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Genotoxic Effects of Spent Engine Oil (SEO)-Polluted Soils on Vernonia Amygdalina Del.

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Abstract: ***The general toxicity and genotoxic effect of spent engine oil (SEO) - polluted soils on Vernonia amygdalina root tips were investigated in this study. Stems of V. amygdalina were cultivated in various concentrations (5ml, 10ml, 15ml, 20ml and 25ml) of spent engine oil (SEO)-polluted soils respectively with non polluted-soil as control. After two weeks, five root lengths and five shoot lengths of V. amygdalina were measured from each test concentration of spent engine oil (SEO)-polluted and control soils respectively, using a well calibrated ruler. Subsequent measurements were taken per week for another three weeks and at the end of the fifth week five root tips from each stem of V. amygdalina were harvested and processed using the aceto-orcein squash technique at week two. Two tests (proximate analysis on the experimental plants and soil analysis) were equally carried out. The mitotic index (M.I) of the effect of each spent engine oil (SEO) product was determined from 500 cells and was 8.80 for the control. The M.I. for spent engine oil (SEO) - polluted soils had values of 5.57 (5ml), 5.23 (10ml), 4.38 (15ml), 5.13 (20ml) and 5.08 (25ml). The mitotic indices produced by the test substances (spent engine oil) were found to be lowest at 15ml of spent engine oil (SEO) (4.38). The microscopic studies showed several chromosomal abnormalities which included stickiness of chromosomes, bridges, vagrant chromosomes, c-metaphase among others. The effects of treatment on the test plants showed some phytotoxicity with chlorosis and mottling of leaves being the most prominent. The implication of these observations in the possible use of V. amygdalina in phytoremediation of spent engine oil – polluted soils is discussed.***
Keywords: Chromosomal abnormalities, genotoxic effects,, phytotoxicity, Spent engine oil (SEO), Vernonia amygdalina.

I. INTRODUCTION

The contamination of the natural environment by petroleum derived substance contributes to the degradation of land (Njoku *et al*., 2009). Changes in some soil properties resulting from contamination of soil with petroleum-derived substance bring about some changes in the biological composition of soil. This can lead to water and oxygen deficits as well as to a shortage of available form of nitrogen and phosphorus (Njoku *et al*., 2009; Wyszkowska and Kucharski, 2000). Oil pollution prevents normal oxygen exchange between soil and atmosphere due to hydrophobic properties of oil (Njoku *et al*., 2009; Odjegba and Sadiq, 2002). The indiscriminate disposal of spent engine oil by motor mechanics is a common source of spent engine oil contamination of soil in countries like Nigeria that do not enforce strict compliance to environmental laws (Stephen *et al*., 2011; Ogbo *et al*., 2009). Contaminants present in the soil can enter the food chain and seriously affect animal and human health (Stephen *et al*., 2011; Khan, 2005).

Spent engine oil (SEO) is typically referred to as used motor oil that have been collected from mechanical workshops, garages, and industry sources such as hydraulic oil, turbine oils, process oil and metal working fluids. Spent oil can also originate at seaports from ocean-going vessels which can contain salty sea water, heavy and intermediate fuel oil along with various heavy metals common to such fuel oil (Olufemi and Oladeji, 2008). Once the spent engine oil is generated there are equipments and procedures in which the used oil can be tested and this helps us to know how the spent engine oil would assume different classification where they can be used again or the kind of handling safety that will be applied when blending it for other purposes (Olufemi and Oladeji, 2008). Spent oil is a common and toxic environmental contaminant not naturally found in the environment (Njoku *et al*, 2009). Since it is liquid, spent engine oil will migrate into the environment and eventually finds its way to contaminate either water or soil. Spent engine oil can be properly analyzed to suit other purposes ranging from generation of electricity, uses in industrial burners, mixing with asphalts for paving space waters in automotive bays, etc, to mention but few (Olufemi and Oladeji, 2008). Spent engine oil (SEO) needs to be analyzed because of the components which have very crucial importance in any processing plant and in the value added product composition. Challenge of processing spent engine oil into value product is very great. It is also significantly different from processing of crude oil (Olufemi and Oladeji, 2008).

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Pollution of the soil with petroleum and it's derivatives is often observed in municipal soils and in areas where petroleum and natural gas are obtained (Njoku *et al*., 2009; Nwakanma *et al*., 2011). Waste lubricating oil creates unsatisfactory conditions for plant growth ranging from heavy metal toxicity to insufficiency in aeration of the soil (Stephen *et al*., 2010). Ogbo *et al.* (2009) reported that oil contamination causes slow rate of germination in plants. The aim of this work was to evaluate the genotoxic effects of spent engine oil (SEO)-polluted soils on *Vernonia amygdalina*

II. MATERIALS AND METHOD

The field work for this study was carried out in a screen house located at the Botanical Garden of the Department of Biological Science, Yaba College of Technology, Yaba. Lagos while the Laboratory work was carried out at Lab. 218 of the Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos.

A. Plant samples

24 sacs were prepared with 10.85kg of soil (sandy loamy soil) following the method of Njoku *et al*, (2009) and Nwakanma *et al*., (2011). Four sacs were not polluted at all. Hence, they served as control. The remaining 20 sacs were divided into five sub-groups of four flower sacks each. The test concentrations included: 5ml, 10ml 15ml, 20ml and 25ml respectively, four sacs for each concentration in the spent engine oil (SEO) served as replicates (Njoku *et al*, 2009a).

The pollutants were mixed with the soil using the hand fork before planting. Planting was done between 6pm and 7pm so as to prevent quick evaporation of petroleum samples after application. Five plant stem samples of *Vernonia amygdalina* were introduced into the soils polluted with spent engine oil (SEO) respectively as well as the controls. The plant samples were watered regularly to ensure survival of the plant sample during the period of study. On the second week after planting, five root-tips of the plant were collected from each stem of *Vernonia amygdalina* plants grown on both polluted soil and control. These root-tips were fixed and then taken for cytological examination. The first root lengths of *Vernonia amygdalina* for both polluted and control soils were measured after two weeks of planting using a well calibrated ruler (Njoku *et al*, 2009; Nwakanma *et al*., 2011). This was done by carefully up-rooting the plant samples using a hand fork. The roots were then washed or rinsed using bore-hole water so as to remove sand particles before measurements were taken as described by (Njoku *et al*, 2009a; Nwakanma *et al*., 2011). Subsequently, root measurements were taken per-week for another four weeks. Five shoot lengths were also taken from each replicate of each concentration of spent engine oil (SEO) - polluted soils respectively as well as from the control.

B. Squash Technique

On the fifth week, final root lengths were measured. The root-tips of both *Vernonia* plants grown on polluted soils and those plants grown on control soils were randomly selected by cutting them off the plant samples with a sharp razor blade or forceps. They were sliced as described by Nwakanma *et al*, (2009). Thereafter, the roots were fixed in freshly prepared 1:3 acetic acid/75% alcohol (V/V) for at least 24 hours and then stored in 70% alcohol under refrigeration until when required (Nwakanma and Okoli, 2010). For control purposes another group of randomly selected roots were taken and placed in a wash glass bottle. Five root tips were taken from each plant, after which they were cut and fixation was carried out as previously described above. (Nwakanma and Okoli, 2010) The root-tips were hydrolyzed in IN HCL at 60^0 C for 5 minutes (Abu and Ezengwu, 2008). This was done to soften the root tissues (Ukaegbu and Odeigah, 2009) as well as to facilitate the disintegration of the middle lamella of the cell staining. This treatment precedes their stabilization before squashing was done (Nwakanma and Okoli, 2010). Subsequently, the roots were placed on a slide and the terminal root tips (1-2mm) were cut off (Olusegun *et al*, 2010) using a sharp razor blade and used for slide preparation (Nwakanma and Okoli, 2010). The rest of the root material was removed from the slide and excess HCl was removed by blotting with a piece of blotting paper (Olusegun *et al*, 2010). For examination of mitotic chromosomes root tips were squashed in FLP – Orcein (2g of Orcein solution) following the method of Nwakanma and Okoli, (2010). The materials (root-tips) were squashed directly by tapping with the blunt end of a ball pen to cause the root-cells to spread out properly (Nwakanma and Okoli; 2010). The slides were then covered with a cover slip and the root cells were allowed to absorb stain for 5 – 10 minutes. Afterwards, the root cells were blotted of excess orcein solution by placing layers of blotting paper on the cover slip and pressing slightly down with the thumb (Olusegun *et al.*, 2010).

The slides were viewed under X40 magnification; the frequencies of mitotically dividing cells were scored by sampling portions of slides which showed un-ambiguity in the configurations of mitotic cells. The mitotic index was defined as the ratio of dividing cells to the total number of cells examined for each treatment (Nwakanma and Okoli, 2010). All slides were coded / labeled and examined blind. The mitotic index (MI) was determined by examination of 500 cells per concentration (100 cells per slide).

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(Olusegun *et al.*, 2010) Characterization of mitosis and chromosomal aberrations were scored in 100 cells per slides. (Nwakanma and Okoli, 2010).

C. Proximate and Soil Analysis

Proximate analysis and TPH analysis on the experimental plants in addition to soil TPH analysis on the experimental soil were carried out using classical method in the Chemistry Department, University of Lagos, Akoka, Lagos.

D. Statistical Analysis

Results were analyzed statistically using student General Linear Model (GLM) which incorporates the univariate analysis of variance (ANOVA) and the pair wise t test comparison at ($P<0.05$).

III. RESULTS AND DISCUSSION

Data on the mean root lengths, leaf lengths, and leaf width of *Vernonia amygdalina* cultivated in spent engine oil-polluted soils during the experiment is shown on Table 1 while the microscopic effects are presented in Table 2 and Figure 1. The phytotoxic effects of the treatments are presented in Figure 2 while the results from proximate and soil analysis are presented on Tables 3 and 4.

The results obtained in this study showed that shoot and root growth of *Vernonia amygdalina* stems cultivated in spent engine oil - polluted soils exhibited more rapid growth in all test concentrations compared to control during the early stages of growth (2nd week) but presented generally lower growth compared to control in later stages (5th week) within the study period (Tables 1, 2 and 3) as well as early development of phytotoxic indices such as chlorosis and mottling of leaves (Fig. 2). The results equally indicated that spent engine oil-polluted soils have toxic effect on root tips of *V. amygdalina* from the microscopic point of view and this is also observed to be correct from the macroscopic result

There were root growth for all levels of concentration and the growth rate for each concentration increased per week. The shortest root-lengths were observed in the 10ml of the experimental plants (Table 3). The longest leaf lengths were observed in the 15ml while the lowest leaf lengths were observed in the 25ml experimental plants (Table 4). The 15ml of the spent engine oil-polluted soils have the highest mean leaf width and the lowest is from the 25ml experimental plants (Table 5).

Table 1: Mean Root Lengths, Leaf length and Leaf Width ± Standard Error of *Vernonia amygdalina* in soils polluted with Spent Engine Oil (S.E.O).

	Spent Engine Oil	Spent Engine Oil	Spent Engine Oil
Concentration (ml)	Mean Root length ± SD	Mean Leaf Length ± SD	Mean Leaf Width ± SD
Control	5.53 ± 2.54	5.88 ± 2.91	2.84 ± 1.21
5ml	6.54 ± 3.18	6.05 ± 3.32	2.83 ± 1.60
10ml	5.74 ± 3.21	5.92 ± 3.00	2.87 ± 1.42
15ml	5.91 ± 3.56	6.36 ± 3.32	2.92 ± 1.53
20ml	5.96 ± 3.40	5.91 ± 3.32	2.82 ± 1.58
25ml	5.77 ± 3.07	5.84 ± 2.80	2.62 ± 1.24

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Table 2: Mitotic effects of spent engine oil polluted soil on the root tips of V. amygdalina

Concentration of Spent Engine Oil (S.E.O.)							Stickiness	c-mitosis	Bridges and fragment	Vagrant	Total aberration	Mitotic index
	Number of cell	Dividing cells	P	M	A	T						
Control	500	44	2	22	9	11	0	0	0	0	0	8.8
5ml	467	25	2	10	8	5	2	2	4	5	13	5.57
10ml	440	23	0	11	6	6	3	1	5	6	15	5.23
15ml	437	19	3	7	2	7	6	1	2	3	12	4.38
20ml	429	22	2	7	5	8	5	0	3	5	13	5.13
25ml	413	21	3	5	6	7	3	0	5	6	14	5.08

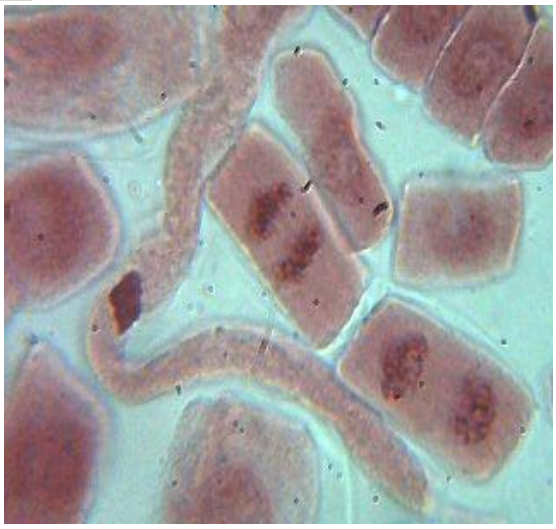
Table 3: Proximate analysis (%) of the experimental leaves of *V. amygdalina*

Parameters	Control	Spent Engine Oil (SEO)			
ml	0	5	10	15	20	25
%						
Moisture content	13.22	15.48	15.49	15.55	15.54	15.56
Total ash	38.42	56.63	58.90	59.03	61.01	62.10
Crude fat	0.950	1.470	1.490	1.700	1.731	1.710
Crude protein	0.69	1.04	1.07	1.04	1.09	1.11
Crude fibre	0.570	0.900	1.020	0.95	0.99	1.34

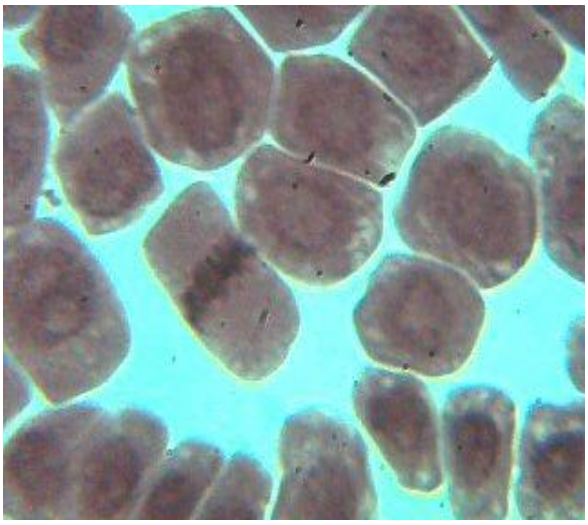
Table 4: Total petroleum hydrocarbon (TPH) analysis of the experimental plants and soil

Concentration	Plants (mg/l)	Soil (mg/l)
Control	0.09	1.14
5ml SEO	0.09	0.54
10ml SEO	0.08	0.92
15ml SEO	0.01	1.02
20ml SEO	0.10	1.06
25ml SEO	0.02	1.07

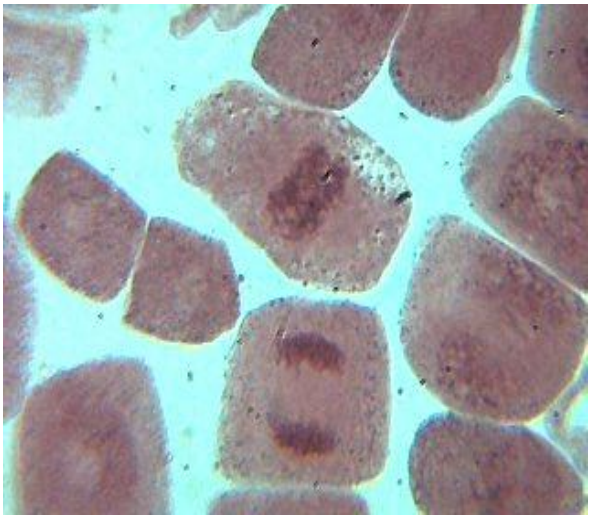
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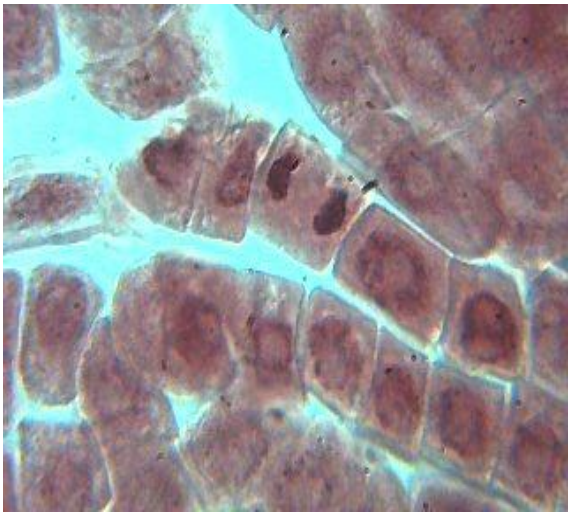
(a) Control (Regular Anaphase)



(b) Control (Regular metaphase)



© Anaphase bridge (20ml S.E.O.)



(d) Sticky chromosome (10ml S.E.O.)

Fig 1 (a – d): Microscopic Effects of Spent Engine Oil (S.E.O) Polluted Soils on *V. amygdalina* Plants.



(a) Normal leaf growth (control)



(b) Normal leaf growth (control)

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(c) Chlorosis in leaf (25ml S.E.O.)



(d) Mottling of leaves (25ml S.E.O.)

Fig 2: Phytotoxic Effect of Spent Engine Oil (S.E.O) Polluted Soils on *Vernonia amygdalina*

The results obtained in this study showed that *Vernonia amygdalina* stems grown in spent engine oil-contaminated soils exhibited early development of phytotoxic indices (chlorosis and mottling of leaves) on the shoot (Fig.1) which agrees with the results of and Agbogidi, (2010) and Nwakanma *et al*., (2011).

The cytogenetic effect of different petroleum product treatments on mitotic division in root tip cells of *V. amygdalina* is given in Table 6. The mitotic index (MI) for the control was 8.8. This was higher from that of all the tested concentrations in spent engine oil-polluted experiments. The highest M.I. from the experiment was from 5ml (5.57) while the lowest was from 15ml (4.38) (Table 6). Similar results were obtained after treating A cepa root cells with textile industrial effluent (Olusegun et al., 2010) and oil field waste water (Odeigah et al., 1997). Inhibition of mitotic activities is sometimes used for tracing cytotoxic substances (Olusegun et al, 2010).

The petroleum product (spent engine oil) studied induced chromosomal aberrations on the root tips of V. amygdalina with laggard chromosome, bridges and fragments, vagrant chromosomes and stickiness of chromosome being the most frequently observed. This suggests that spent engine oil have cytotoxic/genotoxic properties. According to Kong and Ma, (1999), there is a hypothesis that stickiness of chromosome can and may causes incomplete separation of daughter chromosomes as a result of cross linkage of chromo-proteins. The number of aberrant mitotic cells caused by all concentrations of the spent engine oil-polluted soils introduced to the root tip of V. amygdalina apparently was significantly different from that of control. No aberration was observed in V. amygdalina exposed to the control (0ml).

The chlorophyll content of V. amygdalina plant leaf in the polluted soil decreases with increases in concentration of spent engine oil respectively. This is especially notice in the 25ml of spent engine oil (Fig. 1) as it shows marked sign of chlorosis compared to the control. Mottling of leaves where also notice in the 25ml of spent engine oil experimental plants.

Squash technique has been used in screening various types of test substances. Njoku *et al*., (2009) reported the comparative effect of Diesel fuel and Spent Lubricating Oil on the growth of *Zea mays* (Maize). Njoku *et al*., (2011) reported Phytotoxicity Assay of Crude Oil Using Different Accessions of *Sorghum bicolor*. Odeigah *et al*, (1997b) reported genotoxicity of oil field waste water and Nwakanma *et al*., (2011) also reported genotoxic Effects of Diesel and gasoline-polluted soils on *Vernonia amygdalina* which is closely related to this study. According to Abu and Ezeugwu, (2008), monitoring of hazardous waste is vital for sustaining the required legal compliance and in real terms prevents irreversible health and ecological damage.

IV. SUMMARY AND CONCLUSION

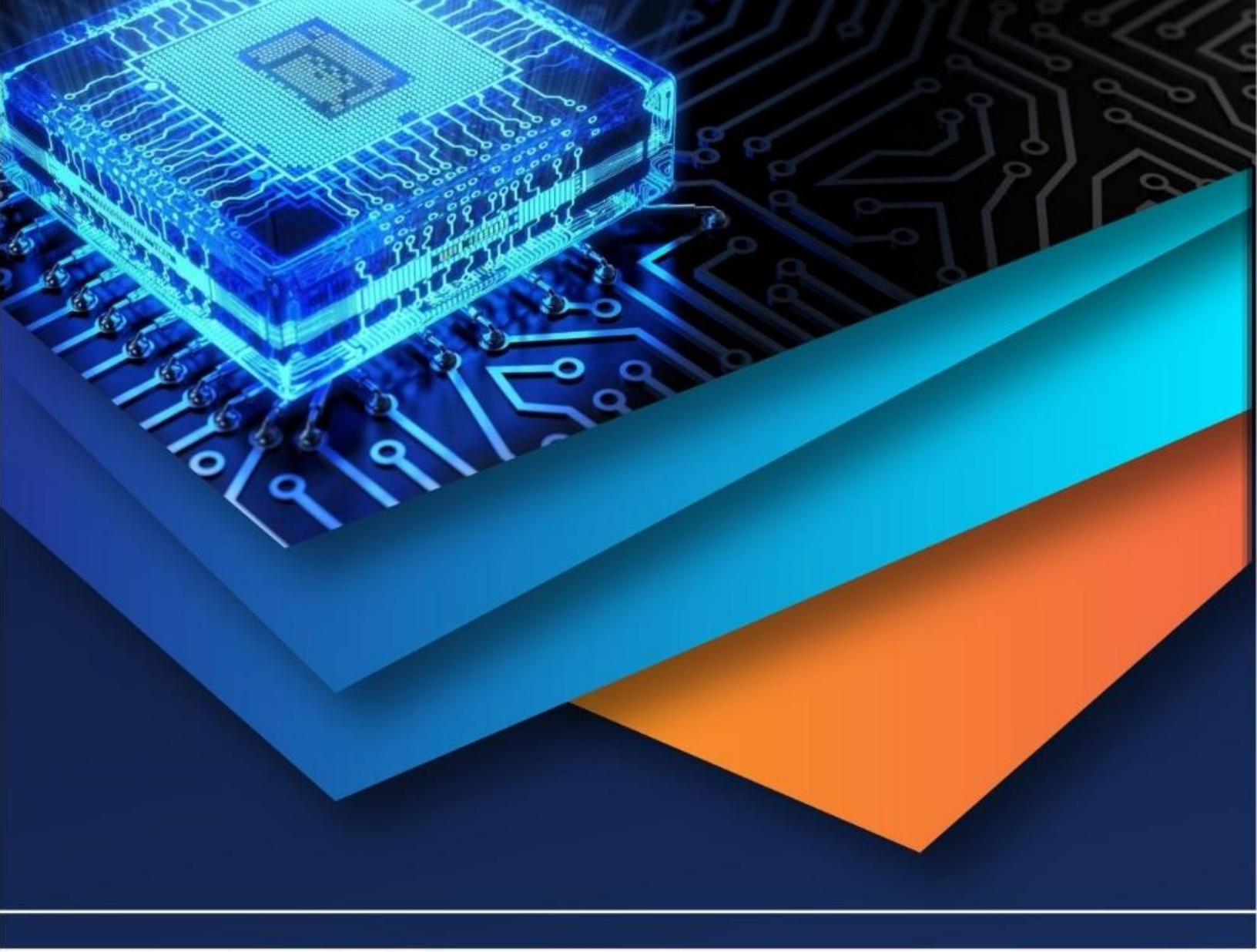
It is hoped that results obtained here with those from related studies as presented in this study may lead us to conclude that spent engine oil in high concentrations constitute environmental hazards. The macroscopic and microscopic results from the spent engine oil-polluted soils in this study show to some extent, that *V. amygdalina* in spite of the genetic aberration can survive on soils polluted with spent engine oil and that such plant (*V. amygdalina*) can be used as a biosensor in monitoring genotoxic effects of petroleum and its by-products such as spent engine oil which will be a helpful tool in bioremediation of SEO - polluted soils.

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