GENOTOXIC EFFECT OF SPENT ENGINE OIL – POLLUTED SANDY SOIL ON SOYBEAN (*Glycine max*)

Angela Chika UDEBUANI[*], Sixtus Ike ONYEMERENECHE[*], Priscilla Nkeonye ABARA[**], Ogochukwu Chidinma UKACHUKWU[*], Henry Uzoma ANUFORO[**]

[*]*Department of Biotechnology, Federal University of Technology, Owerri, Nigeria*
[**]*Department of Biology, Federal University of Technology, Owerri Nigeria*
Correspondence author: Henry Uzoma ANUFORO, Federal University of Technology, Owerri, School of Biological Sciences, Department of Biology, P. M. B. 1526, Owerri, Nigeria, phone: +2348066583404, henry.anuforo@futo.edu.ng

Abstract. There is need for more records that aim to expose the effect of spent engine oil (SEO) on crop production. Hence using molecular and biotechnological methods, the genotoxic effect of spent engine oil – polluted soil on soybean (*Glycine max)* is documented here. Four kilograms (4 kg) of soil obtained at a depth of 1 – 20 cm from agricultural farmland was polluted with different concentrations (4 %, 6 % and 8 %) of spent engine oil, then planted with 4 seeds of *Glycine max* for 21 days. Results indicate a reduction in pH level of polluted soil to 5.43 ± 0.02, 5.38 ± 0.02 and 5.13 ± 0.02 in 4 %, 6 %, and 8 % SEO polluted soil samples respectively, when compared with 6.55 ± 0.20 in control (unpolluted) soil. The mineral components, THC, SOC, organic matter, and the concentration of potentially toxic metals (in the order Pb>Fe>Mn>Cd>Cr) were higher in the polluted soils. The *G. max* planted on polluted soil also experienced reduced growth factors, including leaf number, plant height, change in leaf colour from green to yellow, and mortality (only observed in 8 %). The molecular analysis indicated an increase of *G max* chromosomal breaks, shown by higher number of DNA bands, which reduced in quantity and purity relative to increasing level of dicharge of SEO. This result suggests a possible compromise of the plant DNA and function by SEO; thus indiscriminately discharging this pollutant in the environment portends a genotoxic effect on legumes and crop production.

Key words: Genotoxicity; lubricant; potentially toxic metals; DNA bands; soil pollution.

INTRODUCTION

The indiscriminate disposal of used engine lubricants and/or spent engine oil (SEO) in the environment, constitutes a major source of pollution which could affect most life forms [36]. Spent engine oil arises from operated engines of automobiles, tractor engines, generators and jet engines. Components of spent engine oil include potentially toxic metals, low to high molecular weight compounds, additives, decomposition products and lubricants. These substances are harmful to soil, plants and even humans [20]. Studies have shown that when the oil is discharged on soil, it spreads vertically and horizontally into the soil medium, before being degraded by soil microorganisms [6, 21].

Disposal of spent engine oil on soil does not only affect the soil physical and chemical properties of the soil [22], but crop production, as well [23]. For instance, at higher concentrations, there was delay in seed germination [16] and a drastic reduction in productivity on farmland [26, 35]. Spent engine oil induced loss in DNA integrity of cultivated crops; though more research is needed to know the level of genotoxicity. In view of the grave ecological and health consequences that may arise from discharging SEO, there is need for more surveillance and ecological monitoring [5]. Monitoring of chromosomal alterations and breakage in animals has been reported as parameters in determining genotoxic potentials of environmental contaminants [15, 33].

When SEO is washed into nearby gardens and farmlands, it may persist and finally get absorbed by plants. The mutagenic and carcinogenic chemicals could bioaccumulate in the body through the consumption of polluted crop. Thus, this study explored the effect of SEO-poluted sandy soil on soybean (*Glycine max*). *G. max,* which abundantly grows near locations of spent engine oil pollution, makes it a model plant to test the genotoxic effect of spent engine oil. *G. max* is an important source of beverage to human beings, as well as, protein component for formulation of animal feeds. Thus alteration of its DNA could reduce the nutritional quality of the products.

MATERIALS AND METHODS

Sample collection

Soil sample for nursery was obtained from the botanical garden of School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Nigeria, with no known history of pollution. Spent engine oil (SEO) used in the treatment was collected from an automobile workshop in Nekede Mechanic Village, Owerri-West Local Government Area, Imo State, Nigeria. SEO sample was discharged from cars whose engines were serviced after four months of operation, as suggested by Osubor and Anoliefo [27].

Soil treatment, seed planting and measurement of plant growth parameters

Topsoil was collected using a surface sterilized auger at depth of 1 – 20 cm, prior to airdrying. Afterward, the topsoil (4 kg) was sieved using 2 mm sieve into 12 different labeled and perforated polythene bags. Nine of the bags containing the soils were divided into 3 groups, while the rest were the control. Pollution using SEO was conducted on 1 bag from each group at 4 %, 6 %, 8 % and 0 % (control), calculated with the formula below.

$$\%\ Concentration = \frac{Volume\ of\ SEO}{Weight\ of\ soil} x \frac{100}{1}$$

After planting of 4 Soybeans into the soils, growth parameters of seedlings, such as plant height, leaf number, leaf colour and mortality were determined on days 7, 14 and 21, and results obtained were appropriately recorded.

Determination of physicochemical properties of the soil samples

Soil pH was determined by vigorously shaking 1:2.5 ratio of soil-water suspension and reading the pH with a calibrated pH meter probe (PH-2602). Distribution of soil particle size was determined according to the Bouyoucos hydrometer method [7]. Mixture of sodium hexametaphosphate and sodium carbonate (8 g/L) was used to disperse dried soil sample in < 2 mm mesh size. After addition of 5 mL of distilled water, it was stirred, the content transferred into a measuring cylinder, and results were recorded after 40 seconds. Proportion of silt, sand and clay in the samples were extrapolated based on textural triangle [36]. Soil organic carbon (SOC) content was determined by the $K_2Cr_2O_7$ – H_2SO_4 oxidation procedure, using diphenylamine as an indicator, and 0.5 M $FeSO_4$ solution as the titrant, in line with Walkley and Black [37]. Results from SOC content was multiplied by 1.724 to derive soil organic matter content. Total nitrogen content was determined by semi-micro-Kjeldahl method [13]. Total potassium content of soil sample was analyzed using flame photometry method [13]. After wet digestion of soil sample with H_2SO_4 – $HClO_4$, the colorimetric method was used to determine total phosphorous content [10]. Exchangeable magnesium, calcium and sodium cations were extracted following the ammonium acetate extraction method of Peng *et al.* [29].

Determination of total hydrocarbon content of the soil samples

Total hydrocarbon content of soil was determined by a slight modification of Agbaji *et al.* method [1]. Initially, UV-Visible spectrophotometer (Agilent, Cary 55B) was used to plot a standard regression curve of absorbance against concentration. Triplicate results of different known concentrations of petroleum hydrocarbons in soil samples, measured at 450 nm wavelength was used. Using a weighing balance, 2 g of the soil samples were weighed into a 100 mL beaker, and 20 mL of dichloromethane was added to extract the hydrocarbon content. After shaking vigorously for 30 min, the liquid phase was separated by filtering through cotton wool packed in a funnel. Resulting filtrate was analyzed at 450 nm, using UV-visible spectrophotometer. In each case, corresponding THC was extrapolated from the prepared reference curve.

Digestion of soil samples and determination of toxic metal concentrations

Digestion of soil samples for determination of concentrations of lead (Pb), cadmium (Cd), chromium (Cr), iron (Fe) and manganese (Mn), was done as described by Akinyele and Shokunbi [3], and slightly modified by Anuforo *et al.* [4]. Using a weighing balance, 1g of the dried soil sample was weighed into a crucible and dried to ash in a muffle furnace, by stepwise increment of temperature to 500 °C within an hour. The furnace was maintained at the temperature for 12 h. The resulting residue was dissolved in 1M HNO_3 and then filtered through Whatman No 42 filter paper, before making it up to the mark in a 25 mL volumetric flask. Measurement of the potential toxic metal concentrations of the digested sample were done using Flame Atomic Absorption Spectrophotometer (FAAS). Airacetylene flame and hollow cathode lamps (HCL) were used as sources of light and measurements were taken at appropriate wavelengths (nm), slit (nm) and lamp current (mA). All reagents used were of analytical grade.

Plant DNA extraction

DNA was extracted from the leaves in line with Chen *et al.* methods [9]. Briefly, preparation of samples was done by grinding 100 mg of freeze-dried tissues in a ceramic mortar. The resultant was lysed by adding 450 µL of pre-heated plant extraction buffer in a test tube. Incubation was conducted at 65 °C for 20 min in a water bath, and mixed by occasionally inverting the tubes. Afterward, tubes were removed and allowed to cool for 2 min, before addition of 200 µL of ice-cold 5 M potassium acetate. After cooling, tubes were incubated to allow for precipitation of proteins. After 20 min, the tubes were centrifuged at 10,000 rpm for 10 min and then the supernatant was transferred into freshly labeled tubes. To further precipitate protein, 450 µL of mixture of chloroform and isoamylalcohol (24:1) was added and gently homogenized before centrifuging at 10,000 rpm for 10 min. Resulting supernatant was transferred into freshly labeled tubes. To precipitate the DNA, ice-cold isopropanol was added, gently swirled and incubated at -80 °C for 15 min before spinning in the centrifuge at 10,000 rpm for 10 min. Then, the supernatant was decanted, and DNA pellets were washed using 400 µL of 70 % ethanol, before centrifuging at 10,000 rpm for 10 min. Following decanting of supernatant, the pellet was thoroughly air-dried. Then 60 µL of ultra-pure water was added to the tube to re-suspend the DNA followed by addition of 2 µL RNase and incubation at 37 °C for 30-40 min.

Analysis of the plant DNA quality

Agarose gel (0.8 %) was used to analyze DNA quality and removal of RNA in line with Chen *et al.* method [9]. Gel was prepared by boiling 0.8 g of agarose powder in 100 mL of 1X SBE and cooling to about 60 °C. Then 5 µL ethidium bromide was added and it was gently swirled to mix, before pouring into gel tray and allowing to solidify. Caution was taken to exclude air bubbles while casting the gel. Then mixture of 3 µL of loading dye and 3 µL of DNA was briefly spun to concentrate it. Six microlitre (6 µL) of this mix was loaded on to the gel and it was run for 60 min at 80 V, before visualizing the bands under UV light in a trans-illuminator.

Nanodrop quantification of the plant DNA

DNA concentration was quantified using DNA-50 option of the Nanodrop spectrophotometer 2000 (Thermo Scientific). On the desktop of the computer system connected to the Nanodrop machine, the ND 1000 programme was loaded. "Nucleic Acid" option was selected on the pop-up window, for DNA sample. The Nanodrop pedestal was cleaned with water and a KimWipe. Then 2 µL of water was loaded in the pedestal and "Okay" was selected on the programme. "Blank" was selected to calibrate the machine. On the "Sample Type" section, DNA-50 was selected, and 2 µL of extracted DNA was loaded into pedestal. The machine was allowed to measure the absorbance at 260 nm and 280 nm, results were recorded, and used to determine the concentrations. To determine the purity of DNA samples, ratio of about 1.8 for absorbance measured at A260/280 nm is generally considered as "pure" for DNA.

Data analyses

Mean and standard deviation of all data obtained in this study were analyzed using Microsoft Excel 2010 version.

RESULTS

Physicochemical analysis of the soil samples

The results of the physicochemical properties and total hydrocarbon contents quantified from the analysis of the polluted and unpolluted soil samples are presented in Table 1. Textural evaluation indicated a higher sand percentage in both the polluted and unpolluted soil samples. Percentage clay content of the polluted samples was lower compared with the unpolluted soil sample. The least percentage level of sand, clay and pH content was in the 8 % polluted soil. The percentage soil organic carbon and organic matter were higher in all polluted samples, when compared with the unpolluted ones. However, the percentage nitrogen and phosphorus was higher in the unpolluted soil sample. The mineral nutrient showed a higher percentage of Na, N and P in the unpolluted soils, while Ca, Mg, and K were higher in the polluted soil samples. Total hydrocarbon content was higher in all polluted soil samples than in unpolluted soil sample, where it was not detected.

Toxic metal concentration in soil samples

Toxic metal concentration of the polluted and unpolluted soil samples are shown in Table 2. From the result, the 8 % pollution level experienced the highest concentration of all the toxic metals measured, although Cr was least abundant. Toxic metals of the polluted soils were higher than the control, and decreased in the following order Pb>Fe>Mn>Cd>Cr, when compared with the control (Fe>Pb>Mn>Cd>Cr). This implied that SEO added more Pb to the polluted samples. Soil has high retention capacity for lead and iron, compared to other heavy metals. This could be attributed to fact that Pb sticks to organic matter in the soil, and maybe responsible for lead contamination of food.

Growth performance of *G. max* on polluted soil samples

Performance of *G. max* cultivated on polluted and unpolluted soil samples for 3 weeks, are shown in Table 3. From the results, it is evident that there was rapid growth of plants cultivated on unpolluted soil, from 25 ± 1 to 34 ± 3.6 cm in 21 days. *Glycine max* planted on the polluted soil showed a retarded growth rate compared to the unpolluted soils. There was a reduction in the number of leaves, and a gradual change in color of leaves, from green to greenish-yellow of plants, on the polluted soils. Results also showed the death of two replicates of plants on the 8 % polluted soil in week 1 (7 days), and none in subsequent weeks.

Quality of DNA extracted from *G. max*

Number of bands in *G. max* DNA formed from gel electrophoresis after day 7, 14 and 21, are shown in Table 4. The increase in number of bands increased with % pollution. The concentration (4 %) produced an increase in the number of bands from 2 to 4 in the 7th and 21st weeks, while the highest number of 3 to 5 bands was observed in the 8 % polluted soil, when compared with the control.

Table 1. Physiochemical properties and total hydrocarbon content of the polluted and unpolluted soil.

Parameters	4 %	6 %	8 %	Control
% Sand	92.21 ± 0.08	91.51 ± 0.04	91.22 ± 0.01	93.28 ± 0.05
% Silt	6.07± 0.01	8.05± 0.01	8.10± 0.01	2.08 ± 0.03
% Clay	1.96± 0.01	0.96± 0.01	0.76± 0.01	6.53± 0.001
pH in 1:2.5 H_2O	5.43 ± 0.02	5.38 ± 0.02	5.13 ± 0.02	6.55 ± 0.2
% Organic carbon	1.56 ± 0.01	1.60 ± 0.01	1.61 ± 0.003	1.13 ± 0.001
% Organic matter	2.78 ± 0.02	3.07 ± 0.005	3.32 ± 0.01	1.94 ± 0.001
% Nitrogen	0.11 ± 0.002	0.10 ± 0.002	0.09 ± 0.003	0.17 ± 0.001
% Phosphorus	11.16 ± 0.1	8.61 ± 0.02	5.55 ± 0.05	16.55 ± 0.03
Ca (Cmol/kg)	8.93± 0.02	9.68± 0.1	12.51± 0.04	7.54± 0.01
Mg (Cmol/kg)	2.27± 0.01	2.54± 0.03	4.67± 0.02	2.04± 0.01
K (Cmol/kg)	0.14± 0.003	0.17± 0.001	0.27± 0.003	0.10± 0.01
Na (Cmol/kg)	0.14± 0.003	0.15± 0.003	0.18 ± 0.001	0.11± 0.001
THC	20.24 ± 0.6	35.52 ± 0.5	57.12 ± 0.03	8.31±0.01

Note: Values are mean ± SD of triplicate determinations

Pictorial description of the agarose gel electrophoresis of DNA collected from *G. max* grown of the polluted soils, are shown in Figure 1. After day 7, lane D (8 %) had the highest number of bands (3). Lanes B (4 %) and C (6 %) had 2 bands each, when compared with only 1 band from lane A (control). Lanes B, C, and D had equal number of bands (3), when compared with the only 1 band from lane A. At day 21, lanes C and D had the highest bands (5), lane B had 4, while the least number of bands (1) was seen in lane A (control).

Table 2. Toxic metals content of soil samples polluted and unpolluted with spent engine oil.

Heavy Metals	4 %	6 %	8 %	Control
Pb	0.244 ± 0.001	0.324 ± 0.003	0.442 ± 0.005	0.113 ± 0.001
Cd	0.076 ± 0.001	0.081 ± 0.001	0.110 ± 0.01	0.066 ± 0.002
Cr	0.043 ± 0.002	0.050 ± 0.001	0.051 ± 0.001	0.034 ± 0.003
Fe	0.164 ± 0.002	0.172 ± 0.002	0.194 ± 0.001	0.152 ± 0.002
Mn	0.082 ± 0.001	0.096 ± 0.001	0.129 ± 0.002	0.073 ± 0.002

Note: Values are mean ± SD of triplicate determinations

Table 3. Performance of *Glycine max* plant on spent engine oil polluted and unpolluted soil.

Parameters	4 %	6 %	8 %	Control
Duration (days)	7	7	7	7
Plant height	18.33 ± 0.58	18	17	25 ± 1
Leaf Number	10.33 ± 0.58	10.33 ± 0.58	9	13.33 ± 0.58
Leaf Colour	Light green	Greenish yellow	Greenish yellow	Deep green
Mortality	ND	ND	2	ND
Duration (days)	14	14	14	14
Plant height	20.83 ± 0.76	19.33 ± 0.58	18.5	29 ± 1
Leaf Number	10	8.33 ± 0.58	6	15.67 ± 1.2
Leaf Colour	Greenish yellow	Greenish yellow	Greenish yellow	Deep green
Mortality	ND	ND	ND	ND
Duration (days)	21	21	21	21
Plant height	20.5 ± 1.5	19.33 ± 1.2	17.5	34 ± 3.6
Leaf Number	8 ± 2	8 ± 1.7	6	23 ± 3.6
Leaf Colour	Greenish yellow	Greenish yellow	Greenish yellow	Deep green
Mortality	ND	ND	ND	ND

Note: Values are mean ± SD of triplicate determinations, ND = Not detected.

Table 4. Number of DNA bands of *Glycine max* planted on unpolluted and SEO-polluted soil on agarose gel electrophoresis.

Parameters	Day 7	Day 14	Day 21
Control	1	1	1
4 %	2	3	4
6 %	2	3	5
8 %	3	3	5

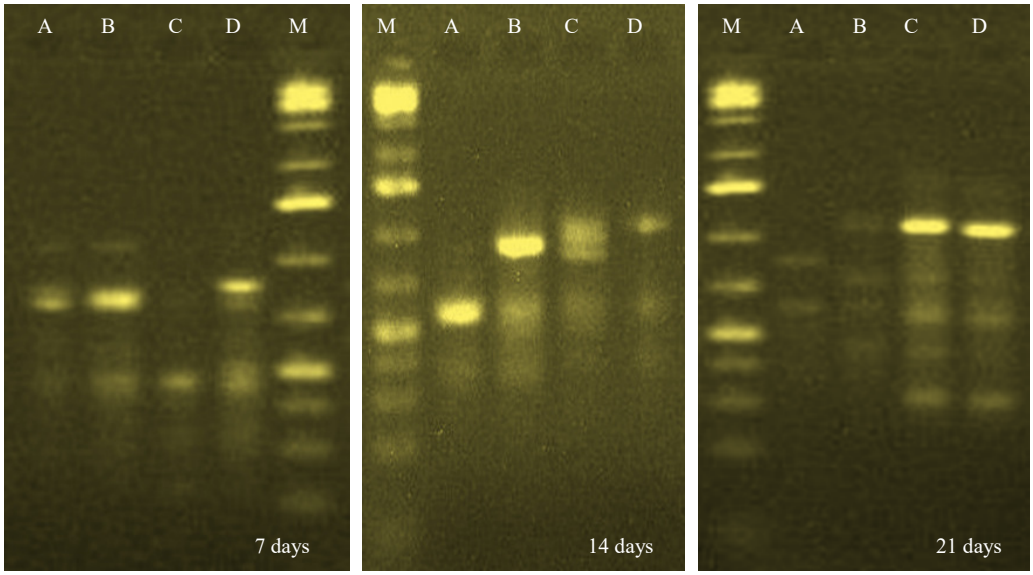

Figure 1. DNA profiles of *Glycine max* grown on spent engine oil – polluted soil, after 7 days, 14 days and 21 days of pollution, on agarose gel electrophoresis. **Note:** A = DNA band of *Glycine max* on unpolluted soil; B = DNA band of *Glycine max* on 4 % concentration of SEO polluted soil; C = DNA band of *Glycine max* on 6 % concentration of SEO polluted soil; D = DNA band of *Glycine max* on 8 % concentration of SEO polluted soil; M = 1 kb plus DNA ladder (marker).

Percentage purity of DNA bands obtained from *G. max* from polluted and unpolluted soils, are shown in Figure 2. Results obtained showed a higher percentage purity of DNA from *G. max* of the unpolluted (1.87), when compared with the polluted ones. Increasing % pollution caused a reduction in percentage purity of DNA. The 4 % pollution level had the highest purity level when compared with the least observed in the 8 % pollution level.

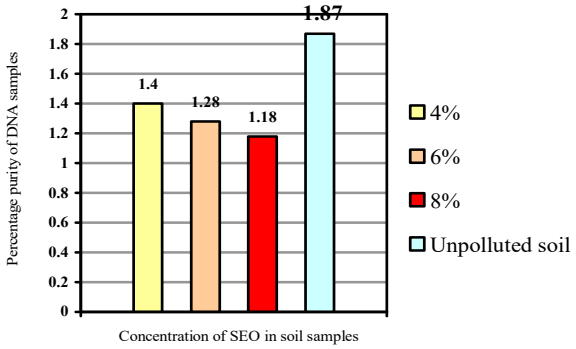

Figure 2. Percentage purity of DNA samples from *G. max* grown on different concentrations of SEO polluted and unpolluted soils.

Quantity of DNA extracted from *G. max*

Nanodrop spectrophotometer analyses of quantity of DNA obtained from *G. max* grown on polluted and unpolluted soils for 21 days are shown on Table 5. Results showed that the quantity of DNA was higher (512.6 ng/µL) in *G. max* of the unpolluted soil, when compared with the polluted ones. The quantity of DNA decreased with increased % level of pollution. Thus, *G. max* grown on 4 % level of polluted soil had second highest quantity (210.4 ng/µL) of DNA, while the least was observed (103.00 ng/µL) in 8 % concentration level.

Table 5. Nanodrop spectrophotometer assessment of the quantity of DNA of *G.max* grown on the polluted and unpolluted soils.

Concentrations of SEO in soil samples	Concentration of nucleic acid (ng/µL)
4 %	210.4
6 %	128.5
8 %	103.0
Unpolluted	512.6

DISCUSSION

It was observed that pollution of soil with SEO did not affect the textural class of the soil. Consequently, the physical properties of the polluted and unpolluted sandy soils were unaffected. Similar finding that spent lubricating oil did not change the physical properties of the soil pH, % silt, % clay and % sand was previously reported by Lale *et al.* [16]. This observation is also similar to the finding of Onweremadu [24] who reported that pollution does not affect the textural class of soil. However, chemical properties of the soil samples showed that SEO – polluted soils had lower pH, making them more acidic. These results agreed with the reports of Udebuani *et al.* and Umunnakwe *et al.* [34, 36]. It is known that pH influences oil decomposition. Otitoju [28] reported that the acidic condition of soil reduces microbial activity, thereby reducing the rate of degradation of pollutant. Earlier report has shown that the organic matter content of studied soil samples was in the range of 1.68 to 2.60 % [25]. This is related to range of 1.94 ± 0.001 to 3.32 ± 0.01 recorded in this study. Meanwhile, Umunnakwe [36] had also reported an increase in total organic carbon content of soil in Orji Mechanic Village, Imo State, Nigeria. Mineral nutrients tend to be higher in polluted soil, showing that the nutrients were unused. One of the adverse effects of oil is that it makes nutrients abundant in the soil, but renders them inaccessible to plant roots. Umunnakwe [36] reported that pollution of soil with SEO affects the physical, chemical, biological and toxic metal levels. However, in their study, Lale *et al.* [16] observed that pollution of soil with spent lubricating oil did not significantly affect soil micronutrients concentration, but significantly increased the total hydrocarbon content of the polluted soil. This is in line with the findings of this study.

Studies have shown that Pb in the soil affects metabolic and biochemical activities necessary for normal growth, and development, of plants, thereby reducing their growth [32]. Lead induces oxidative stress to higher plants [30]. Results of this study indicated that the number of leaves and plant heights of *G. max* decreased in the polluted soils. This could result from inability of the plants to absorbed adequate amount of nutrients, and oxygen, needed for normal metabolic activities. These results were corroborated by the findings of Agbogi and Edema, and Bremner and Mulvaney [2, 18]. Greenish – yellow colouration of leaves was observed in *G. max* grown on polluted soil, when compared with the control. This result is similar to the finding of Uhegbu *et al.* [35], who suggested that the yellowing of leaves is a result of an interference with chlorophyll biosynthesis. The main possible reason is the deprivation of plants by SEO through locking up the essential nutrients required for the biosynthesis of chlorophylls. Death of plants observed in this study could be as a result of the inability of the plant to draw nutrients from the soil, and the inhibition of photosynthetic potentials [14]. Reduction of oxygen content of the soil, which could impede respiration of root cells, could also be implicated in the death of plants. It is thought that further mortality of plants during the period of treatment discontinued because plants were able to adapt to adverse effects of the pollutant. Nwakanma [19] reported an initial increase in shoot and root growth for *Vernonia amygdalina* grown on SEO – polluted soils, which decreased in later stages of growth, compared with the control. Lale *et al.* [16] reported that spent lubricating oil-polluted soil significantly decreased the height of plants, number of leaves, shoot dry weight and laminar leaf area.

This study observed higher number of DNA bands in *G. max* grown on polluted soil, compared with the control. DNA damage has been found to characterize exposure to crude oil [11, 12]. Specifically, DNA strand and chromosomal breakage are used to establish genotoxicity [33]. Increased number of bands recorded from agarose gel analyses of the DNA obtained from the polluted plants showed chromosomal breakage, which is a result of exposure to pollution by engine oil. Exposure to petroleum contamination causes an increase in number of single strand breakage, and variations in DNA cells content [33]. Results of quantification of DNA and percentage purity for *G. max* grown on polluted soil samples, provided some evidence that the genetic integrity of *G. max* was compromised. This suggests that spent engine oil possesses genotoxic potentials. Several researchers reported DNA damage in some species of plants exposed the to pollutant [17, 31]. Reports by Nwakanma [19] had revealed chromosomal aberrations, including bridges, laggard chromosome, vagrant chromosomes, fragments, and stickiness of chromosome in root tips of *V. amygdalina,* grown on SEO – polluted soil, confirming the cytotoxic/genotoxic potentials of the pollutant.

Spent engine oil altered the chemical properties of the treated soil of this study, thereby reducing the growth parameters of the test plants. However, the oil did not affect the physical content of the soil. Therefore, arable lands should be protected from the indiscriminate disposal of spent engine oil, to guarantee high agricultural outputs, and food security. This study also established that spent engine oil, indiscriminately disposed in the environment, is genotoxic to *G max* plant. This is evident by the breakages, reduction of concentration and purity levels of DNA samples of *G. max* plants grown on the SEO – polluted soils. This observation of genotoxic effect from SEO on the test plant, and perhaps other species of plants, is of great concern to the entire ecosystem and human health. This is because of the grave health challenges associated with alteration of structural and compositional integrity of most plant DNA.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Agbaji, J.E., Nwaichi, E.O., Abu, G.O., (2020): Optimization of bioremediation-cocktail for application in the eco-recovery of crude oil polluted soil. AAS Open Research, 3(7): 1-25.

[2] Agbogi, M.O., Edema, N.E., (2003): Effects of crude oil and its water-soluble fractions on the growth parameters of *Panicum repens* Linn. Nigeria Journal of Ecology, 6: 1-4.

[3] Akinyele, I.O., Shokunbi, O.S., (2015): Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples. Food Chemistry, 173: 682-684.

[4] Anuforo, H.U., Akujobi, C.O., Umeh, P.K., Ejimadu, P.I., (2020). Pattern of distribution and concentration of selected heavy metals in farmlands near roadsides in Owerri, Nigeria. Analele Universităţii din Oradea, Fascicula Biologie, 27(1): 32-38.

[5] Ayoola, O.S., Akaeze, C.O., (2012): Genetic evaluation and toxicity of spent engine oil on *Clarias gariepinus*. Research Journal of Environmental Toxicology, 6: 133-141.

[6] Bhattacharya, M., Biswas, D., Santanu, S.S., Datta, S., (2015): Biodegradation of waste lubricants by a newly isolated *Ochrobactrum* sp. Biotechnology, 5: 807-810.

[7] Bouyoucous, G.J., (1951): A recalibration of the hydrometer method for making mechanical analysis of soils. Agronomy Journal, 4: 434-438.

[8] Bremner, J.M., Mulvaney, C.S., (1982): Nitrogen - total. pp. 595-624. In Page, A.L., Miller, R.H., Keeney, D.R., (eds.): Methods of Soil Analysis: Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin.

[9] Chen, H., Rangasamy, M., Tan, S.Y., Wang, H., Siegfried, B.D., (2010): Evaluation of five methods for total DNA extraction from western corn root worm Beetles. PLos ONE, 5(8): 119-163.

[10] Comings, K.J., Booth, D.B., Horner, R.R., (2000). Storm water pollutant removal by two wet ponds in Bellevue, Washington. Journal of Environmental Engineering, 126: 321-330.

[11] Deasi, S., Verlecar, X., Ansari, Z., Jagtap, T., Sarkar, A., Vashistha, D., Dalal, S., (2010): Evaluation of genotoxicity responses of *Chaetoceros tenuissimus* and *Skeletonema costatum* to water accommodated fraction of petroleum hydrocarbons as biomarker of exposure. Water Research, 44: 2235-2244.

[12] Goanvec, C., Theron, M., Lacoue-labarthe, T., Poirier, E., Guyomarch, J., Le-Floch, S., Laroche, J., Nonnotte, L., Nonnotte, G., (2008): Flow cytometry for the evaluation of chromosomal damage in turbot *Psetta maxima* (L) exposed to the dissolved fraction of heavy fuel oil in sea water: a comparison with classical biomarkers. Journal of Fish Biology, 73: 395-413.

[13] Huang, K., Li, Y., Hu, J., Tang, C., Zhang, S., Fu, S., (2021): Rates of soil respiration components in response to inorganic and organic fertilizers in an intensively-managed Moso bamboo forest. Gerderma, 403: 115212.

[14] Hussion, O., (2013): Redox potential (EH) and pH as drivers of soil/plant/microorganism systems: A transdisciplinary overview pointing to integrative opportunities for agronomy. Plant soil, 362(1): 389-417.

[15] Jha, A.N., (2004): Genotoxicological studies aquatic organisms. Mutation Research-Fundamental Molecular M, 552(1-2): 1-17.

[16] Lale, O.O., Ezekwe, I.C., Lale, N.E.S., (2014): Effects of spent lubricating oil pollution on some chemical parameters and growth of cowpea (*Vigna unguiculata* Walpers). Resources and Environment, 4(3): 173-179.

[17] Li, T., Zhang, M., Lu, Z., Herman, U., Mumbengegwi, D., Crittenden, J., (2016). Effects of heavy metals from soil and dust source on DNA damage of the *Leymus chinensis* leaves in coal-mining area in northwest China. PLoS ONE, 11(12): e166522.

[18] Njoku, K.L., Akinola, M.O., Busari, T.O., (2012): Effect of time of application of spent oil on the growth and performance of maize (*Zea mays*). African Journal of Environmental Science and Technology, 6: 67-71.

[19] Nwakanma, N.M.C., Ikegwu, E., Osaigbovo, E.J., (2018): Genotoxic effects of spent engine oil (SEO) – polluted soils on *Vernonia amygdalina* Del. International Journal for Research in Applied Science & Engineering Technology, 6(XII): 564-570.

[20] Nwite, J.N., Alu, M.O., (2015). Effect of different levels of spent engine oil on soil properties, grain yield of maize and its heavy metal uptake in Abakiliki, Southeastern Nigeria. Journal of Soil Science and Environmental Management, 5(4): 44-51.

[21] Okon, J.E., Mbong, E.O., (2013): Effects of nutrient amendment of spent engine oil polluted soil on some growth parameters of *Abelmoschus esculentus* (L.) moench in South-South Nigeria. Bulletin on Environmental Pharmacology and Life Science, 2(5): 75-78.

[22] Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O., Emede, T.O., (2007): The effects of spent engine oil on soil properties and growth of maize (*Zea mays* L.). Journal of Applied Science and Environmental Management, 11(3): 147-152.

[23] Olayinka, B.U., Arinde, O.O., (2012): Effect of spent engine oil on germination and seedling growth of groundnut (*Arachis hypogaea*). Insight Ethnopharmacology, 2(1): 5-9.

[24] Onweremadu, E.U., (2009): Magnesium content of two soil groups in Southeastern Nigeria in relation to selected pedological properties. American-Eurasian Journal of Sustainable Agriculture, 3(3): 481-486.

[25] Opeyemi, A.O., Adewunmi, B.I., Oluwaseyi, A.I., (2020): Physical and chemical properties of soils in Gambari Forest Reserve near Ibadan, South Western Nigeria. Journal of Bioresource Management, 7(2): 57-67.

[26] Osuagwu, A.N., Nwofia, G.E. (2014): Effect of spent engine oil on the germination ability of eleven accessions of African yam bean seeds (*Sphenostylis sternocarpa* Hochst ex A. Rich) Harms. Journal of Agriculture and Veterinary Science, 7(1): 59-62.

[27] Osubor, C.C., Anoliefo, G.O., (2003): Inhibitory effect of spent lubricating oil on the growth and respiratory function of *Arachis hypogaea* L. Benin Science Digest, 1: 73-79.

[28] Otitoju, O., Udebuani, A.C., Ebulue, M.M., Onwurah, I.N., (2017): Enzyme-based assay for toxicological evaluation of soil ecosystem polluted with spent engine oil. Journal of Agriculture and Ecology Research International, 11(3): 1-13.

[29] Peng, J.F., Song, Y.H., Yuan, P., Cui, X.Y., Qiu, G.L., (2009): The remediation of heavy metals contaminated sediment. Journal of hazardous materials, 161(2-3): 633-640.

[30] Quereshi, M.I., Abdin, M.Z., Qadir, S., Iqbal, M., (2007): Lead induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Biology of Plant, 51: 121-128.

[31] Sebbio, C., Carere, C., Nascetti, G., Bellisario, B., Mosesso, P., Cimmaruta, R., Angeletti, D., (2014): Interspecies variation in DNA damage induced by pollution. Current Zoology, 60(2): 308-321.

[32] Strubinskai, J., Hanaka, A., (2011): Adventitious roots system reduces lead uptake and oxidative stress in sunflower seedlings. Biological Plant, 55: 771-774.

[33] Theodorakis, C.W., Bickham, J.W., Donnelly, K.C., McDonald, T.J., Willink, W.P., (2011): DNA damage in cichlids from an oil production facility in Guatemala. Ecotoxicology, 21: 496-511.

[34] Udebuani, A.C., Okoli, C.I., Nwigwe, H., Ozoh, P.T.E., (2011): Effects of spent engine oil pollution on arable soil of Nekede Mechanic Village Owerri, Nigeria. International Journal of Natural and Applied Sciences, 7(3): 257-260.

[35] Uhegbu, F.O., Akubugwo, E.I., Iweala, E.J., Uhegbu, O.C., (2012): Impact of spent engine oil on soil and growth of *Zea mays* seeds. Scientific Journal of Environmental Sciences, 1(1): 1-8.

[36] Umunnakwe, J., Aharanwa, B.C., Njoku, R.E., (2020): Impact of used motor oil on the soil qualities of Orji Mechanic Village, Owerri, Nigeria. International Journal of Engineering Technologies and Management Research, 7(2): 1-12.

[37] Walkley, A., Black, I.A., (1934): An examination of the digestates method for determining soil organic matter and propose modification of the chronic acid titration method. Soil Sciences, 37: 29-38.

Received: September 9, 2022
Accepted: October 31, 2022
Published Online: November 2, 2022
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House