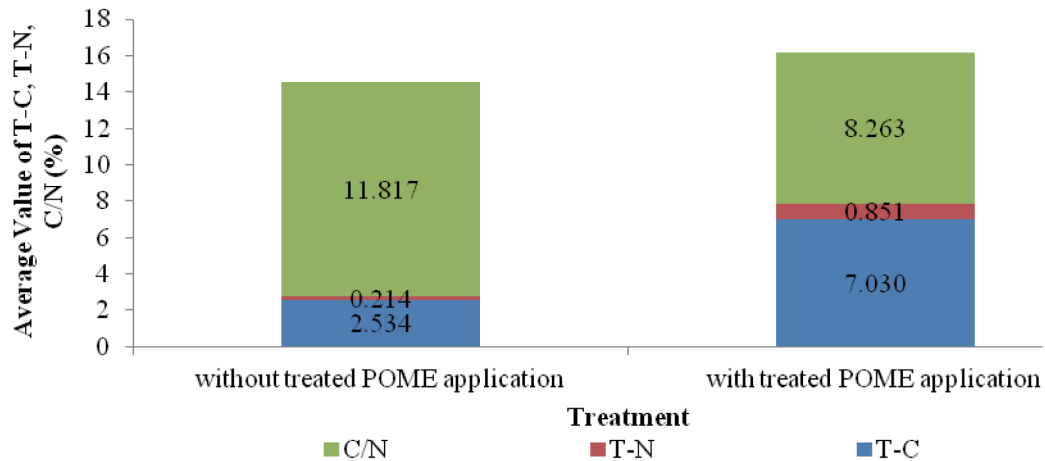
$$\text{ENIU}_k = a + b \log(\text{ET}_k/\text{ET}_{std}) + c [\log(\text{ET}_k/\text{ET}_{std})]^2 \quad (1)$$

where  $\text{ET}_k$  represents the elution time of a quinone species

$k$ , and  $\text{ET}_{std}$  represents the elution time of standard quinone. The constants are shown as  $a$ ,  $b$ , and  $c$ , which are empirically obtained for each HPLC system [11].

The amounts of quinone were calculated from the peak area based on the mole absorption coefficients (ubiquinones: 14.4 1/(mM·cm), menaquinones: 17.4 1/(mM·cm) and plastoquinones: 15.3 1/(mM·cm)) [12]. The quinone mole fraction was calculated as a ratio of the quinone content in the species  $k$  to the total quinone content. The abbreviations of quinone types are ubiquinone: UQ, menaquinone: MK, plastoquinone: PQ and vitamin K1: VK1. The nomenclature of bacterial quinone is as follow: the abbreviation for the type of quinone followed by a dash and the number of isoprene units in its side chain. For example, UQ-10 denotes a ubiquinone with 10 isoprene units in its side chain, and MK-9 (H2) shows a menaquinone with 9 isoprene units in its side chain and one of the double bonds in the side chain is saturated with two hydrogen [6].

In order to evaluate the changes of microbial community structure in the soils with and without treated POME application, the diversity ( $DQ$ ) and bioenergetic divergence ( $BD_q$ ) indices of respiratory quinone profile were calculated. These indices were calculated by the following equations:



**Fig. 3** Average value of Total C, Total N, and C/N ratio in soils without and with treated POME application.

$$DQ = \left( \sum_{k=1}^n (\sqrt{f_k}) \right)^2 \quad (2)$$

$$BDq = \left( \sqrt{UQ} + \sqrt{MK} + \sqrt{PQ} + \sqrt{VK1} \right)^2 \quad (3)$$

where,  $f_k$  is the mole fraction of quinone species  $k$ ;  $n$  is the number of quinone species with the mole fractions higher than or equal to 0.001; UQ, MK, PQ, and VK1 indicated the molar fraction of ubiquinones, menaquinones, plastoquinones, and vitamin K1, respectively;  $f_{ki}$  and  $f_{kj}$  are the mole fractions of quinone species  $k$  for  $i$  and  $j$  samples, respectively. On the basis of the fluctuations in dissimilarity of quinone profiles extracted from one soil, dissimilarities greater than 0.2 indicate a statistically significant difference in the quinone profile between two samples [13].

#### Total C and total N

The total carbon and nitrogen of soils were determined through dry combustion, using an Elementar Vario EL analyzer (Type CCA-1111 CE, Made by Eyla, China), from 5.0 mg (+0.1 mg) soil samples. This method use dry oxidation or combustion method.

#### Productivity of palm oil

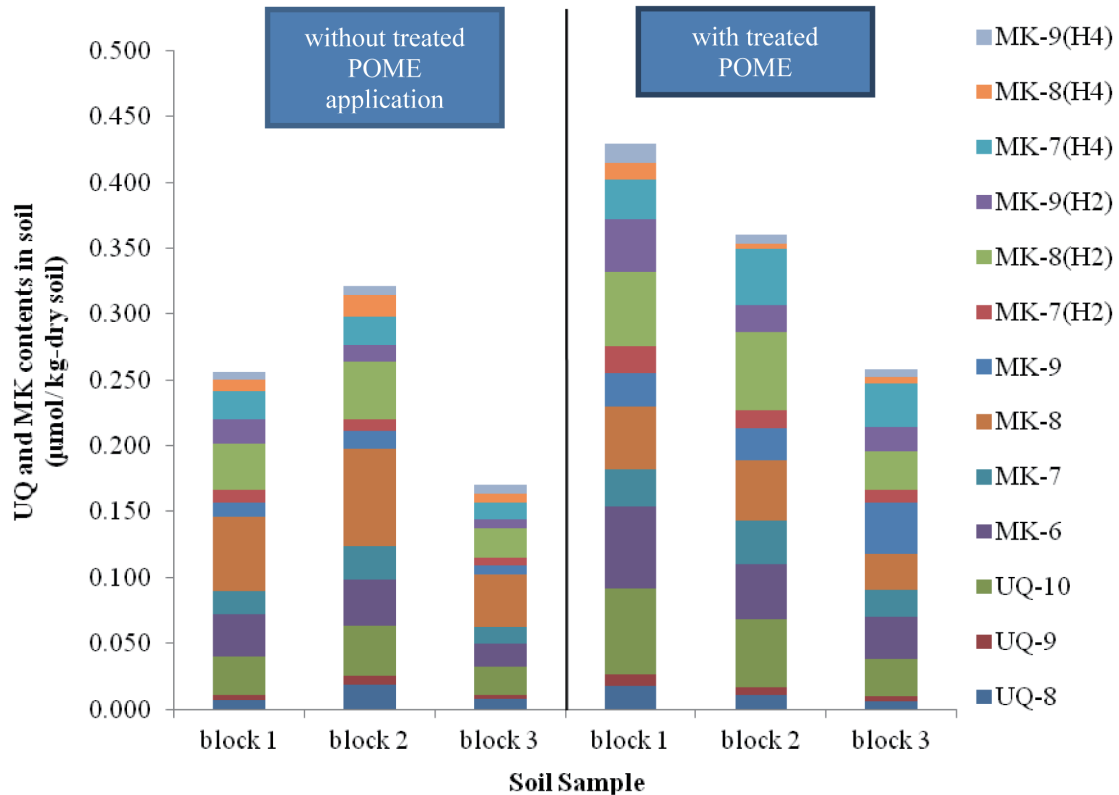
The data of productivity, number of bunches/tree, and average weight of palm oil fruit bunches were collected from records of the company (PTPN VII Unit Usaha Bekri in Sinar Banten Village).

## RESULTS AND DISCUSSION

### Microbial community structure

In the POME application, the treated POME with BOD lower than 5,000 mg/l was used as source of water and nutrient for the plants. The effluent was also expected to improve the soil properties and physical structure of soils, such as increasing permeability of soils, increasing moisture of soils, improving pH of soils and enriching content of organic compounds [2]. In this research, differences in soil characteristics without and with treated POME application were observed. The results showed that total C and total N contents in soils without treated POME application were 2.53% and 0.21%, respectively. These values are much lower than the total C and total N contents in soils with treated POME application which were 7.03% and 0.85%, respectively (**Fig. 3**).

The effluent was also expected to increase the activity of soil microflora and microfauna [13]. The activity of soil microflora and microfauna can be seen from the microbial community structure using the quinone analysis. The microbial community structure and biomass concentration are the important factors for the organic pollutant degradation capacity in the ecosystem. The quinone of microorganism is one of the bacterial respiration elements and has a very important role as electron transporter [9]. The quinone profile was presented as molar fraction of each quinone type. This has been approved as the simple mean and useful for the analysis of microorganism population of a mixed culture [4–6]. Some changes of microorganism population may not cause a change of quinone profile, because different bacteria could have the similar dominant quinone [7]. However, every



**Fig. 4** Average value of UQ and MK in soils without and with treated POME application in each plantation land block.

transformation of quinone profile is a transformation of bacterial population [4–6]. Indexes of the microbial community structure characteristic of a mixed culture using quinone profile include: (a) type and number of quinone species, (b) dominant quinone species and its molar fraction, (c) ratio of UQ and MK in the palm oil fraction, (d) diversity and equalization of quinone, (e) bioenergetic divergence index, and (f) amount of total quinone [4–6]. There are many quinones used as a sign of chemotaxonomic of systematic quinone, but it was also as a method to characterize the microorganism population in environment from quantity, quality and activity aspects [4].

**Figure 4** shows the contents of UQ and MK in soils without and with treated POME application in three points of each plantation land block. The results show that plantation soils of with and without treated POME application contain three types of UQ which are dominated by UQ-10 and 10 types of MK which are dominated by MK-8. UQ-10 contains few types of bacteria (*Acidiphilium*, *Agrobacterium*, *Ochrobactrum*, *Sphingomonas*), and few types of fungi (*Aspergillus/Neosartorya*, *Paecilomyces*, *Penicillium/Talaromyces*) [13]. MK-8 contains some bacteria (*Aeromonas*, *Proteus*, *Enterobacter*) [13].

The results of this research also show that there was a difference in microbial community structure in soils with and without treated POME application. **Figure 5** shows the comparison of the average values of UQ and MK in soils with and without treated POME application and **Fig. 6** shows the content of each quinone species in soils with and without treated POME application. **Figures 5 and 6** show the average values of UQ and MK contents in soils, which were observed from 18 samples (three points at three blocks for each treatment). Total UQ, MK and ratio of UQ/MK of soils with treated POME application were higher than that without treated POME application. Total UQ and MK of soils without treated POME application were 0.045  $\mu\text{mol/kg-dry soils}$  and 0.204  $\mu\text{mol/kg-dry soils}$ , respectively, whereas total UQ and MK of soils with treated POME application were 0.074  $\mu\text{mol/kg-dry soils}$  and 0.301  $\mu\text{mol/kg-dry soils}$ , respectively. The UQ/MK ratio of soils without treated POME application was 0.220, whereas UQ/MK ratio of soils with treated POME application was 0.249. These data show that the treated POME application has a potential to increase the quinone content in the soil. The quinone content can be used as an indicator of the amount of microorganism biomass [14].

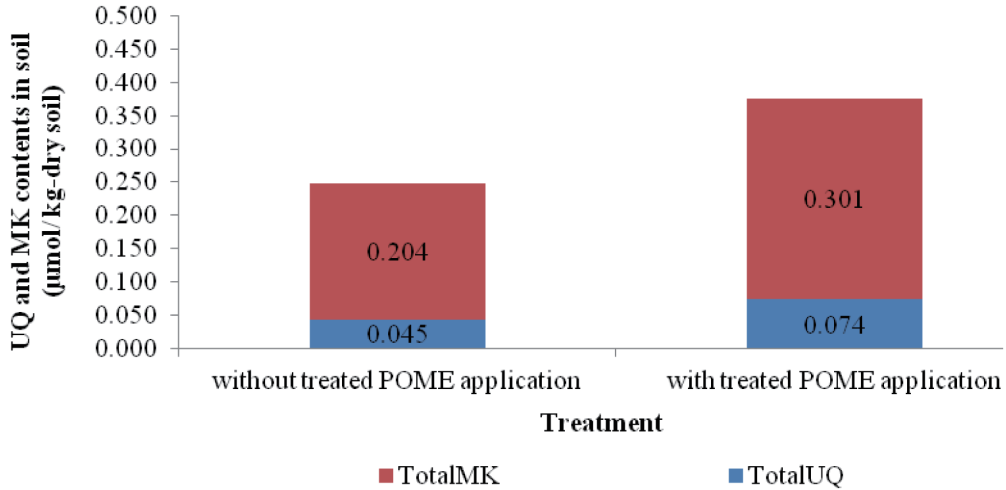


Fig. 5 Average value of UQ and MK in soils for each treatment (without and with treated POME application).

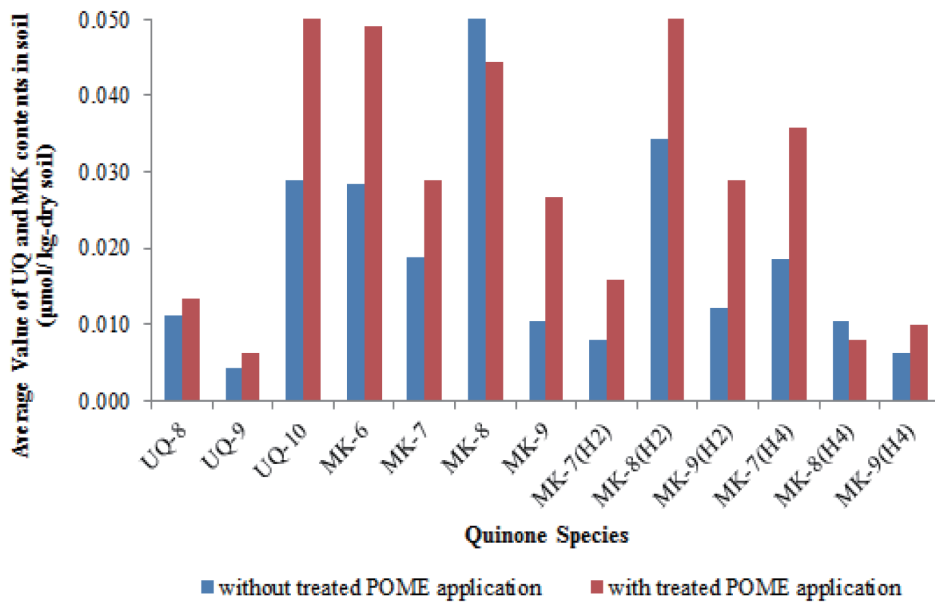


Fig. 6 Comparison on content of each quinone species in soils without and with treated POME application.

Figure 7 shows the percentage of UQ and MK in soils with and without treated POME application. Figure 7 shows that the percentages of UQ-8, UQ-9, MK-8, and MK-8 (H4) in soils have decreased after treated POME application, meanwhile percentages of UQ-10, MK-6, MK-7, MK-9, MK-7 (H2), MK-8 (H2), MK-9 (H2), MK-7 (H4), and MK-9 (H4) have increased. The results also show that the DQ and BDq are 11.32 and 0.87, respectively, for soils with treated POME application and 11.21 and 0.86, respectively, for soils

without treated POME application.

The diversity of microorganism has an important role on plant nutrient supply and absorption, because it provides a biochemical lane which can be counted to check the enzyme, antibiotic and another useful molecule. The existence of UQ in soils with treated POME application provides more energy needed by the microorganism for growth and cell maintenance. The UQ also functions as antioxidant, which protects cells from a damage that is caused by harmful mol-



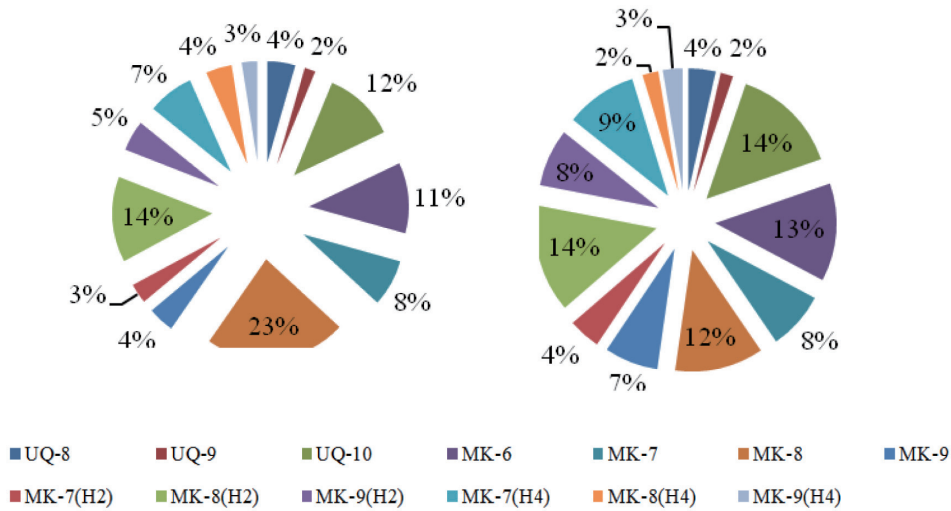


Fig. 7 Percentage of each quinone species in soils without and with treated POME application

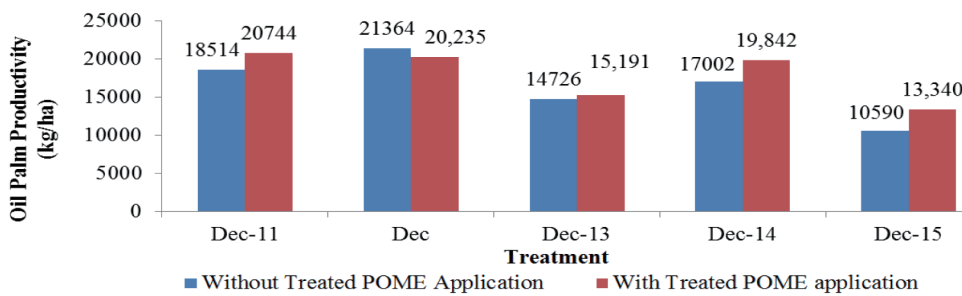


Fig. 8 Oil palm productivity without and with treated POME application.

ecules. Consequently, microorganism in the soils with the application of treated POME lead to better soil properties and physical structure, resulting in increased productivity of the oil palm plantation.

**Productivity of oil palm plantation**

Observations at PTPN VII Unit Usaha Bekri show that the use of treated POME as land application has an effect to the productivity, number of bunches/tree, and average weight of palm oil fruit bunches. The ratio of productivity, bunches/trees number, palm oil fruit bunches average area with treated POME application and without application are shown in Figs. 8, 9, and 10.

The effluent application as treated POME generally increased the productivity, bunches/trees number and average weight of palm oil fruit bunches, compared with that without treated POME application. These data correlate positively

with the quinone research data. The soils with treated POME application, that contain higher UQ, MK and UQ/MK ratio, have more improved characteristics and structure. Application of treated POME can lead to increased productivity, bunches/trees number and average weight of palm oil fruit bunches. Better soil characteristics and structure lead to improved conditions of mineralization process in the soils. The effluent used for treated POME application contains some essential elements providing the soils with N, P, and K. The application of treated POME provides N, P, and K which are important nutrients for oil palm growth.

**CONCLUSIONS**

The total UQ, the total MK and UQ/MK ratio of soils with treated POME application are higher than those of soils without treated POME application. It indicates that treated

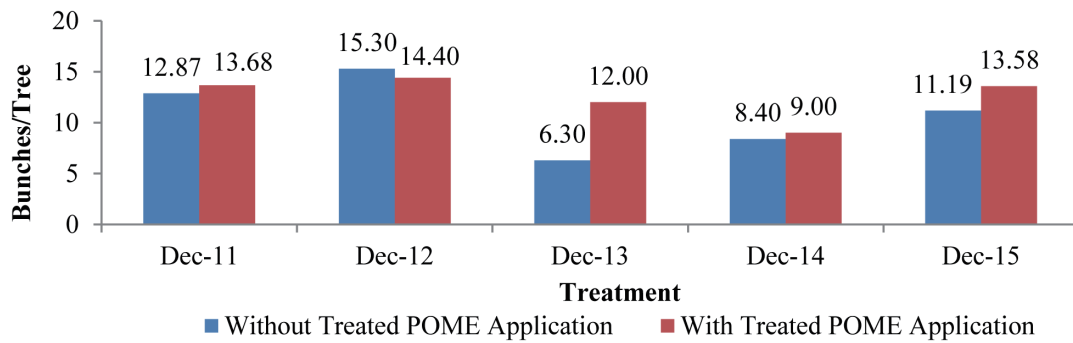


Fig. 9 Number of bunches per tree without and with treated POME application.

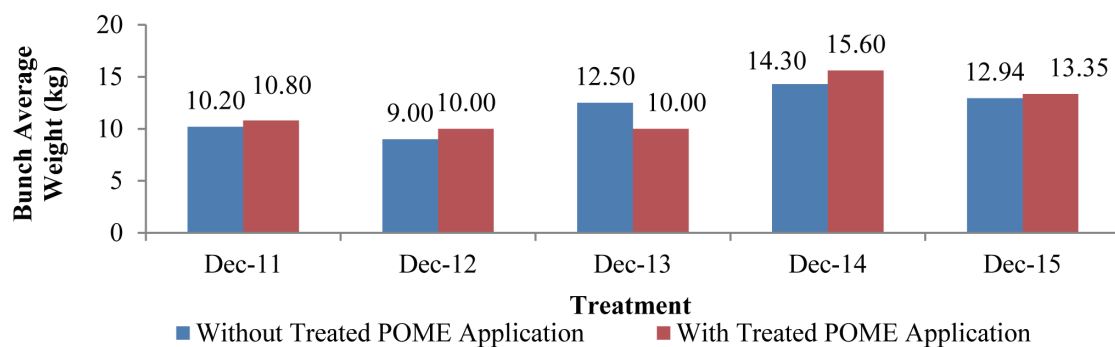


Fig. 10 Weight of bunches without and with treated POME application.

POME application can improve soil health condition in the oil palm plantation. The treated POME application increased the amount of microorganism but did not change the diversity of microorganisms in soils.

The oil palm plantation area applied with treated POME have generally the higher productivity, number of bunches per trees and average weight of fresh fruit bunches.

## REFERENCES

- [1] Indonesia Ministry of Agriculture: Kelapa Sawit Sumbang Ekspor Terbesar Untuk Komoditas Perkebunan. <http://ditjenbun.pertanian.go.id> [accessed in December, 2013, in Indonesian].
- [2] Rahardjo PN: Studi Banding Teknologi Pengolahan Limbah Cair Pabrik Kelapa Sawit. *J. Tek. Ling.*, **10**, Hal 09–18, 2009. [in Indonesian]
- [3] Hasanudin U: Study on microbial community structure and pollutant purification in coastal sediment. PhD thesis, Department of Ecological Engineering Toyohashi University of Technology, Japan, 2005.
- [4] Hiraishi A: Isoprenoid quinones as biomarkers of microbial populations in the environment. *J. Biosci. Bioeng.*, **88**(5), 449–460, 1999. doi:10.1016/S1389-1723(00)87658-6
- [5] Hu HY, Nozawa M, Fujie K, Makabe T, Urano K: Studies of microbial acclimation to hard chemicals on the basis of respiratory quinone profiles and kinetic analyses. *Water Sci. Technol.*, **34**(5–6), 249–256, 1996.
- [6] Hu HY, Lim BR, Goto N, Fujie K: Analytical precision and repeatability of respiratory quinones for quantitative study of microbial community structure in environmental samples. *J. Microbiol. Methods*, **47**, 17–24, 2001. doi:10.1016/S0167-7012(01)00286-X
- [7] Hiraishi A: Respiratory quinone profiles as a tools for identifying different bacterial populations in activated sludge. *J. Gen. Appl. Microbiol.*, **34**, 39–56, 1988. doi:10.2323/jgam.34.39
- [8] Hasanudin U: Aplikasi quinone profile untuk analisis perubahan perilaku mikroba di lingkungan. Jurnal FMIPA. Universitas Lampung, Lampung, 2007. <http://jurnal.fmipa.unila.ac.id/index.php/sains/article/view/274> [accessed in March, 2015, in Indonesian].



- [9] Hu HY, Fujie K, Nakagome H, Urano K, Katayama A: Quantitative analyses of the change in microbial diversity in a bioreactor for wastewater treatment based on respiratory quinones. *Water Res.*, **33**, 3263–3270, 1999<sup>a</sup>. doi:10.1016/S0043-1354(99)00044-5
- [10] Hu HY, Fujie K, Urano K: Development of a novel solid phase extraction method for the analysis of bacterial quinines in activated sludge with a higher reliability. *J. Biosci. Bioeng.*, **87**(3), 378–382, 1999<sup>b</sup>. doi:10.1016/S1389-1723(99)80049-8
- [11] Tamaoka J, Fujimura YK, Kuraishi H: Analysis of bacterial menaquinone mixture by high performance chromatography. *J. Appl. Bacteriol.*, **54**, 31–36, 1983. doi:10.1111/j.1365-2672.1983.tb01297.x
- [12] Kröger A: Determination of contents and redox states of ubiquinone and menaquinone. *Methods Enzymol.*, **53**, 579–591, 1978. doi:10.1016/S0076-6879(78)53059-0
- [13] Fujie K, Hu HY, Tanaka H, Urano K, Saitou K, Katayama A: Analysis of respiratory quinones in soils for characterization of microbiota. *Soil Sci. Plant Nutr.*, **44**(3), 393–404, 1998. doi:10.1080/00380768.1998.10414461
- [14] Katayama A, Fujie K: Characterization of soils microbiota with quinone profile. In: Bollag J-M, Stotzky G. (eds.), *Soils biochemistry*, Marcel Dekker, Inc., New York, USA, vol. 10, pp. 303–334, 2000.