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Effect of Produced Formation Water on Soil Nitrifying Bacteria and Earthworms

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ABSTRACT: The research work was carried out to investigate the effects of produced formation water on soil nitrifying bacteria and earthworm using standard methods. The microbial analysis of produced formation water samples showed the presence of *Pseudomonas aeruginosa*, *Bacillus cereus*, *Micrococcus luteus and Yeast and Aspergillus niger*. Physicochemical parameters of produced formation water (PFW) analysed shows neutral pH (6.33), high electrical conductivity (960.6 S/cm), lower than accepted temperature (28.46^{o}C), total dissolved solids (TDS) (756mg/l), dissolved oxygen of 4.6mg/l and higher than accepted of nitrate (52.mg/l), sulphate, lead, chromium, cadmium and copper. The PFW treatment was observed to increase nitrate content and optical density of receiving soil. The treatment progressively decreased the *Nitrobacter* count across the experimental duration period (seven days) and decreased earthworm survival as the % treatment increases. This study revealed that produced formation water negatively impacts the receiving soil and its organisms in several ways. Hence, regulatory bodies could strengthen its sensitization to the public and private enterprises and the general populace on the damage of discharge of produced formation water.

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Produced water is often generated during the production of oil and gas from onshore and offshore wells. Formation water is seawater or fresh water that has been trapped for millions of years with oil and natural gas in a geologic reservoir consisting of a porous sedimentary rock formation between layers of impermeable rock within the earth's crust (Collins, 1975). Produced formation water is a by-product of oil and gas hydrocarbons production from underground reservoirs (Atuanya and Obikudo, 2004). Several substances or waste materials introduced into the environment as a result of petroleum exploration and exploitation activities may be toxic and persist in their immediate environments (Ajuzie and Osaghae, 2001). According to Atuanya and Obikudo (2004), during the production of oil and gas, water is co-produced in sufficient amount. Atuanya and Obikudo (2004) described that produced formation water, which is a natural water layer formed in oil and gas reservoir, being denser lies under the hydrocarbons. It was however described that oil reservoirs frequently contain large volume of water, and that gas reservoirs tend to produce only small quantities (Atuanya and Obikudo, 2004). To obtain maximum oil recovery, additional water is often injected into the reservoirs to help force oil to the surface. Veil *et al*. (2004) also stated that fresh water, brine/seawater, and production chemicals sometimes are injected into a reservoir to enhance both recovery rates and the safety of operations and these surface waters and chemicals sometimes penetrate to the production zone and are recovered with oil and gas during production. Produced water (formation and injected water containing production chemicals) has been

documented to represent the largest volume waste stream in oil and gas production operations on most offshore platforms (Krause, 1995). Produced water may account for 80% of the wastes and residuals produced from natural gas production operations (McCormack *et al.,* 2001). Produced water is a complex mixture of dissolved and particulate organic and inorganic chemicals. The physical and chemical properties of produced water vary widely depending on the geologic age, depth, and geochemistry of the hydrocarbon-bearing formation, as well as the chemical composition of the oil and gas phases in the reservoir, and production chemicals added to the production. Produced formation water are effluent discharge from the production of oil and gas. Effluent is a liquid waste produced from human activities. Domestic commercial and industrial activities are some major human activities that generate effluent (Albert and Tanee, 2016). According to Ajuzie and Osaghae (2011), these effluents can be harmful to the environment as these effluents often contain chemicals that can alter the physical and chemical properties of soil, resulting in destruction of soil ecological balance. Most environmental regulatory agencies in countries that have significant offshore oil and gas production place limits on the concentration of petroleum (usually measured as total oil and grease) that can be present in produced water destined for ocean discharge. It is stipulated that produced water be treated before ocean discharge to avoid the harmful effects that the chemicals in the waste waters may have on the receiving environments. However, not every production plant is critical about this activities. Udochukwu *et al.* (2017) thereby described that the final destinations of persistent contaminants from this effluent discharge to water bodies is often the soil, thereby rendering the soil environment hazardous to soil biological sentiments. Soil biological sentinels are indicator organisms in the soil that are pointer to soil contamination and pollution (Udochukwu *et al.,* 2017). An indicator species is any biological species that defines a trait or characteristics of the environment (Udochkwu *et al*., 2017). Indicator organisms usually indicate an environmental condition including pollution, species competition or climate change. Indicator species are very sensitive in the region where they are found but can be used as a monitoring tool for ecological balance by ecologist. Earthworm and nitrifying bacteria (*Nitrobacter* and *Nitrosomonas*) are the major soil sentinels (Udochukwu *et al.,* 2017). Caranto and Lacaster (2017) described nitrification, an important player in agricultural systems to aid soil fertility and ecological balance, as the biological oxidation of ammonia or ammonium to nitrite followed by the oxidation of the nitrite to nitrate. Nitrification also plays an important role in the removal of nitrogen from municipal wastewater (Kuypers *et al.,* 2011). These *Nitrobacter* and *Nitrosomonas,* used in this study, are the major well known nitrifying bacteria that carry out nitrification process in the soil. Earthworms are simple cylindrical, coelomate and segmented burrowing animals which are hermaphrodites. Some species of earthworm include *Agastrodrilus, Pluvialus sp, Lumbricus sp, Allolobophora* etc. (Liebieke *et al.,* 2015). They are largely regarded as perhaps the most important soil organisms in terms of their influence on organic matter breakdown, soil structural development and nutrient cycling. Earthworms' activities aerate the soil and are conducive to mineralization of nutrient and their uptake by vegetation. They are sentinels that can be used to assess toxicity in terrestrial system as they are sensitive to contaminants (Liebieke *et al*., 2015). Based on the foregoings, this study investigated the effects of produced formation water (PFW) on soil nitrifying bacteria and earthworms.

MATERIALS AND METHODS

Sample collection: Three (3) samples each of produced formation water (PFW) were collected directly from three selected categories of oil and gas reservoirs in Utorogu and Olomoro fields of Shell Petroleum Development Company (SPDC) operations. These categories include: a) produced formation water samples from oil well reservoirs that received injection gas in Olomoro field, (b) produced formation water samples from gas well reservoirs in Utorogu field on land, and (c) produced formation water samples from onshore oil well that is not receiving injection gas in Olomoro field. The samples were collected at an interval of seven-days for each reservoir sample. The samples were collected with sterile 250ml bottles with stopper and transported to the laboratory for further analysis. Soil samples were collected in sterile polyethylene bags at a depth of 0-15cm. The effluents samples dissolved oxygen and Biological oxygen demand were fixed on site by adding 1.0ml each of wrinkler's solution and taken to laboratory for further preparation. The earthworms (*Lumbricus terrestris*) were collected from University of Benin botanical farm. The worms were collected according to the method described by Spiegel (2002). They were collected by digging and hand sorting from sub surface litters and taken to the laboratory for identification. They were then washed and left on moist filter paper for 12hours to void their gut contents. The earthworms used were selected based on maturity (shown by the presence of clitellum and liveliness).

Physicochemical Analyses of Produced Formation Samples: Produced formation water samples were

analysed for the following physicochemical parameters: pH, temperature, conductivity, total solid, total dissolved solid, turbidity, total suspended solid, chemical oxygen demand (COD), biochemical oxygen demand (BOD) was determined and dissolve oxygen (DO). pH and conductivity of PFW samples were carried out using Jenway pH and conductivity meter (45a O model). Temperature test was determined using a mercury-in-slot thermometer at 100ºC. Total solid, total dissolved solid (TDS) and total suspended solid (TSS) were determined using gravimetric methods, according to APHA (1993). Turbidity was measured using a program 96 Bach colorimetric method while dissolved oxygen (DO) was determined on a dissolved oxygen meter according to APHA (1993). Chemical oxygen demand (COD) was determined using potassium dichromate titrimetric method while biochemical oxygen demand (BOD) using dissolve oxygen, 5-day incubation method according to APHA (1993).

Isolation and Enumeration of nitrifying bacteria: The method used for the isolation of bacteria (*Nitrosomonas and Nitrobacter*) from soil was adopted from Colwell and Sambrusky (1972). *Nitrosomonas* was isolated using winogradsky metium for nitrification phase 1, which is made up of (NH4) $2SO_4$, 2.0g, K_2KPO_4, 1.0g, $MgSO_4$.7H2O, 0.5g, NaCl, 2.0g, $FeSO_4.7H_2O$, 0.4g, $CaCO_3$, 0.01g, agar 15.0g, distilled water, 1000ml. *Nitrobacter* was isolated using Winogradsky medium phase 2(KNO_2 0.1g, Na_2CO_3 1.0g, NaCl 0.5g, $FeSO_4$. $7H_2O$ 0.4g, agar 15.0g, distilled water 1000ml). The sterilized media are aseptically dispensed into sterile petri-dishes after cooling to about 45ºC. The petri dishes containing solidified nutrient medium were then inoculated with serially diluted soil samples and incubated aerobically for 4 days at room temperature (28±ºC). After incubation, growth of bacterial colonies were observed, and number of bacteria colonies was counted and expressed in colony forming unit per gram (cfu/g). Standard incocula were prepared from the stock cultures.

Estimation of heterotrophic bacterial and fungal counts from PFW samples: PFW samples were collected from the sterile container. One lillilitre (1ml) of the sample was measured into 9ml of sterilized distilled water. The solution was then serially diluted to 10^{-3} (3-fold). 0.1ml of the 10^{-3} dilution was used for the estimation of aerobic heterotrophic bacterial and fungal population by spread plate inoculation into appropriate media. This plating was done in duplicates. Nutrient agar and potato dextrose agar were used for bacterial and fungal enumeration respectively.

Characterization and identification of bacteria isolates from the PFW samples: To obtain pure culture, each discrete colony was sub-cultured and stored on agar slant at 4ºC for further characterization. The isolates were identified by their morphological, cultural and biochemical characteristics. Cultural characteristics of the bacterial isolates were observed on nutrient agar plates. The pure cultures were characterized using different methods of microscopic observations including Gram reaction and motility test. The cultural characteristics observed on solid media include colony shape, size, margin, surface, colour, elevation and transparency. Isolates were identified using standard morphological characteristics and identification keys for bacteria as described by Bergey and Holt, (1993). Biochemical characterization of the isolates includes coagulase test, indole test, oxidase test, catalase test, urease test, citrate, glucose and sucrose utilization and Gram staining.

Preparation of diluents for toxicity tests: Sodium nitrite (0.25mg NaNO2/I, Winigradsky broth) and ammonia sulphate (5.0mg (NH4)2SO4/I Winogradsky broth) diluents for *Nitrobacter* and *Nitrosomonas* respectively were adopted. The diluent were sterilized at 121ºC and 15 PSI for 15 minutes.

Preparation of formation water concentration for Nitrobacter toxicity tests: PFW samples concentration of 100, 150, 200 and 250 ml/L were used to determine median lethal concentration (LC50) for *Nitrobacter* sp. A control experiment was also set up.

Nitrobacter and Nitrosomonas acute toxicity tests: To each of the PFW samples concentration (90ml) was measured into 250ml volumetric flask, 10ml of bacteria (*Nitrobacter* and *Nitrosomonas*) standard inoculum was aseptically inoculated. The nitrite content of the medium containing both PFW concentration and the bacteria inoculum was determined using spectrophotometer. This was sub-cultured at first day. Following incubation of the diluents containing the toxicant and the standard inoculum, they were sub-cultured at one day interval for four days. Sub-culturing was done by spread plate method on winogradsky media. Phase I was used for *Nitrosomonas* and phase 2 for *Nitrobacter*. Sub-cultured plates were incubated at room temperature (28 + or -2ºC) for 72 hours. The number of colonies observed after incubation for both *Nitrobacter* and *Nitrosomonas* were counted and recorded for each plate. The percentage inhibition of bacterial growth (Log survival) was plotted against test concentration. A control experiment was also set up by inoculating the diluents without toxicant. The percentage growth

inhibition was determined by comparing the bacterial growth of the treated with the control. The percentage inhibition/deviation was calculated using the formulae below (described by Grunditz and Dalhammar, 2001).

$$Inhibition\ (\%) = \frac{C_{ref} - C_{sample}}{C_{ref}} x100$$

Where C_{ref} is colonies in control. C_{sample} is colonies in treated sample.

Earthworm bioassay: The earthworms were obtained and maintained in the laboratory using the procedure described in ASTM standard E-2172-01 (ASTM, 2001). Soil samples were obtained from the organism's habitat. Five concentration (100, 150, 200 and 400ml/kg) of the PFW samples were prepared for the definitive test. Five hundred grams (500g) of the soil were mixed with various test concentration of the PFW samples. The soil samples were inoculated with earthworm (one worm per treatment). The worms were weighed before inoculation and the survived worms were also weighed after test period. The worms used for the experiment weighed between 500mg and 700mg. Before inoculation, the worms were placed on the moist filter paper in covered petri dishes for 24hrs to void their gut contents. During this test period, earthworms were not fed so that the effect of PFW samples on the growth of the earthworms will be evident (Udochukwu *et al.*, 2017). Distilled water (50ml) was added to the soil mixture to achieve 45% moisture content. A control containing only soil, water and the test organisms was also set up. The distribution of the test organisms among the test chambers was randomized. Earthworms' survival and body weight were determined at 1, 4, 7 and 17days interval after inoculation. Death was the primary criterion used in this test guideline to evaluate the toxicity of the test substance (Atuanya and Tudararo-Aherobo, 2014). However, in addition to death, weight loss, behavior symptoms and pathological symptoms were recorded. Mortality was assessed by emptying the test medium on a glass or other inert surface and the earthworms were sorted from test mixture and their reactions were tested by a gentle mechanical stimulus. Any adverse effects were noted and reported. The 14th day test would be unacceptable if more than 30% of the control organisms died or the total mean weight of the earthworms in control chambers declined significantly (by 30%) during the test.

Statistical analysis: The data obtained from the different parameters were subjected to Statistical Package for Social Sciences (SPSS), using Analysis of Variance. The probability level used for statistical significance was $p<0.05$ for the test.

RESULTS AND DISCUSSION

Table I shows the physicochemical properties of produced formation water collected from three oil reservoirs. Table 2 shows the total heterophic bacterial count of PFW samples from three oil reservoirs. The bacterial count ranged from 2.18 x 10^3 cfu/ml to 2.22 x 10^3. cfu/ml. Table 3 shows the total fungal count of PFW samples from the three sampling points. The count ranged from 4.60 x 10^2 cfu/ml to 5.00 x 10^2 cfu/ml.

Table 1: Physicochemical properties of PFW samples from three oil and gas reservoirs

PARAMETERS	A	B	C	MEAN
pH	6.34	6.34	6.31	6.33
EC (S/cm)	954	975	953	960.6
Temperature (ºC)	28.5	28.5	28.4	28.46
Total Solid (mg/l)	250	150	200	200
Total Dissolved Solid (mg/l)	770	760	738	756
DO (ppm)	4.6	4.8	4.4	4.6
Nitrate (mg/l)	51.0	52.1	53.2	52.1
Nitrate (mg/l)	43	42	45	43
Sulphate (mg/l)	33	37	34	35
Lead (mg/l)	50	51	52	51
Chromium (mg/l)	66	67	68	67
Cadmium (mg/l)	31	32	33	32
Copper (mg/l)	25	22	22	23

The LC50 and EC50 values of *Nitrobacter* sp is presented on page 4. The bacteria isolated include *Pseudomonas aeruginosa, Baccilus cereus* and *Micrococcus luteus.* The characteristics of fungi isolates from PFW samples. The fungi isolates are Yeast and *Aspergillus niger*. Table 5 contains the toxicity test; the effect of % PFW treatment on dumpsite soil nitrate (mg/l). The optical density (at 600nm) of dumpsite soil samples with % treatment of PFW is presented on Table 6 the optical density ranged from 0.191 (Day 1, 0% treatment) to 0.714 (Day 1, 75% treatment). The Nitrobacter count (cfu/ml) with PFW treatment observed over a period of 7 days is presented on Table 7. The mean count ranged from 2.0 x 10^2 cfu/ml (Day 3, 75% treatment) to 1.07 x 10^3 cfu/ml (Day 1, 75% treatment) at Table 8 shows

earthworm bioassay observed for 7 days with % PFW treatment. The main weight (N) of the earthworm ranged from 0.58 (Day 1) to total death (from day 2 onward, especially on 75% treatment).

Table 2: Total heterotrophic bacterial count of PFW samples from three oil and gas reservoirs

Samples	Count (cfu/ml)
A	2.20×10^3
B	2.18×10^3
C	2.22×10^3

Table 3: Total fungal count of PFW samples from three oil and gas reservoirs

Samples	Count (cfu/ml)
A	4.60×10^2
B	5.00×10^2
C	4.60×10^2

Table 4: LC50 and EC50 values for *Nitrobacter sp*

Incubation time (hrs)	LC50	EC50
24	22.15	78.82
48	16.04	108.49
72	14.88	122.61
96	11.56	139.47

The result of the investigation of the effect of produced formation water on soil nitrifying bacteria and earthworms showed that PFW exhibit great modification on the survival of earthworms as well as the load and activity of soil nitrifying bacteria. The result showed the pH of all the analysed formation water samples were fairly neutral, with mean value of 6.33. This does not agree with the findings of Isehunwa and Onovae (2011) who reported that salinity is higher in produce formation water than some sea water. However. The mean pH value (6.33) recorded for all samples was within the World Health Organization (WHO) acceptable limit of 6.00-9.00 for waste water to be discharged into the environment (WHO, 2004). Also mean electrical conductivity (EC) of 960.6 S/cm was obtained from the PFW samples. The high levels of (EC) in the formation water could be ascribed to the high levels dissolved ions in the water. The mean temperature of the water samples was 28.46^0C. This mean value is below the WHO acceptable limit of 40^0C. The mean total dissolved solids (TDS) value (756mg/l) recorded in these samples fell below the WHO acceptable limits of 2000mg/l. This result is in line with the one observed by Atuanya and Tudararo-Aherobo, (2014) from the oily sludge discharge of Nigerian petroleum refinery. The mean dissolved oxygen obtained from these samples was 4.6mg/I, which is lower than WHO standard of 5.0mg/l. This can be accounted for by the increased biological activities of the produced water, in which oxygen is being utilized. Also, the mean nitrate content of 52.1 mg/l was higher than the WHO standard of 45mg/l. This high value is due to the organic content of the produced formation water. The content of sulphate, lead, chromium, cadmium and copper were found to have higher values than the WHO specifications. The bacteria and fungi count obtained from the PFW samples, which ranged from 2.18×10^3 cfu/ml and 4.60×10^2 to 5.00×10^2 cfu/ml respectively, indicated high microbial load in formation water. The microbial analysis of produced formation water samples showed the presence of pseudomonas *aeruginosa*, *Bacillas cereus* and *Micrococcus luteus*. These three bacterial species isolated from PFW in this study are common bacteria isolated from soil, and adaptable organisms found in various habitats. The fugal species isolated from the water samples are Yeast and *Asperigillus* niger.

Table 5: Toxicity Test, Effect of % PFW treatment on Nitrate (mg/l)

PFW	Treatment	Day 1	Nitrate (mg/l) Day 4	Day 7
0%		30.14	21.74	7.58
		31.21	22.06	5.93
		30.56	20.77	5.61
	Mean	30.64	21.52	6.37
25%		50.45	41.74	27.22
		51.23	41.74	28.51
		50.67	38.51	29.16
	Mean	50.78	40.66	28.30
50%		70.73	60.77	45.61
		70.15	64.35	47.54
		70.67	69.56	45.29
	Mean	70.52	64.89	46.15
75%		80.99	74.64	75.29
		81.39	74.80	77.87
		81.33	76.25	79.48
	Mean	81.24	75.23	77.55

Table 6: Optical Density at 600nm of soil with % treatment of PFW

PFW	Treatment	Day 1	Nitrate (mg/l) Day 4	Day 7
0%		0.184	0.283	0.220
		0.194	0.280	0.215
		0.195	0.291	0.216
	Mean	0.191	0.284	0.217
25%		0.197	0.440	0.311
		0.200	0.416	0.325
		0.189	0.424	0.321
	Mean	0.195	0.427	0.319
50%		0.200	0.577	0.402
		0.204	0.534	0.402
		0.205	0.572	0.408
	Mean	0.203	0.561	0.404
75%		0.249	0.604	0.549
		0.237	0.624	0.555
		0.228	0.620	.0551
	Mean	0.714	0.616	0.552

The result of the test of the effect of PFW on soil nitrate (Table 5) shows that nitrate increased progressively with increase in % PFW applied, while the nitrate content decreased across the days. The increase in nitrate observed with increased percentage of PFW applied is consistent with the fact that PFW is obtained from the processing of oil, which is rich

hydrocarbon source. The decrease in nitrate across the days can also be explained from the fact that their nitrate denitrifiers such as *Micrococcus*, Bacillus and *Pseudomonas aeruginosa* use this nutrient for their nitrate *demand* for carbon and energy source, especially under anerobic conditions, which has been reported by Oboh *et al.* (2006). While plants use nitrateas a nitrogen source in the formation of protein. Akaochere *et al*. (2008) also reported that the effects of oil spills on soil can lead to an enrichment of the oil degrading microbial population. The optical density of receiving soil samples measured at 600nm was also observed to increase with increase in percentage of formation water applied (as presented on Table 6). This is consistent with the findings of Sebiomo *et al*. (2010) who reported that optical density increases with exposure to hydrocarbon or lubricating oil (in his case from non-mechanic workshops). This is because in population of biotic agents triggered by the nutrient in the formation water. Several studies have demonstrated correlations between optical density and microbial load. This current findings also agree with these studies as soil microbial load was seen with increase in % of PFW treatment, and reduced progressively as the contact days increases (as seen in Table 7).

Table 7: *Nitrobacter* count (cfu/ml) with PFW treatment observed over a period of 7days

		Day 1	Day 4	Day 7
0%		$9.2x10^2$	$4.2x10^2$	$2.4x10^2$
		$9.2x10^2$	$4.2x10^2$	$2.8x10^2$
		$8.4x10^2$	$4.0x10^2$	$2.4x10^2$
	Mean	$8.9x10^2$	$4.1x10^2$	$2.5x10^2$
25%		$9.0x10^2$	$5.0x10^2$	$3.6x10^2$
		$8.4x10^2$	$5.0x10^2$	$4.0x10^2$
		$8.8x10^2$	$5.6x10^2$	$3.4x10^2$
	Mean	$8.7x10^2$	$5.2x10^2$	$3.7x10^2$
50%		$8.4x10^2$	$3.8x10^2$	$2.6x10^2$
		$9.2x10^2$	$3.4x10^2$	$3.8x10^2$
		$9.x10^2$	$4.2x10^2$	$3.2x10^2$
	Mean	$8.9x10^2$	$3.8x10^2$	$3.2x10^2$
75%		$1.14x10^2$	$5.4x10^2$	$2.0x10^2$
		$1.04x10^2$	$5.0x10^2$	$2.2x10^2$
		$1.04x10^2$	$4.6x10^2$	$1.8x10^2$
	Mean	$1.07x10^2$	$5.0x10^2$	$2.0x10^2$

According to Neff *et al*. (2011), biomarkers may be used to indicate: an organism has been exposed to a specific or group of chemicals, an organism is affected by a contaminant and is responding to it, or the organism has been injured. The bacteria count of soil samples with PFW treatment observed over a period of 7 days which is presented on Table 7 shows a progressive reduction of the Nitrobacter count from day 1 to day 7 of the 75% of PFW treatment, while the highest count across all the treatment was observed on day 1. This shows over 80% reduction of the bacteria load just after 7 days of exposure to PFW. It is however expected to have more lethal effect should the soil organisms be exposed to the treatment for a longer period and consistently. This result portrays that the important nitrification activity of the nitrifying bacteria which enhances soil fertility may be hindered in an eco-system polluted with produced formation water. Earthworm bioassay was observed for 7 days with different percentage formation water treatment (Table 8).

Table 8: Earthworm bioassay observed for 7 days with % PFW treatment

DAYS		Weight(% Formation water) 0%	25%	50%	75%
DAY 1	Replicate 1	0.46	0.20	0.85	0.50
	Replicate 2	0.27	0.09	0.46	0.45
	Replicate 3	0.35	0.15	0.42	0.39
	MEAN	0.36	0.15	0.58	0.45
DAY 2	Replicate 1	0.45	0.22	0.39	-
	Replicate 2	0.39	0.25	0.12	-
	Replicate 3	0.24	0.09	0.34	-
	MEAN	0.36	0.19	0.28	-
DAY 3	Replicate 1	0.23	0.26	0.28	-
	Replicate 2	0.28	0.17	-	-
	Replicate 3	0.31	-	-	-
	MEAN	0.27	0.14	0.09	-
	Replicate 1	0.42	0.25	0.35	-
DAY 4	Replicate 2	0.18	0.27	-	-
	Replicate 3	0.41	-	-	-
	MEAN	0.34	0.17	0.12	-
	Replicate 1	0.24	0.22	-	-
	Replicate 2	0.40	0.24	-	-
DAY 5	Replicate 3	0.28	-	-	-
	MEAN	0.31	0.15	-	-
	Replicate 1	0.29	0.23	-	-
	Replicate 2	0.34	0.25	-	-
DAY 6	Replicate 3	0.28	-	-	-
	MEAN	0.30	0.16	-	-
	Replicate 1	0.39	0.19	-	-
	Replicate 2	0.22	0.21	-	-
	Replicate 3	0.28	-	-	-
	MEAN	0.30	0.16	-	-
	Replicate 1	0.39	0.19	-	-
DAY 7	Replicate 2	0.22	0,21	-	-
	Replicate 3	0.41	-	-	-
	MEAN	0.34	0.13	-	-
Survival Rate		100%	57%	29%	11%

The results from the bioassay revealed that produced formation water could kill or reduce the growth of earthworms. The weight of the earthworms and the survival rate were determined. There was a progressive reduction in earthworm weight over time in all soil except in the control (without PFW treatment-%). From 25% treatment, a steady decline in survival rate of the earthworms was seen, from 57% to 11%. The earthworms were observed to be completely dead at 50% treatment from day 2 (even in triplicates). There was evident change in weight of earthworms during the test across all PFW preparations, and weight can be said to be a more sensitive parameter than survival in accessing pollutant effect in soils

earlier reported by Udochukwu *et al* (2017). Gazali (2017) reported that the organic and inorganic compounds in produced water have higher toxicity compared to that of crude oil, and the discharge of these toxicity compared to that of crude oil, and the discharge of these toxic constituents and contaminants to the aquatic life and agricultural resources by altering the natural state of the aquatic environment. It is necessary to treat produced water before discharge to avoid the harmful effects that the chemicals in the waste waters may have on the receiving environment. According to Neff *et al*. (2011), treatment removes solids and dispersed oil, suspended solids, scales, and bacterial particles, as well as most volatile hydrocarbons and corrosive gases.

Conclusion: It is evident from this study that produced formation water impacts the receiving soil and its organisms in several ways; from increasing nitrate content, increasing soil optical density, inhibiting earthworm survival to modifying soil nutrient and balance. Although the soil is subject to self-initiated bioremediation a while after exposure to formation water or other pollutants, the soil may suffer grievously if it is consistently exposed to such discharges.

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