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PALM OIL MILL EFFLUENT EFFECT ON SOIL FERTILITY: A LONGITUDINAL ASSESSMENT OF *ZEA MAYS* PLANT

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Abstract

This research evaluate the growth of maize (*Zea mays*) on palm oil mill effluent (POME) contaminated soil. The physiochemical properties and heavy metal loads of the soil samples and POME were determined. The growth rates of *Zea mays* was evaluated on shoot length, leaf length, root length, chlorophyll content, germination time, germination percentage and biomass.The palm oil mill effluent were mixed with the contaminated soil, the un-mixed soil was used as control. Soil dehydrogenase and phosphatase activities were assayed in contaminated and un-contaminated soil using standard methods. The plants were irrigated by serially diluting POME samples. The K^+, Ca^{2+}, Mg^{2+}, cation exchange capacity and phosphorus content of the POME contaminated soil were significantly ($P< 0.05$) lower than the control. There was no significant $P > 0.05$ difference in the C, N, Na^+ and organic content of the soil samples. The heavy metals in the POME showed Zn, Cu, Ni and Fe, but Pb, Cd and Cr were absent. POME contaminated soil and the control showed Pb, Zn, Ni and Fe, while Cd and Cr were not detected. The activities of dehydrogenase (0.13±0.57) and phosphatase activity (0.38±0.22) in POME contaminated soil decreased significantly $p \leq 0.05$ compared to the dehydrogenase (0.76±0.11) and phosphatase activity (1.35±0.36) in control soil. POME is a potent pollutant to inhibiting the growth of *Zea may*.

Keywords: *maize (Zea mays), POME, soil enzymes, soil pollution and soil fertility*

Introduction

Investigation on the germination of maize (*Zea mays*) grown on palm oil mill effluent (POME) contaminated soil was carried out. The physiochemical properties and heavy metal loads of the contaminated soil samples with POME were determined. Prior to planting of the maize, 400 g of the soil samples were mixed thoroughly with 200ml of POME and allowed to stand for 7 days with the control which received 200ml of distilled water before analysis. Dehydrogenase and phosphatase activities were assayed. From the onset of germination, the germinating zea may were irrigated with serially diluted POME samples as follows: 0%, 100%, 80%, 60%, 50%, 30% and 10% in a completely randomized design in triplicates. The performance and growth rates of the Zea mays were

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evaluated based on: shoot length, leaf length, root length, chlorophyll content, germination time and germination percentage and biomass. This ultimately provide information on the consequences of POME on soil ecosystem, to bring about the treatment model of POME before discharge and prevent the incident of soil ecosystem annihilation.

Oil palm (*Elaeis guineensis*) is one of the species of palm oil commonly called African oil palm or "macaw fat" (Obahiagbon, 2012). It is the primary source of palm oil. It originated from West and South Africa, specifically Angola and Gambia. The oil palm grows in warm climates 500 meters above sea level. This tropical plant is majorly grown for its industrial production of vegetative oil (Corley and Tinker, 2003). They are single stemmed plant and grow 20m tall. The leaves are pinnate and reach 3-5m (Nwaugo et al., 2008). Ehirim and Odii (2004) reported that economic crop provides the source of livelihood, allowing numerous small landholders to partake in the economy. Despite their usefulness to the economic growth of a nation, its processing technique has also contributed to server environmental pollution emanating from the large quantity of liquids and solids waste produced during the oil extraction process see figure 1 and 2.

Figure 1. *Remnants of fresh POME to be discharged*

Figure 2. *Discharged of treated POME on farmland*

These waste comprise of fibrous material (i.e. empty fruit bunches, palm pressed fibres and palm kernel shell and use fibrous materials (i.e. palm kernel cake and palm oil mil effluent (POME) (Cheah, 2003). During oil extraction process, the waste liquid generated is called POME. It is harmful to the soil ecosystem if discharged untreated into the environment (Verla et al., 2014).

POME is also a residual waste liquid (liquid left after degumming) that is left after recovery of oil (Nwoko et al., 2012). Fresh POME is a thick brown- like/ colloidal mixture of water, oil and fine suspended solids. It is hot (80ºC - 90 ºC) and possesses a very high biochemical oxygen demand, with acidic pH of 4 – 5 (Md Din et al., 2006; Nwoko et al., 2012). Due to the large volume of water used during oil processing, it was estimated that a milling plant with a capacity of 10 tons fresh fruit per hour may require water treatment plant that is equivalent to that needed by a population of million inhabitants (Brezing, 1986).

In Nigeria, palm oil extraction is dominated by subsistence farmers who use the crude method of extraction. POME generated is always poured away unguided into the environment. Especially farmlands close to the oil mill (Ogboghodo et al., 2001). The unrecoverable oil in the POME mixes with water, will float to the surface, forming a wide-spread film and making atmospheric oxygen unable to permeate the soil. The water body may become depleted with dissolved oxygen (Chavalparit, 2006). Communities located close to palm oil processing plant may suffer from odour emissions generated from the aged POME due to the release of hydrogen sulphide and malodorous gases into the environment (Hartley, 2004).

Materials and methods

Collection of the Palm Oil Mill Effluents. Palm oil mill effluent was collected at Ugochukwu Ndiogbuonyeoma M.P.C.S. Oil processing mill at Ndiogbuonyeoma Ndibeuche Arondizuogu in Okigwe Local Government Area of Imo state, Nigeria. The effluent was passed through a sieving cloth to remove debris, stones and suspended solids before transporting to the planting site and laboratory for analysis.

Collection of the Soil Samples. The soil samples for the planting of the maize were collected from University of Nigeria Botanical Garden 0-15 cm depth. The soil was handpicked to remove debris and large particles. A 200 ml of the POME was thoroughly mixed with 400 grams of the soil and left for 7 days to allow for mineralization and equilibration before analysis.

Preparation of the maize grains for planting. The maize grains were bought from Ogige market in Nsukka, Enugu State. Floatation technique was used to check for viability of the maize grain. The grains were soaked in water for 30 minutes, to remove the floated ones, while the descended ones were consider viable and used for the planting.

Experimental Design. The maize seeds were raised in a perforated polyethylene bag containing 2 kg of agricultural soil and a total of 200 ml of the effluent, clean water or the combination of the two were used in watering the potted plant at the interval of three days throughout the duration of the experiment. Seven groups were made and each group has 7 representation arranged in triplicate with each bag having 7 seeds planted (Table 1).

GROUPS	TREATMENT
1	treated with 100% WATER
2	treated with 100% POME
3	treated with 80% POME : 20% WATER
4	treated with 60% POME : 40% WATER
5	treated with 50% POME : 50% WATER
6	treated with 30% POME : 70% WATER
7	treated with 10% POME : 90% WATER

Table 1
Soil samples with zea maize plants treated with POME and water.

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After 7 days from the planting date, measu-rements were done on: shoot length, leaf length, leaf and width. The plants were up rooted after 22 days for biomass (wet-weight and dry weight), root length, chlorophyll content and heavy metal determination on the leaves of the maize plant.

Determination of total nitrogen. Total nitrogen in POME contaminated soil and control soil were determined using the macro - Kjeldahl method described by Bremner and Mulvaney, (1982).

Determination of available phosphorus. Available phosphorus in POME contaminated soil sample and control soil sample were determined by Vanadomolybdo-phosphoric acid colorimetric method described by Kitikum et al. (2000).

Determination of Sodium and Potassium. The exchangeable cations determined include: calcium (Ca), magnesium (Mg), Sodium (Na), and potassium (K). They were determined using modified methods described by Kitikum et al., (2000).

Determination of Cation Exchange Capacity (CEC). Cation exchange capacity was determined according to the method described by Chapman (1965).

Determination Soil Organic Carbon and Organic Matter. Organic carbon / organic matter were determined using dichromate oxidation method of Walkey and Black (1934) as modified by Nelson and Sommers (1982).

Determination of heavy metals in maize leaves in POME contaminated soils and control soil. The leaves were freshly cut, rinsed in deionised water, dried with unused cotton wool and labelled. They were packed in a thick brown envelope, after which it was oven dried over night at 110^{o}C. They were homogenised and analysed for Lead, Zinc, Cadmium, Chromium, Copper, Nickel and Iron after digestion according to the AOAC (1995) protocol.

Determination of the Chlorophyll Content in the Maize leaves. Chlorophyll concentration was determined using the method described by Wellburn, 1994.

Determination of the Soil Dehydrogenase Activity. Soil dehydrogenase activity was determined using Alef (1995). The soil dehydrogenases convert 2, 3, 5 - tripheyl tetrazolium chloride to formazan with optimum absorbance at 546nm. Dehydrogenase activity in soil was determined by estimating the rate of production of triphenyl formazan (TPF) from tri-phenyl tetrazolium chloride (TTC).

Determination of Soil Phosphatase Activity. Soil phosphatase activity was determined using Alef (1995).

For germination time, the potted plants where routinely checked and the rate limiting day it took the last seed per treatment to sprout was noted.

The germination percentage was calculated using the equation 1

$$\text{Germination \%} = \frac{NS}{TNS} \times 100 \qquad [1]$$

Where:
NS = Number of seeds germinated in each treatment;
TNS = Total number of seeds planted in the same treatment

Determination of the plant biomass (wet-weight and dry weight) (gram). The freshly uprooted maize plants were rinsed with distilled water and damped with dry cotton wool. They were weigh, followed by summation. They were packed in a thick brown envelope oven dried over night at 40^{o}C. Thereafter, the weight of each plant in a given treatment was taken, followed by summation of each plant value. Biomass content (g) was determined by differences in the wet and dry weights.

Statistical Analysis. Investigations were carried out in triplicate, and data presented as mean ± standard deviation using descriptive statistics. One Way Analysis of Variance (ANOVA) was used to compare mean difference between samples. Significance was accepted at $p < 0.05$.

Results and discussions

Heavy metal analysis of POME, POME contaminated soil, control soil and the maize leaves.

The heavy metals on POME, soil contaminated with POME, control soil and maize leaves were determined and presented in table 2 and 3. The Fe^{3+} concentration was 28.00 mg/kg higher in POME, soil contaminated with POME, 5.11 mg/kg and control soil 2.93 mg/kg. Other metals such as Zn^{2+}, Cu^{2+}, and Ni^{2+} were detected in POME as: 1.41 mg/kg, 2.10 mg/kg, and 0.27 mg/kg respectively. Pb^{2+} was undetected. In POME contaminated soil, Zn^{2+} had 3.90 mg/kg, Cu^{2+} 0.20 mg/kg, Ni^{2+} 0.40 mg/kg and Pb^{2+} 0.40 mg/kg separately. The control soil, gave Pb^{2+}, Zn^{2+}, Cu^{2+}, and Ni^{2+} as 0.50 mg/kg, 0.76 mg/kg, 0.20 mg/kg, respectively. Cd^{2+} and Cr^{2+} were undetected in all the test samples. Previous authors have reported the range of heavy metals detected in POME to be; Fe, 75-164 mg/l, Cu, 0.8-1.6 mg/l, Cd, 0.01-0.02 mg/l, Cr, 0.05-0.43 mg/l (Wood et al., 1979), Fe, 205 mg/l, Cu, 1.0 mg/l, Cd and Cr was undetected (Borja et al., 1996), Ni, 0.68-0.88 mg/l, Fe, 2.85-5.61 mg/l, Cu, 1.21-1.45 mg/l, Cd, 0.03±0.00 mg/l, Cr, 2.01±0.08 mg/l (Verla et al., 2014). The variations of each element in POME, when compare to previous reports, POME contaminated soil and control soil were due to various factors such as trace metal contents of the palm fruits, the nature of parent materials and contamination from the engine during digestion (Abulude et al., 2007). The hardness of the water used during processing was a contributing factor of heavy metals (Adeyeye and Ayejuyo, 2002). The presence of soil microorganisms may alter the concentration of individual heavy metal. For instance, *Pseudomonas putida* have the capacity to degrade toxic compounds in the soil. The decrease in the performance of maize planted in POME contaminated soil may be as a result heavey metals present in POME, which inhibited the maize growth. By affecting dehydrogenase and

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phosphatase. The negative influence of some heavy metals on the activity of soil enzymes was reported by Leiros *et al.*, (1999).

Table 2. *Atomic absorption spectrophotometer analysis of POME, POME contaminated soil and Control soil. Values expressed in mg/kg.*

Sample ID	Pb^{2+}	Zn^{2+}	Cd^{2+}	Cu^{2+}	Cr^{2+}	Ni^{2+}	Fe^{3+}
POME	ND	1.41	ND	2.10	ND	0.27	28.0
POME contaminated soil	0.40	3.90	ND	0.20	ND	0.40	5.11
Control soil	0.50	0.76	ND	0.20	ND	0.55	2.93

ND = Not Detected

Contrary to the opinion of Li et al., (2005), he reported an increase in activities of dehydrogenase is proportional to increased concentrations of the pollutant. Though he worked on the effect of petroleum-contaminated wastewater, which may serve as carbon source. Thus, the reduction in the enzyme activities as observed ascribed to the effect of heavy metals on some soil microorganisms. Nies, (1999) and Deng and Tabatabai, (1995) have reported that metal ion may inhibit enzyme reactions by reacting with the protein active groups of the enzyme or with the enzyme substrate complex.

The heavy metal analysis of the maize leaves in table 3 showed that cadmium and chromium were undetected in all the samples. Lead and copper concentrations considerably above the FAO (2005) permissible limits of 0.01 mg/kg to 0.20 mg/kg. Zinc and iron concentrations were below the FAO (2005) acceptable limits of 5.00 mg/kg to 425.00 mg/kg. Nickel concentration in maize leaves was above the FAO (2005) acceptable limit (0.20mg/kg) and nickel concentration in maize leaves was below the FAO (2005) acceptable limit of 0.20mg/kg.

Groups	Pb	Zn	Cu	Ni	Fe	Cd	Cr
1	0.3	ND	0.7	0.65	3.21	ND	ND
2	ND	ND	ND	0.45	6.38	ND	ND
3	0.2	0.12	ND	0.23	4.21	ND	ND
4	0.4	0.04	0.5	0.13	6.83	ND	ND
5	0.2	0.02	0.3	0.13	4.50	ND	ND
6	ND	0.03	0.4	0.08	4.01	ND	ND
7	ND	0.07	0.6	0.12	2.13	ND	ND

ND = Not Detected

Table 3
Atomic absorption spectrophotometer analysis of heavy metals (mg/kg) for the maize leaves of the seven treatment

In table 4, there was significant decrease ($P < 0.05$) in the dehydrogenase and phosphatase activities of POME contaminated soil relative to the control soil sample. The significant decrease in dehydrogenase and phosphatase activities may

be attributed to exposure of the soil enzymes systems to more organic matter in the effluent (Sinsabaugh et al., 1991).

Sample ID	Dehydrogenase (µg/g)	Phosphatase (µg/g)
Control	0.76±0.11	1.35±0.36
POME treated soil	0.13±0.57	0.38±0.22

N = 2 - Results expressed in mean ± SD.
Significant at (P < 0.05) - Values are means of triplicates ± SD

Table 4.
Soil enzyme activity of non POME contaminated soil and POME contaminated soil in µg/g dwt, where dwt is dry weight of 1 g moist soil.

The influence of POME on the chlorophyll content, shoot length, leaf length, leaf width, root length, biomass, and germination rate (germination time and germination percentage)on maize were determine and presented in tables 5, 6,7, 8, 9 and 10 respectively. The biomass, chlorophyll content, germination rate (germination percentage and germination time) of the maize planted with 100% W was significantly higher (P < 0.05) than the other treatments except in the case of the root length measurement; where the maize planted in 100%P was significantly higher (P < 0.05) than other treated samples. The performance of the maize growth in varying concentrations of the effluent, showed that an increase in the concentration of the POME is associated with a decrease in the performance of the maize growth. This agreed with Madaki and Seng, (2013) who reported the effect of untreated sugar mill effluent at variable concentrations (0, 25, 50, 75 and 100 %) on germination, speed of germination, peak value and germination value of peanut (*Arachis hypogea*) and green gram (*Vigna radiata*). It was reported the germination percentage decreased with the increasing concentration of effluent in all the tested seeds, while the germination speed (%), peak value and germination increased from control to 25 % and 50 % concentration and decreased from 50 % to 75% and 100 % effluent.

GROUP	Germination time	Germination (%)
1	5.00 ± 0.00^{a}	87.70 ± 0.17^{f}
2	7.00 ± 0.58^{c}	68.30 ± 0.36^{a}
3	7.00 ± 0.00^{c}	69.30 ± 0.20^{b}
4	7.00 ± 0.00^{c}	74.70 ± 0.26^{c}
5	6.00 ± 0.00^{b}	75.50 ± 0.35^{d}
6	6.00 ± 0.58^{b}	77.50 ± 0.26^{e}
7	5.00 ± 0.00^{a}	87.70 ± 0.26^{f}

n = 7
Results expressed in Mean ± SD
Different letters in each column indicate significant differences at $P < 0.05$.

Table 5
Germination time and germination percentage of each treatment

There was a significant (P < 0.05) decrease in the chlorophyll a and chlorophyll b content of maize leaves in other groups relative to the control. The reduction in

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chlorophyll content results from the Cu-induced inhibition of aminoleaevulinic acid-dehydratase, reported by Scarponi and Perucci (1984). Izawa, (1977) suggested that the inhibition of chlorophyll was due to induced inhibition of electron transport System in PS-I.

The significant fall in the chlorophyll content under the higher percentage of effluent concentration was due to inhibitory effect of toxicants of effluent on chlorophyll synthesis in exposed plant.

Table 6
Chlorophyll 'A' and 'B' content of the maize leaves.
Values expressed in mg/g

GROUP	Chlorophyll A	Chlorophyll B
1	1.19 ± 0.03^{g}	0.68 ± 0.00^{g}
2	0.66 ± 0.19^{a}	0.40 ± 0.00^{a}
3	0.68 ± 0.10^{b}	0.42 ± 0.00^{b}
4	0.78 ± 0.08^{c}	0.47 ± 0.00^{c}
5	0.84 ± 0.00^{d}	0.51 ± 0.00^{d}
6	0.88 ± 0.02^{e}	0.53 ± 0.00^{e}
7	0.89 ± 0.01^{f}	0.55 ± 0.00^{f}

n = 7
Results expressed in Mean ± SD
Different letters in each column indicate significant differences at $P < 0.05$

The root length of maize planted in 100% POME was significantly higher ($P < 0.05$) than other treatments. The mechanisms plants use to withstand harsh conditions; deep roots penetration and development is a positive attribute that give plant advantage over toxic compounds. The assumption is that the maize seeds may have concentrated its energies towards root development.

Table 7. *Shoot length (cm) of each treatment in different days*

GROUP	1st day of PPM after 5 days of planting	2nd day of PPM after 8 days of planting	3rd day of PPM after 11 days of planting	4th day of PPM after 14 days of planting	5th day of PPM after 17 days of planting
1	2.80 ± 0.50^{c}	4.40 ± 0.00^{cd}	7.13 ± 0.31^{c}	10.60 ± 0.10^{c}	12.23 ± 0.58^{d}
2	1.53 ± 0.15^{a}	3.50 ± 0.00^{a}	5.47 ± 0.75^{a}	8.63 ± 0.15^{a}	10.50 ± 0.10^{a}
3	1.70 ± 0.10^{ab}	3.53 ± 0.58^{a}	5.97 ± 0.12^{ab}	9.00 ± 0.58^{b}	10.67 ± 0.50^{ab}
4	1.97 ± 0.06^{b}	3.70 ± 0.30^{ab}	6.00 ± 0.20^{ab}	9.13 ± 0.58^{bc}	11.03 ± 0.12^{bc}
5	2.80 ± 0.10^{c}	4.47 ± 0.58^{d}	6.70 ± 0.80^{bc}	9.27 ± 0.58^{c}	11.17 ± 0.58^{c}
6	2.53 ± 0.15^{c}	3.87 ± 0.95^{abc}	6.93 ± 0.58^{c}	10.13 ± 0.58^{d}	12.23 ± 0.15^{d}
7	2.83 ± 0.56^{c}	4.00 ± 0.20^{abc}	6.46 ± 0.71^{c}	10.03 ± 0.58^{d}	12.17 ± 0.67^{d}

n = 7
Different letters in each column indicate significant differences at $P < 0.05$.
Letter 'a'<'b'<'c'<'d' - PPM = Post Planting Measurement

The soil properties of the POME contaminated soil decreased while the plant growth and its performance were reduced with increasing effluent concentration.

Table 8. *Leaf length (cm) of each treatment in different days*

GROUP	1st day of PPM after 5days of planting	2nd day of PPM after 8 days of planting	3rd day of PPM after 11 days of planting	4th day of PPM after 14 days of planting	5th day of PPM after 17 days of planting
1	7.61 ± 0.23^{f}	9.70 ± 0.40^{b}	12.70 ± 0.85^{c}	14.65 ± 0.91^{c}	16.27 ± 0.58^{d}
2	5.03 ± 0.06^{a}	8.10 ± 1.20^{a}	9.10 ± 0.10^{a}	12.37 ± 0.15^{a}	14.63 ± 0.58^{a}
3	5.50 ± 0.17^{b}	9.93 ± 0.65^{b}	9.93 ± 0.15^{ab}	12.37 ± 0.15^{a}	15.20 ± 0.10^{b}
4	6.40 ± 0.00^{c}	9.10 ± 0.30^{ab}	10.03 ± 0.25^{ab}	12.7 ± 0.10^{ab}	15.33 ± 0.58^{c}
5	7.73 ± 0.12^{f}	10.00 ± 1.10^{b}	10.27 ± 1.70^{ab}	14.50 ± 0.17^{c}	16.27 ± 0.58^{d}
6	6.87 ± 0.12^{d}	9.67 ± 0.06^{a}	11.03 ± 0.58^{b}	13.00 ± 0.10^{ab}	16.23 ± 0.58^{d}
7	7.27 ± 0.58^{e}	0.13 ± 0.85^{ab}	11.00 ± 0.10^{b}	13.20 ± 0.17^{b}	16.17 ± 0.12^{d}

n = 7

Different letters in each column indicate significant differences at $P < 0.05$.

Letter 'a'<'b'<'c'<d<'e'<'f' - PPM = Post Planting Measurement

Table 9. *Leaf width (cm) of each treatment in different days*

GROUP	1st day of PPM after 5 days of planting	2nd day of PPM after 8 days of planting	3rd day of PPM after 11 days of planting	4th day of PPM after 14 days of planting	5th day of PPM after 17 days of planting
1	0.93 ± 0.58^{d}	1.13 ± 0.58^{c}	1.17 ± 0.15^{c}	1.43 ± 0.12^{b}	1.53 ± 0.58^{d}
2	0.70 ± 0.00^{a}	0.77 ± 0.58^{ab}	0.70 ± 0.10^{a}	0.80 ± 0.10^{a}	0.83 ± 0.58^{a}
3	0.70 ± 0.10^{a}	0.70 ± 0.10^{a}	0.77 ± 0.58^{a}	0.80 ± 0.10^{a}	0.87 ± 0.58^{a}
4	0.80 ± 0.00^{b}	0.87 ± 0.58^{b}	0.87 ± 0.58^{a}	0.90 ± 0.00^{a}	0.90 ± 0.10^{a}
5	0.83 ± 0.58^{bc}	0.73 ± 0.58^{a}	0.87 ± 0.58^{a}	0.93 ± 0.58^{a}	1.20 ± 0.10^{c}
6	0.90 ± 0.00^{cd}	1.03 ± 0.58^{c}	1.07 ± 0.15^{c}	1.33 ± 0.58^{b}	1.43 ± 0.15^{d}
7	0.92 ± 0.40^{cd}	0.90 ± 0.17^{c}	1.17 ± 0.15^{c}	1.40 ± 0.17^{b}	0.50 ± 0.10^{d}

n = 7

Different letters in each column indicate significant differences at $P < 0.05$.

Letter 'a'<'b'<'c'<'d' - PPM = Post Planting Measurement

GROUP	BIOMASS
1	96.80 ± 0.01^{g}
2	50.87 ± 0.01^{a}
3	63.84 ± 0.01^{b}
4	74.46 ± 0.15^{c}
5	81.38 ± 0.02^{d}
6	82.86±0.03e
7	85.30 ± 0.21^{f}

Table 10

Biomass (g) of each treatment

n = 7 Results expressed in Mean ± SD

Different letters in each column indicate significant differences at $P < 0.05$.

Due to the high concentrations of oil and gum in the oil palm processing effluents, discharging into the environment promiscuously on farmlands and water bodies could cause pollution. Therefore best approaches to treating this effluent before discharging should modelled.

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Conclusions

The investigation has proof undoubtedly the consequence of discharging untreated POME on Agricultural land. It elevated level of heavy metals is a threat to the soil ecosystem and soil properties. Though some vital elements in POME may exert positive effects the growth and nutrient uptake by the maize plant.

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