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Eco-toxicity assessment of fossil fuel tainted soil before phytoremediation trial

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Abstract

The research objective was to assess the extent of fossil fuel toxicity in the contaminated soil for an improved appraisal before phytoremediation trial and eco-safety of the soil. We used Bonny light fossil fuel to contaminate soil at different percentage levels of concentrations (5%, 3%, 1% and 0%). We assessed earthworm weight change and survival rate and plant response of *Zea mays* to the various treatments. Our results show that soil treated at concentration level of 5% had the highest earthworm mortality rate while the control soils had the highest earthworm survival rate. In addition, there was a noticeable change in the body weight of surviving earthworms in the 5% and 3% treated soil. Germination, shoot length and root length of the experimental plant was not totally inhibited in all the different concentrations. There was greater germination percentage obtained in 3%, 1% and 0% fossil fuel concentrations. The study verifies that high fossil fuel contamination percentage affects the biota of the soil by inhibiting plant germination and growth as well as reducing the survival and body mass of earthworms in the soil. The petroleum oil percentages utilized in this investigation can support phytoremediation trial and proposes that petroleum oil soil contamination ≤ 3% is ecologically safe for plants and animals' survival as well as development.

Keywords: 'Fossil fuel', Petroleum oil, *Zea mays* L., Eco-toxicity and Phytotoxicity.
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Introduction

Fossil fuel (crude oil) production has remained the mainstay in the Nigeria's economy for decades (Inegbedion et al., 2020). The daily barrels of crude it produces place Nigeria as the largest producer of fossil fuel in Africa and the 6th largest in the world (Osuji and Onojake, 2004). A reservoir of crude oils is found across nine southern states of Nigeria collectively known as Niger delta states. In recent times, crude oil has been found in other states in the country (Ajiya, 2023) Fossil fuel spillage and gas flaring are two common occurrences in this region and they affect the quality of human lives, soil health, water quality and ecological organisms such as plants, animals and microorganisms (Ogboghodo et al., 2004; Nwaichi, et al., 2015. Fossil fuel contains a multifarious mix of organic compounds of varied molecular mass. Components of petroleum with lower log (K_{ow}) seem have greater lethal prospects compared to those with high up log (K_{ow}) (Frick et al. 1999). The toxic ability hydrocarbons in fossil fuels are mainly between C_{10}-C_{19} compounds and they are usually characterized with lower boiling points (Brils et al., 2002; Joner et al., 2001; Neff et al., 2000).

The eco-toxicity level of crude oil related pollutants in soil is of great concern to stakeholders (Ebulue, 2022). Pollutant's concentrations and the type of pollutants in topsoil can be characterized by analyzing chemically but when it comes to the overall soil quality, chemical analysis alone cannot reflect its true eco-toxicity level (Zawierucha et al., 2022). However, few studies have reported that biological methods seem to be more appropriate in determining the probable risk of pollutants in soil on the environment and its ecology (Tang et al., 2011). The use of biological organisms as an assay to measure the toxicity of contaminated soil has gained global responsiveness over preceding few decades (Cruz et al., 2013). In ecological risk assessment, toxicity assessment of the tainted soil with assay of biological organisms could provide significant information as regards its 'characterization procedure' as it has proven to be effective for a compound mixture of 'contaminants' such as petroleum hydrocarbons (Cruz et al., 2013). Ecotoxicological diagnosis can be done using soil animals and plants as test organisms. The use of Earthworm as a test organism for this bioassay has been standardized by 'International Organization of standardization (ISO) in 1993 (ISO11268-1)' (OECD, 2000) and gained extensive researched even at the subcellular levels (Owojori et al., 2009; Tang et al., 2011). In earthworms, the polyaromatic hydrocarbons present in petroleum contaminants damages the metabolism of glucose by enhancing fatty acid metabolism and alters the tricarboxylic acid intermediates (Tang et al., 2011). The need for an eco-toxicity test before phytoremediation trial is imperative because it tells if the phytoremediation will be successful since toxicity is concentration dependent (Kirk et al., 2002; Tang et al., 2011). For an organism to successfully remediate a contaminant, it must first survive the concentration of that contaminant (Bala et al., 2022). The toxicity of plant test consists of elongated roots, germination of seeds and growth of the plants. The criterion for plants to be used for ecotoxicological test is sensitivity of the plant to the presence of toxic substances that interferes with the 'survival during the early seedling development when numerous physiological processes are taking place' (Cruz et al., 2013). There have been reports on the toxic ability of fossil fuel oil on aquatic organisms in water (Perkin et al., 2005; Martinez-Jeronimo et al., 2005) but few documentations on the eco-toxicity of fossil fuel oil on topsoil with earthworm as well as *Zea mays*.

The research objective was to assess the extent of fossil fuel toxicity in the contaminated soil by examining the germination, root elongation, hypocotyl elongation of *Zea mays* as well as the survival of the earthworm for an improved appraisal before phytoremediation trial and eco-safety of the soil.

Materials and Methods

Study Area

The research took place in a screen house in the botanical garden at the Department of Plant Biology and Biotechnology, University of Benin (6.397149, 5.6149716).

Preparation and Analysis of Contaminated Soil

The fossil fuel used for the preparation of the contaminated soil is bonny light fossil fuel obtained from Emreke-kokori flow station Ugheli Delta State, Nigeria. Soil was gotten from the Department of Soil Sciences, Agriculture Faculty, University of Benin at a depth of 0-15 and 15-30cm. Determination of pH of the soil was by adding up 25ml of distilled water to 10g air dried topsoil sample in about 50ml beaker (ASTM, 1995). The suspension temperature was read with a thermometer (Model; St. 250g). The suspension was mixed intermittently for 20minutes before reading with a pH meter (Model; St. 250g).). For electrical conductivity, 10g of air-dried soil was placed in 100ml beaker, then 25ml distilled water was added after which we stirred at a repeated cycle four times on 40minutes intervals before measuring the suspension temperature with a thermometer. To get the conductivity of the soil suspension, the electrode was rinsed with the soil suspension solution. Characterization of the soil was done using hydrometer method (Sugita and Marumo, 2001). After characterization of the homogenized soil, the soil was filtered through a 2mm sieve to separate debris in the form of stones, plant residues after which proportions were placed in different bowls and mixed with 5%, 3% and 1% fossil fuel. 125cm of fossil fuel divided by 2500g of soil multiply by 100% gave 5% concentration of fossil fuel. The test control soil representing 0% (ctrl) was quartz sand with particle size ≤ 2 mm (Cruz et al., 2013). All were in triplicates.

The percentage concentration of fossil fuel was calculated by applying the formula beneath.

$$\% \text{ conc. of fossil fuel in the soil} = \frac{Volume\ of\ fossil\ fuel\ (cm3)}{Weight\ of\ soil\ (g)} \times 100.$$

Phytotoxicity Test

Maize (*Zea mays*) used for the experiment was acquired from Agriculture Faculty, University of Benin. It was chosen because it is suitable for the climate, easily accessible, ephemeral, a positive review from several researchers and a good representative of the poaceae family (Balasubramaniyam, 2015; Charlotte, 2016; Zand and Muhling, 2022). Seed viability was tested by simple floatation technique and only viable seeds were used for testing. Soil of about 250g was placed in a 180 mm diameter container with adjusted humidity of about 50%. Twenty grains (seeds) were placed in the soil. Seeds were cultivated on 5%, 3% and 1% fossil fuel tainted soil. The control (0%) was quartz sand with particle size ≤ 2 mm. The germination of seeds was determined by virtual emergence of seedlings and after 96 hours, numbers of germinated seeds were recorded in each treatment with three replicates. The phytotoxicity test was 'based on ISO (International Organization for standardization) and OECD (Organisation for Economic Co-operation and Development) method'. Germinated seedlings in different concentrations of the tainted soil were juxtaposed with controls and values recorded as a percentage relative to the controls. After 96 hours, seedlings were pulled out and cleaned thoroughly with tap water to separate any form of soil debris. The longest main root was measured with the help of a meter rule measured in mm, obtained value served as root length measurement. Shoots of the plant were measured as well with a meter rule.

Earthworm Acute Toxicity Test

Standard earthworm studies were carried out on contaminated and uncontaminated soil according to OECD methods (OECD, 2004). Prepared artificial soil according to OECD guidelines was used as control. Prepared soil was put in a glass bottle about 400g for each concentration. Humidity was adjusted to 50% by covering with net to prevent direct water loss by evaporation or evapotranspiration. Ten adult earthworms (*Eisenia fetida*) were handpicked during the rainy season, washed with distilled water, and cleaned, group weighed, and the average value weight recorded with a weighing balance. The grouped earthworms were placed into the different treatment bottles thereafter, the bottles were wrapped with net to allow gaseous exchange then placed in a dark chamber. The mean earthworm weight was 0.515±0.025g. Deceased earthworms were checked after day ten. The basis for certifying the earthworm dead was by no reaction to prickling. At the end of the experiment, the weight of the surviving earthworms was taken, average values were calculated. All concentrations were tripled, and mean value recorded. The percentage survival of earthworm was calculated by the formula below.

$$\% \text{ survival} = \frac{number\ of\ surviving\ earthworm\ in\ the\ contaminated\ soil}{number\ of\ earthworms\ place\ in\ the\ contaminated\ soil}\ x\ 100$$

Percentage change in the weight of earthworm was calculated using the formula below.

$$\% \text{ change in weight} = \frac{initial\ weight - weight\ of\ surviving\ earthworm}{initial\ weight\ of\ earthworm}\ x\ 100$$

Statistical Analysis

For percentage germination, emergent plants, root length and shoot length data were analyzed with one-way ANOVA using GraphPad prism and Microsoft Excel for graphics.

Results and Discussion

Soil Physicochemical Characteristics

The soil was characterized based on gravimetric and physicochemical properties. As depicted in Table 1 beneath, pH of soil was slightly acidic (5.29) with high electrical conductivity 280.1µs/cm, percentage of total organic carbon (TOC) and total organic nitrogen (TON) stayed

0.215 and 0.853 respectively. The exchangeable acidity of the soil was 0.713 while the obtained values of Na. K, Ca, NO_3 and PO_4 were 10.5mg/kg, 18.0 mg/kg, 12.5 mg/kg, 123.6 mg/kg, and 161.2 mg/kg correspondingly. The soil had a high percentage of sand followed by slit and a very low percentage of clay. These soil parameters are very important for successful phytoremediation. The bioavailable metals were Fe (14.63mg/kg), Cd (0.913 mg/kg), Mn (9.56 mg/kg), Pb (1.02 mg/kg), Cu (1.61 mg/kg) and Zn (5.23 mg/kg).

The character (texture) of soil can influence phytoremediation by impacting the biological availability of the pollutant. Clay textured soil is adept in holding together molecules more easily than either silt or sandy textured soil (Brady and Weil, 1996). Hence, the biological availability of contaminants is reduced in topsoil with high clayey matters. In view of this, Carmichael, and Pfaender (1997) reported that bigger particle sized soil like sand naturally possess greater mineralization of PAHs than topsoil with tinner particles, reasons been that the biological availability of pollutants in sand is higher. The type of soil can also influence quality and quantity of exudates from the roots of plants which might impact the effort of phytoremediation (Frick et al., 1999).

Soil pH significantly affects the biological availability of heavy metals in topsoil by controlling the solubility and hydrolysis of metal hydroxides, carbonate, and phosphates (Nwaichi et al., 2021). The pH value of 5.29 tells us the soil is acidic. This value differs from the findings of Masakorala et al. (2014) who documented a rise in the pH of fossil fuel tainted topsoil. This discrepancy could illuminate from the chemical reaction that occurs between the hydrocarbons and heavy metals in the crude as well as the other elements in the soil. The soil electrical conductivity (EC) was 280.1µS/cm. EC which is a measure of the ionic concentrations in the soil and have been reported not to directly affect plant growth but is useful to signify the amount of available nutrients for plant uptake as well as the salinity level of the soil which inhibits plant growth and disrupt microbial activities (Nwaichi *et al*., 2021). Topsoil with high organic carbon content (>5%) enables strong adsorption of contaminants thereby making the contaminants less available. Hitherto, organic carbon content (1 to 5%) termed moderate leads to limited availability (Otten et al., 1997) while in low organic carbon, we have high contaminant availability and the organic carbon in this study was low. Nitrogen, phosphorus, and potassium are macronutrients necessary for plant growth, nitrogen was found to be below soil agricultural standard while that of phosphorus and potassium was relatively fair (HSE-ENV, 2004)

Table 1: Background means concentration of soil physical and chemical parameters.

Parameters	Mean
pH	5.29
Electric conductivity (µs/cm)	280.1
Total Organic Carbon (%)	0.215
Total organic Nitrogen (%)	0.853
Exchangeable acidity (meq/100g)	0.713
Na (mg/kg)	10.50
K (mg/kg)	18.0
Ca (mg/kg)	12.5
NO_3 (mg/kg)	123.6
PO_4 (mg/kg)	161.2
Heavy metals	
Fe (mg/kg)	2230
Cd (mg/kg)	18.5
Mn (mg/kg)	58.5
Pb (mg/kg)	15.6

Cu (mg/kg)	18.3
Zn (mg/kg)	89.2
Cr (mg/kg)	12.2
Gravimetric parameters	
Clay (%)	3
Sand (%)	91
Slit (%)	6

The toxicity of crude oil differs with the type of hydrocarbon contaminant, concentration of crude oil, type and properties of soil (Kirk et al., 2011). Germination/Emergence, root and shoot growth are good pointers for plants to tolerate and establish in crude oil contaminated soil (Kirk et al., 2011) while percentage survival and change in weight is a good indicator for earthworm toxicity (Ramadass et al., 2016).

Percentage Rate of Germination

Figure 1 shows the percentage germination rate of the *Zea mays* seeds sown in the altered concentrations of fossil fuel in the tainted soil as well as the control. It shows that the control (0%), 3% and 1% treated soil had 96.67%, 91.67% and 90% germination rate respectively. Thus, suggesting a very low phytotoxicity and high germination rate but for the 5% fossil fuel contamination, the toxicity was relatively high with about 18.33% germination. Our results suggest that as the level of oil pollution increases, germination of plants becomes inhibited. Similarly, to our study, Ogboghodo et al. (2004) found that increased concentrations of crude oil affect the germination rate of maize. Tang et al., (2011) also reported increased germination of maize at lower concentration of contamination. My result also conforms to Sorana et al. (2018), they reported less than 50% germination in *Festuca pratensis* and *Lolium perenne* seeds exposed to high crude oil concentration. The reduced germination could be from the verity that unstable fractions of oil have high wetting capacity and incisive influence (Sorana et al., 2018). When it gets in touch with seeds, the hydrophilic fraction of the oil would enter the seeds through its coat and likely kill the embryo (Cruz et al., 2013). The unfavorable environment that fossil fuel generates may also be responsible for the reduced germination observed in the 5% fossil fuel contamination. When fossil fuel contaminates soil, it creates an anoxic environment, reducing leachability as water floats on the surface of the soil thereby reducing water. When water is reduced, toxicity increases as the possibility of dilution is affected (Tang et al., 2011).

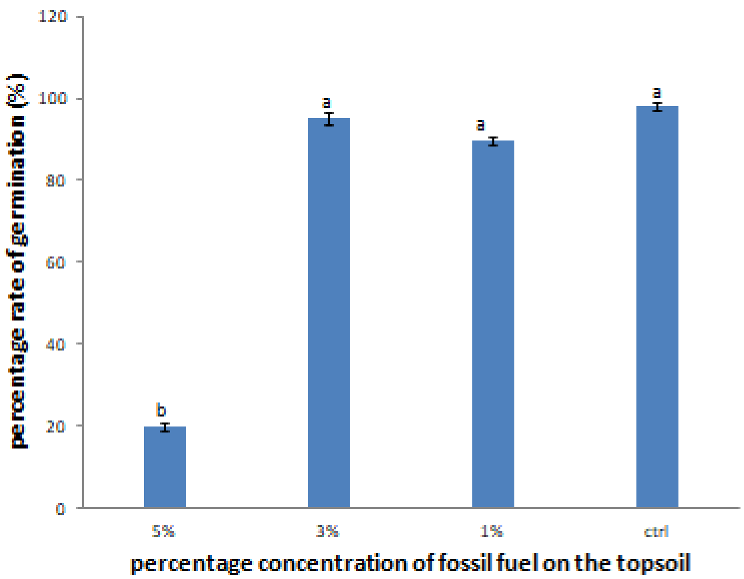

Fig 1: Percentage rate of germination of *Zea mays* in the fossil fuel tainted soil after 4 days. Different alphabets in bar indicates statistically significant. *ctrl is the control with 0% fossil fuel.

Shoot Length of the Zea mays

The chart in Figure 2 shows the result of the shoot length in the different percentage of fossil fuel tainted soil. While the control showed increased shoot length of 75mm, the least shoot length was observed in the 5% fossil fuel treated soil. It was generally observed that concentration of the fossil fuel was inversely proportional to the shoot length.

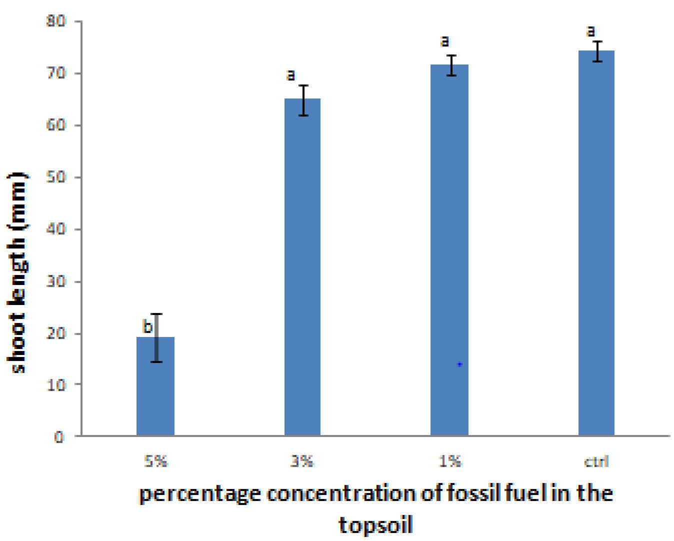

Fig 2: The shoot length (mm) of *Zea mays* in the different concentrations of fossil fuel during the phytotoxicity test. *ctrl is the control with 0% fossil fuel.

Root length of *Zea mays*.

The development of the roots of the plant '*Zea mays'* in the phytotoxicity test seems to follow the same pattern as the shoot length with 5% fossil fuel concentration showing relatively high retardation (Figure 3), having a mean root length of 35mm far less than the control with about 125mm root length. The result shows that 1% and 3% fossil fuel concentration support the elongation of the plant root unlike the 5% fossil fuel contamination. A decline in the length of root and shoot of *Zea mays* was observed with a rise in concentration of fossil fuel. Root is the principal tissue of plants that has direct contact with contaminant (Cruz et al., 2013). The reduction in shoot length in 5% fossil fuel contaminated soil as against the other percentage concentrations coincides with the findings of Cruz et al. (2013). They reported that shoot and root length reduce with increase concentrations of diesel contaminants. Similarly, Tang et al., (2011) also reported a decrease in root length of *Zea mays* with increase in total petroleum hydrocarbon concentration. The toxic effect experienced by the roots and shoots of *Zea mays* in 5% contaminant concentration might be linked to the composition of enormous number of PAHs extant in crudes as well as traces of heavy metals (Cruz et al., 2013). Hence increase in crude oil concentration reduces pH value creating an acidic condition which then upsurges the bioavalability of the heavy metals extant in the oil to the plants (Henry, 1998; Cruz et al., 2013).

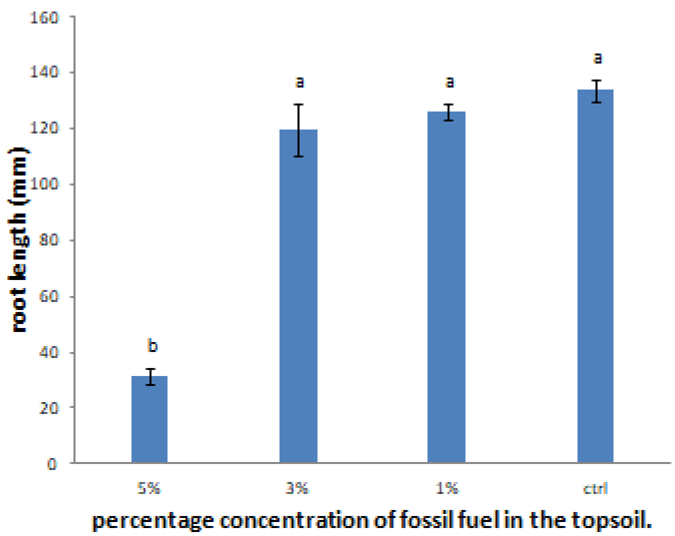

Fig 3: The length of the root of *Zea mays* in the various concentrations of fossil fuel tainted soil. *ctrl is the control with 0% fossil fuel.

Earthworm survival

Earthworm survival was found to be impacted in all the treated soil and the control. Higher percentage survival was recorded in the control (90%) as well as in 1% fossil fuel concentration (70%) and this was significantly different. The survival rate was inversely proportional to fossil fuel concentration in the tainted soil (Figure 4). The use of earthworm soil dwellers for toxicity study is important because it creates paths that increases the perviousness of soil and as well increases biological activities in the soil by transforming large organic matter into bits of fragments and they also secrete nutrient rich materials (Charlotte, 2016). Earthworm survival and change in weight were markedly impacted in the 5% concentration of fossil fuel followed by 3% fossil fuel concentration. Tang et al., (2011) reported increased mortality in oil concentration of 5% and reduced survival rate to less than 40%. Petroleum hydrocarbons have been reported to cause death, swelling, body abrasions, hardening, curling and low fecundity in earthworm (Oboh et al., 2007). Earthworms are porous fragile organisms that can absorb toxic contaminants via their body which brings about its high sensitivity (Oboh et al., 2007). We suggest that one of the reasons while there was loss of body weight of the earthworms and subsequent death is because polyaromatic hydrocarbon compounds from the fossil fuel may have impaired glucose breakdown, with an associated upsurge in fatty acid absorption and changes in tricarboxylic acid (TCA) cycle intermediates in the earthworms (Tang et al., 2011).

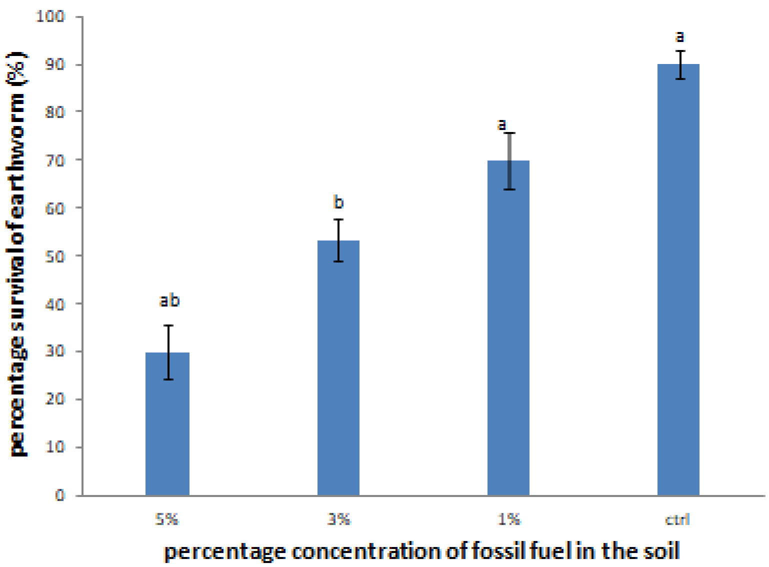

Fig 4: Percentage survival of earthworm in the fossil fuel tainted soil. *ctrl is the control with 0% fossil fuel.

Earthworm Weight Change

The result in Figure 5 shows the change in the weight of earthworm in the different concentrations of the tainted soil. No death was recorded when earthworms were exposed to the control and 1% fossil fuel contamination. Relative loss (death) was detected in the earthworms exposed to 3% and 5% fossil fuel contamination (Figure 5). The loss in 5% contamination was higher than that of the 3% contamination however, the body mass of the few survivals increased. The dead earthworms might have improved nutrients of the soil in which the surviving earthworms used to increase their body mass. The decrease in earthworm weight in the control treatments can be attributed to alterations in living environments. Positive correlation was observed in fossil fuel concentrations and body weight inhibitions showing a significant dose-effect relationship which can be linked to the stress from the fossil fuel leading to a general high rate of weight clampdown. Tang et al., (2011) concurred by reporting suppression of earthworm mass to increase up to 48.91% at TPH content of 1.5%. The earthworm toxicity test revealed that the concentrations in soil exceeding 1% caused a dose-dependent loss in earthworms. That is as the concentration increases, the earthworm decreases.

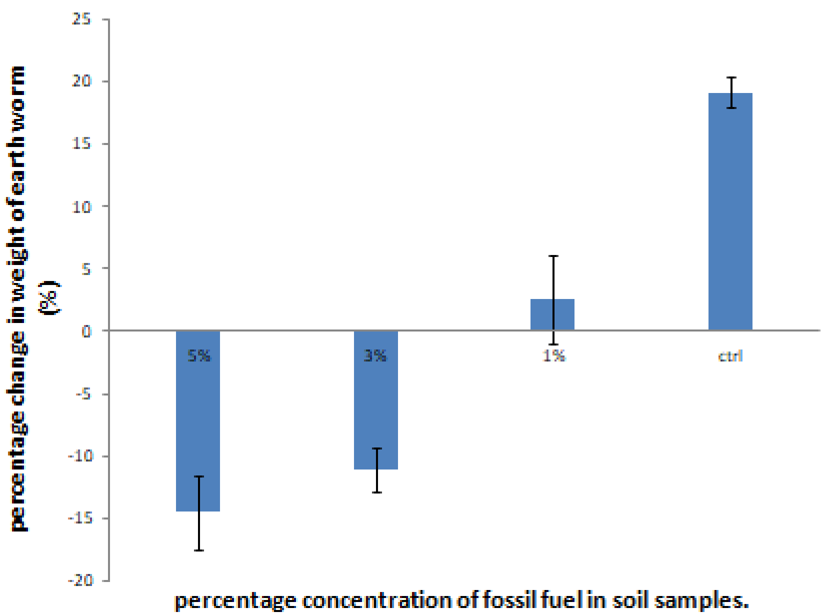

Fig 5: Percentage change in weight of the earthworms after 14 days growth in the fossil fuel tainted soil.

The negative change in weight recorded in 5% and 3% was from increased body mass recorded in the few surviving earthworms in the soil. The initial and final weight of the surviving earthworms was used for the calculation and not as a group. As a group, the weight of the dead worms counters the weight gained by the surviving earthworms. The results are not presented in this manuscript.

Conclusion

The research verified the ecological toxicity of fossil fuel pollutants in soil. High percentage germination was recorded in 3%, 1% and 0% fossil fuel contaminated soil while in 5% an elevated inhibition rate was observed which signifies that very high fossil fuel contamination might not favour the germination of *Zea mays* in fossil fuel contaminated soil which might halt phytoremediation process. To have a successful phytoremediation trial, the test plants need to survive and establish in the contaminated soil so it can remediate the contaminant. We recommend that Bonny light fossil fuel contamination in topsoil of 3% and below is ecologically safe for plants and animals survival and development however further studies can research into the biosafety of biomagnifications and translocation of constituents of the contaminants in lower concentration percentages.

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