Vicia faba seed: A bioindicator of phytotoxicity, genotoxicity, and cytotoxicity of light crude oils

Elaheh Alavi
Islamic Azad University Tehran North Branch
Golnaz Tajadod (tajadodg@gmail.com)
Islamic Azad University Tehran North Branch
Sayeh Jafari Marandi
Islamic Azad University Tehran North Branch
Sedigheh Arbabian
Islamic Azad University Tehran North Branch

Research Article

Keywords: bioindicator, cytotoxicity, genotoxicity, light crude oil, phytotoxicity, Vicia faba

Posted Date: April 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1423421/v1

Abstract

Crude oil contamination is a serious threat to the environment and human health. The present study aimed at assessing the risk of contaminated soil with light crude oil using *Vicia faba* seed. To this end, the seeds were planted in 0 (control), 1, 2, and 4% (w/w) light crude oil-contaminated soils. The seed germination and then the root lengths were measured for phytotoxicity. The mitotic index, chromosome aberrations, and micronucleus formation in the root tip cells were examined for cytotoxicity and genotoxicity. The results of this study showed that light crude oil had toxic effects on *Vicia faba* growth characteristics, even at 1%. The phytotoxicity assay showed that crude oil reduced seed germination and root length, while cytological observations indicated an increase in mitotic index, chromosome aberrations, and micronucleus formation. The light crude oil at 4% induced the simultaneous occurrence of nuclear bud, polyploidy, and micronucleus that may be considered as severe clastogenic and aneugenic effects of light crude oil. Accordingly, *V. faba* can be considered a reliable living system for monitoring light crude oil pollution in soils, even at low concentrations.

Introduction

Crude oil is a primary source of energy for industrial and domestic needs. However, crude oil and derivatives are common types of environmental pollutants all over the world. These contaminants caused serious environmental problems especially in oil producing countries (Anyasi 2018, Noori 2018).

In recent decades, oil pollution has received much attention from different aspects. Many investigations have shown the ability of plants in accumulating petroleum hydrocarbons (PHs) and possibly contaminating food chains. For example, Radwan et al., (2000) identified high molecular weight hydrocarbons in *Vicia faba* plant. They also reported an increasing level of chain hydrocarbons (mainly C_{30}, C_{36}, and C_{40}) in the seeds with an increased level of oil pollution in soil. Gao and Zhu (2004) reported the accumulation of phenanthrene and pyrene in the roots and shoots of 12 plant species, and found a positive correlation between the amounts of pollutants in soil and those accumulated in the plants. Rao et al., (2007) determined some PHs in the roots of *Vicia faba* grown in crude oil-contaminated soils. Anyasi and Atagana (2018) introduced some native plants with the ability of growth in oil-polluted soils and the accumulation of PHs in their roots. The PHs can affect DNA structure, leading to some kind of cancer, suggesting that accumulation of PHs in food chains can pose serious risks to human health (Alkio 2005). Therefore, it is necessary to find easy and effective ways to assess the degree of soil pollution with oil.

Plants can be used as bioindicators for monitoring soil conditions and risks. Bioindicators are living organisms for assessing the environmental risks and also estimating the level of pollution in the environment (Parmar 2016). The sensitivity of plants to pollutants has made them suitable living systems for detecting the phytotoxic, cytotoxic, and genotoxic effects of polluted soils (Cruz 2019, Salazar-Mercado 2019). The growth and development of terrestrial plants are entirely dependent on soil quality; therefore the effects of pollutants may be assessed according to the modifications that occurred

in plant development, structure, and/or life cycles. Exposure to pollutants can impact seed germination and root elongation as critical stages in plant development, and therefore can be used as phytotoxic tests to monitor contaminated soils (Bamgbose 2015, Cruz 2019).

Mitotic index (MI) can be applied for assessing the cytotoxicity of pollutants. The chromosome aberrations (CA), nuclear anomalies, and micronucleus (MN) formation were introduced for assessing the genotoxicity of the contaminants (Cruz 2019). Chromosome adherence, rings, breakage, polyploidy and disturbed telophase are some examples of chromosome aberrations in plants that happen due to changes in chromosome structure or number. The impact of toxic substances on cell cycle can be determined by measuring the MI, while nuclear anomalies are used as indices for the decrease in membrane integrity (Iqbal 2016).

MN is formed from chromosome fragments or complete chromosomes that do not take part in the formation of daughter nuclei during the cell division. Chromosome breakage and disturbance of cell spindle are two main mechanisms for MN formation (Krisch-Volders 2011). Therefore MN formation is considered as a sign of changes in the structure or numbers of chromosomes due to the presence of mutagens and carcinogens in the environment (Youssef 2018).

To evaluate the toxic effects of different compounds and environmental agents, researchers have conducted investigations using root tip meristem cells. They believe these cells are reliable systems for environmental risk assessment (Fatma 2018, Roberto 2016, Salazar-Mercado 2019, Watanabe 2014). The *Vicia faba* root tips were effectively used for examination of the risks of environmental agents. *Vicia faba* has the advantages of having just six pairs of large chromosomes and being easy to grow and handle, which make it a good choice for environmental cytotoxic and genotoxic studies (Youssef 2018). Kanaya et al., (1994) examined the occurrence of CA in *Vicia faba* root tip meristem to assess the clastogenic potential of four coded chemicals, namely azidoglycerol, N-methyl-N-nitrosourea, sodium azide, and maleic hydrazide. Cotelle et al., (2015) concluded the *Vicia faba* MN test is a practical assay for risk assessment of polluted soils. Barbafieri and Giorgetti (2016) examined the toxic effects of boron on *Vicia faba* root tip meristem. Jiang et al., (2019) showed the toxic effects of nano- and micro-plastics using *Vicia faba* root meristem. Most probably, compounds or environmental agents that have cytotoxic or genotoxic effects on the root tip of *Vicia faba* cells can pose serious risks to animal health too (Liu 2017).

The current study aimed to evaluate the toxic effects of light crude oil contamination using *Vicia faba* seeds. To this end, we analyzed seed germination, root elongation, mitotic index, chromosome aberrations, and micronucleus formation in the root tip meristems. We also examined the capability of *Vicia faba* seed as a reliable living system for monitoring crude oil-polluted soils.

Material And Methods

Seed cultivation

Seeds of *Vicia faba* were washed repeatedly and soaked in water for 24 hours; then they were transferred to soil in separate plates. The plates were rectangular cubs with 22 cm× 17 cm× 5 cm dimensions filled with 500 g soil. The soil was a fine mixture of one part sand and two parts clay. For the experimental group, the soils were thoroughly mixed with Iranian light crude oil (bought from National Iranian Oil Company) at 1, 2, and 4% (w/w) to ensure that all soil particles were homogenously contaminated.

In every plate, 12 seeds were planted and covered by a thin layer of soil and let them grow for seven days in a closed room equipped with an air conditioner system at a nearly constant temperature of 27˚C. There were three replicates for the experimental group and control for every test. After seven days, the germinated seeds were taken out of the soil and washed carefully for further assays.

Phytotoxicity assay

Germination percentages were calculated by dividing the number of germinated seeds to total number of seeds and reported as germination percentage according to Thabet et al., (2019). The lengths of the primary roots were measured in centimeter using a ruler.

Cytotoxicity and genotoxicity assays

Similar to other research works (Thabet 2019, Youssef 2018), the cytotoxicity and genotoxicity investigations were carried out using light microscopy techniques. To this end, two following tasks were performed:

Root sample preparations

Roots were washed with distilled water and fixed in a freshly prepared fixative liquid composed of three parts absolute ethanol and one part glacial acetic acid for 24 hours (Youssef 2018). The fixed roots were stored in ethanol 70% and kept at 4°C for subsequent observations. The solvents were all purchased from Merck in reagent grade and used as received without any further purification.

Slide preparations

Slide preparations were done according to Cruz et al., (2019) with slight modifications. To soften roots, they were hydrolyzed with 5.0 N hydrochloric acid solution for 15 to 20 min. Hydrolyzed roots were stained with Schiff reagent for 45 min, then washed with distilled water. Well-stained root tips were squashed on slides using 45% acetic acid under cover glasses, and for every replicate, about 1000 cells were examined using light microscopy (BH2, Olympus) at different magnifications. The number of mitotic cells, different observed anomalies, including chromosome aberrations, and micronuclei were counted and reported in percentages.

Statistical analysis

All the data were reported in means of three replicates with standard deviations. The means of collected data from the experimental and control groups were subjected to one-way analysis of variance (ANOVA)

using SPSS v.16.0. The statistical significance of data sets were performed by the least significant difference (LSD) test at p ≤ 0.05.

Results

Seed germination and root length

Crude oil caused a significant (p ≤ 0.05) monotonic reduction in the seed germination percentage with increasing oil concentration in the soil from 0 to 4% (Table 1). The highest reduction (50.9%) was observed for the contaminated soil with 4% oil, which is almost 42% less than that of control (92.6%). The lowest reduction was recorded for the 1% contamination that was 12% less than control. The root length was also decreased monotonically, although it was not significant for all treated seeds at p ≤ 0.05. There were no significant difference in the root lengths of the seeds planted in the soil with 2 and 4% contamination at $p < 0.05$ (Table 1). The mean of root lengths in the contaminated soil with 1% oil was 28% less than that of control seeds, while that of the soils with 2 and 4% contaminations were 41 and 51% less than that of control seeds, respectively.

Table 1
Phytotoxicity of different percentages of light crude oil on *Vicia faba* seeds. Data are the mean values ± standard deviations. The values with similar superscripts are not significantly different at p ≤ 0.05.

Traits	Light crude oil concentration in soil (%)			
	0% (control)	1%	2%	4%
Seed germination (%)	92.59 ± 1.5^{a}	81.47 ± 3.2^{b}	70.37 ± 4.2^{c}	50.92 ± 1.5^{d}
Root length(cm)	2.86 ± 0.30^{a}	2.23 ± 0.25^{b}	1.43 ± 0.11^{c}	1.1 ± 0.17^{c}

Cytotoxicity and genotoxicity assays

The current study showed that soil contaminated with light crude oil resulted in significant (p ≤ 0.05) increase in mitotic index of *Vicia fba* root tip cells compared with control (Table 2). Although the difference of MI was significant at p ≤ 0.05 within the experimental group, there was no positive correlation with the percentage of light crude oil in the soil. The mean of MI value in the seeds exposed to 1% contamination (8.7%) was 3.19 times more than that of control (2.73%), whereas that of the seeds exposed to 2 and 4% contaminations were 2.28 and 1.75 times more than control, respectively. Increasing the light crude oil concentration in soil from 1 to 2 and 4% resulted in 28.39 and 45.17% reduction in the MI value, respectively (Table 2).

Table 2
Cytotoxicity and genotoxicity of light crude oil at different concentrations on *Vicia faba* seeds. Data are the mean values ± SD. Different lower case superscripts show statistically significance difference at p≤0.05.

Cell indices	Light crude oil concentration in soil (%)			
	0%	1%	2%	4%
MI (%)	2.73 ± 0.68^{d}	8.70 ± 0.43^{a}	6.23 ± 0.30^{b}	4.76 ± 0.60^{c}
CA (%)	0.69 ± 0.04^{c}	2 ± 0.2^{b}	2.48 ± 0.33^{a}	1.81 ± 0.22^{b}
MN (%)	3.33 ± 0.57^{c}	5.66 ± 0.57^{c}	14.66 ± 1.5^{b}	18.66 ± 2.0^{a}

Oil contamination in this study induced the occurrence of anomalies and MN formation in *Vicia faba* L. root tip cells (Table 2). Developed roots under light crude oil contaminations showed chromosome aberrations and anomalies. The frequencies of CA in the seeds grown in the soils contaminated with 1, 2, and 4% oil were 2.9, 3.59 and 2.62 times higher than control, respectively (Table 2). It was found that the percentages of the crude oil in the soils is not the only influential factor on the percentage of CAs as the highest CA was observed in the seeds contaminated with 2% oil, the lower and higher percentages of oil showed about 50% less CA, and still ~ 65% higher than that of control. Also there was no significant difference at p ≤ 0.05 between 1 and 4% soil contamination.

Sticky chromosomes, C-mitosis, breaks, laggards, and vagrants (Fig. 1) as well as bridge formation in anaphase or telophase (Fig. 2) were some kinds of aberrations observed in the seeds exposed to the soil contaminated with different concentrations of oil.

The simultaneous occurrence of nuclear bud, polyploidy, and large MN, was only observed at 4% (Fig. 3), whereas C-mitosis and bridges were common in 1 and 2% (Figs. 1 and 2). The common aberration in control was the anaphase bridge, while other aberrations of polyploidy, multipolar, and diagonal anaphase were not observed in this group. Although the maximum percentage of the aberrations was recorded for 2% (Table 2), but 4% contamination showed more different aberrations (Fig. 3). There was a monotonic increase in the MN formation with increasing concentrations of the oil in the soil. Accordingly, the light crude oil resulted in an increase in the MN formation compared with that of control in all light crude oil concentrations; although the difference was not significant between control and 1% (Table 2). The occurrence of MN in the root tip cells was significantly (p ≤ 0.05) increased in response to the percentages of the crude oil in the soil (Table 2).

Discussion

Seed germination, as a critical phase in plant development, is commonly used as an indicator for risk assessment (Kaur 2017, Luo 2018, Shen 2016, Varjani 2020). It was also the first step in the process of evaluating appropriate plants for remediation of contaminated soils (Dib et al., 2019). According to the present study, the light crude oil caused a significant reduction in the seed germination of the

experimental group (Table 1). This finding is consistent with the results of Lorestani et al., (2014), who reported that 1, 2, 3, and 4% of light crude oil in soil (w/w) reduced the seed germination of *Vicia ervilia*. In contrast to their results, we received a dose dependent response in the seed germination within the experimental group. The dose-dependent response of seed germination in the present study is consistent with the reports of Oyedeji et al., (2015) who showed that increasing crude oil content in the soil resulted in more severe adverse effects on the seed germination of some legume tree species.

Decreased ability of seeds to germinate in hydrocarbon contaminated soils has been reported by other researchers as well. Tran et al., (2018) showed adverse effects of oil pollution on the germination of *Acacia raddiana* and *Acacia tortilis* seeds. Zhu et al. (2018) reported that seed germination of some grass species is negatively affected by crude oil-contaminated soil. They assessed seed germination in weathered crude oil contaminated soil (1 part crude oil, 8.5 parts sandy loam soil) and scored a range of reduction from 4.3 to 100% among examined species. However, Perez-Hernandez et al., (2013) found that heavy crude oil in soil did not have a significant effect on the seed germination of some tropical trees, and even they observed a positive effect on the seed germination rate for some species. Fismes et al., (2002) also found that seed germination and growth of carrot and lettuce were not affected significantly even at high concentrations of polyaromatic hydrocarbons (PAHs) in soil. Accordingly, the impact of oil-contaminated soils on seed germination depends on plant species and the type and concentration of oil (Besalatpour 2008, Fismes 2002, Oyedeji 2015, Perez-Hernandez 2013).

Crude oil is hydrophobic and hence it covers seed coats, and possibly act as a physical barrier to oxygen and water uptakes (Besalatpour 2008, Ighovie 2014). Volatile components of crude oil are accounted for decreasing seed germination (Zhu 2018). Decreased germination can be discussed as a result of overproduction of reactive oxygen species (ROS) too. The level of ROS is elevated as the rate of mitochondrial respiration increases during seed germination (Janku 2019). Also, it is documented that hydrocarbon contamination causes oxidative stress in plants by inducing the production of ROS (Cui 2016, Ghalamboran 2020, Noori 2018); therefore the fail of seeds to germinate can be assumed as a result of ROS accumulation in seed tissues. However, further studies is necessary to find out the level of ROS in seed tissues and embryos of *Vicia faba*.

The reduction of root length in the present study is consistent with the results of other researchers. For example, Shirdam et al., (2009) reported that crude oil decreased the root length of *Kochia scoparia* (L.) Schard and *Linum usitatissumum* L. up to 76.9 and 78.2%, respectively. *Vigna unguiculata* grown in gasoline contaminated soil developed shorter roots than control (Achuba 2018). Cruz et al., (2019) showed that the contamination of soil with petroleum and diesel fuel at 6.8% (w/w) resulted in 75 and 53% reductions in the root and 70% of hypocotyl, respectively. However, according to Hawrot-Paw and Bakowska (2014), there are some plant species that can compensate the decline of root growth and even develop longer roots than unaffected plants. They reported that *Vicia faba* ssp. *Minor* could grow longer roots than control after 14 days of germination at 1% diesel oil. However, the shorter roots in the current study contradict the reports of Lorestani et al., (2014), which surveyed the effect of light crude oil in the

soil at 1, 2, 3, and 4% (w/w) on *Faba vulgaris* and *Vicia ervilia*. They reported longer roots for treated plants at all contaminations compared to control, except at 4% contamination.

Developing shorter roots in the current study can be described as a result of penetration of some hydrocarbons into the seed and or root tissues. A number of researchers have documented the presence of oil-derived hydrocarbons in the roots of plants grown in oil-contaminated soils. For example, Rao et al., (2007) found aliphatic hydrocarbons in *Vicia faba* root, and Gao and Zhu (2004) showed the accumulations of two kinds of polyaromatic hydrocarbons (phenanthrene and pyrene) in the roots of examined plants. They also reported a positive correlation between the amounts of phenanthrene and pyrene in the roots with corresponding concentrations in soils. Inhibition of root growth in the current study can be explained as a result of the low penetration of water in the soil and limited access to water and oxygen because of hydrophobicity nature of crude oil. However, it is documented that hydrocarbons can change the activity of enzymes and reduce the amount of nutrients. For example, Achuba and Iserhienrhien (2018) reported a significant ($p \leq 0.05$) reduction of total sugar, protein, and amino acids in *Vigna unguiculata* seedlings grown in gasoline contaminated soil. They also reported significant decreases for α-amylase and starch phosphorylase activities under gasoline contaminations.

Cytotoxicity and genotoxicity

A cytotoxic compound may increase or decrease the MI values in the root tip meristem (Salazar-Mercado 2019). A reduction in the MI can be attributed to disruption of DNA synthesis and an increase in the MI can be related to the role of pollutants in inducing tumors (Kayumov 2019). The current study showed a significant increase in the MI values for seeds grown in crude oil-contaminated soils. Achuba (2006) reported significant reductions of cell divisions in the root of cowpea seedlings exposed to 1 and 2% crude oil contaminations. Njoku et al., (2011) showed inhibitions of cell divisions in different accessions of *Sorghum bicolor* root tips treated with crude oil. Also, Ma et al., (2014) reported the negative effects of aqueous extracts of crude oil-contaminated soils on the division of root tip meristem of *Vicia faba*. However, Cruz et al., (2019) did not find any significant changes for the MI values in *Allium cepa* roots subjected to petroleum pollution, while they found significant changes in response to diesel contamination. Researchers concluded that inhibition of cell divisions led to reductions in the lengths of the roots. According to the results of the current study, increased MI did not have any positive effects on the longitudinal growth of roots, and the experimental group had shorter roots than the control. It must be taken into account that root elongation depends on various factors such as hormones, expansin proteins, turgor pressure, and enzymes; therefore cell proliferation is not the only reason for root elongation.

Polycyclic aromatic hydrocarbons as a constituent of crude oil can induce cell death in the plants. Alkio et al., (2005) reported cell death in *Arabidopsis* leaves as a result of phenanthrene exposure. Therefore increasing MI values in the current study may be a compensation mechanism that the plant applies to substitute dead cells with the new ones. It should be noted that a negative correlation was found between the MI values and the crude oil concentrations. Doubling the oil contamination, there were 28.39 and

45.17% significant reductions in the MI of the plants exposed to 2 and 4% contaminations compared with 1%, respectively; however, these values were still significantly more than that of control (Table 2).

According to Pena-Castro et al., (2006), petroleum hydrocarbons can up-regulate some genes that act in signal transduction pathways and result in cell division. Therefore, increased values of MI can be assumed to occur due to changes in signal transduction pathways involved in the cell cycle, need to be explored in future studies.

The current study indicated the significant ($p \leq 0.05$) genotoxic effects of light crude oil as it induced MN formation and anomalies, including nuclear buds and CAs in root tip cells. Bridges, vagrant, laggard and sticky chromosomes, breaks, C-mitosis, disturbed polarity, and polyploidies were observed anomalies in the current study (Figs. 1 and 2). The MN formation showed a dose-dependent manner in the current study, while the CA frequencies were not related to the concentration of light crude oil. It may be concluded that the light crude oil had a clastogenic effect at high concentrations, and because of that, cells tried to exclude DNA in the form of MN and nuclear bud. MN and nuclear buds are formed from chromosome fragments and/or whole chromosomes, which left behind in the anaphase and failed to take part in the formation of the daughter nucleus after telophase. They have been proposed as indicators for clastogenic and aneugenic effects of environmental agents (Cruz 2019, Nouairi 2019, Souguir 2013). Studies showed that oil pollution induces oxidative conditions in plants by enhancing the ROS levels. On the other hand, oxidative stress can induces DNA damage and destroy the genetic material (Ei Hajjouji 2007); therefore the clastogenic effects of light crude oil may be attributed to the role of light crude oil in inducing oxidative stress, need to prove in future studies (Ighovie 2014).

Observed aberrations and anomalies in the current study may indicate the effect of light crude oil on the organization of mitotic spindle, which led to the formation of aberrations like C-mitosis and disturbed polarity. Diagonal anaphase and C-mitosis alongside laggards and disturbed chromosome orientations have been attributed to disruption of spindle formation because of the changed activity of cyclin-dependent kinases (Fatma 2018). Bridges are clastogenic aberrations that may result from a disruption of the chromatin structure and or a chemical interaction with spindle proteins and microtubules (Thabet 2019). They are suggested as typical signs of the genotoxic effects of a contaminant that can lead to cell death (Bhat 2019, Ma 2014, Youssef 2018). Light crude oil may act on spindle formation and mitosis by affecting gene expression. Fatma et al., (2018) discussed disoriented chromosomes during metaphase and anaphase as an impact of a pollutant on genes responsible for spindle formation.

The genotoxic effect of oil pollution and occurrence of different anomalies at different contaminations in the current study are compatible with the findings of Njoku et al., (2011), which surveyed the toxic effects of crude oil on Sorghum bicolor accessions seeds (0, 2, 4, 6, and 8% by volume of crude oil in distilled water) and recorded different kinds of aberrations at different concentrations. It is also consistent with the findings of Ma et al., (2014), who performed a toxicity test by exposing *Vicia faba* root tips to various water extracts of petroleum-contaminated soils. We found an incidence of nuclear buds, polyploidies, and significant induction of MN formation only at 4% contamination. This observation can be discussed as

aneugenic and clastogenic effects of light crude oil and cell tendency to the elimination of exceeding DNA in the form of buds and or MN (Fernandes 2007). Also, it can explain what we observed about the decrease of CA at 4% contamination compared with 2%, despite the increase of light crude oil contamination in soil.

According to the results, the maximum value for MI was at 1% contamination, while the highest percentages of anomalies and MN formation were recorded in 2 and 4% contaminations, respectively. The lack of the same trend for these three indexes in response to crude oil shows that different points of the cell cycle of *Vicia faba* root tip cells have been affected by light crude oil in the current study.

Conclusion

Vicia faba root tip cells can be used as living systems for monitoring soil contamination with light crude oils. It is a biocompatible method for environmental risk assessment and can be carried out with minimum facilities, equipment and materials. In the current study, different parameters in *Vicia faba* were considered for evaluation of phytotoxic, cytotoxic, and genotoxic effects of contaminated soils with light crude oil. Seed germination and root length were selected for assessing phytotoxic effects, while root tip cells of *Vicia faba* were used as cellular and genetic materials for surveying cytotoxic and genotoxic impacts of soils contaminated with light crude oil. The light crude oil showed harmful dose-dependent effects on seed germination and length of root. It also increased mitotic index, chromosome aberrations, and micronucleus frequencies in the root tip meristems. Accordingly, the seed germination and the frequency of micronucleus formation can be proposed as reliable bioindicators for using *Vicia faba* to determine and estimate the levels of light crude oil contamination in soils.

Statements And Declaration

Acknowledgement

The authors wish to acknowledge the proof reading and editing services by Hamyarapply Group.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent to Publish

The participants has consented to the submission of the manuscript to the journal.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Elaheh Alavi. The first draft of the manuscript was written by Elaheh Alavi

and then commented on previous versions of the manuscript by Golnaz Tajadod, Sayeh Jafari Marandi, Sedigheh Arbabian. All authors read and approved the final manuscript for submission.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Availability of Data and Materials

All the data and materials can be provided upon request.

References

1. Achuba FI (2006) The effect of sublethal concentrations of crude oil on the growth and metabolism of cowpea (Vigna unguiculata) seedlings. Environ 26:17–20
2. Achuba FI, Iserhienrhien LO (2018) Effects of soil treatment with abattoir effluent on morphological and biochemical profiles of cowpea seedlings (V. unguiculata) grown in gasoline polluted soil. Ife J Sci 20:51–59
3. Alkio M, Tabuchi TM, Wang X, Colon-Carmona A (2005) Stress responses to polycyclic aromatic hydrocarbons in Arabidopsis include growth inhibition and hypersensitive response-like symptoms. J Exp Bot 56:2983–2994
4. Anyasi RO, Atagana HI (2018) Profiling of plants at petroleum contaminated site for phytoremediation. Int J Phytoremediat 20:352–361
5. Bamgbose I, Anderson TA (2015) Phytotoxicity of three plant-based biodiesels, unmodified castor oil, and diesel fuel to alfalfa (Medicago sativa L.), lettuce (Lactuca sativa L.), radish (Raphanus sativus), and wheatgrass (Triticum aestivum). Ecotoxicol Environ Saf 122:268–274
6. Barbafieri M, Giorgetti L (2016) Contaminant bioacailability in soil and phytotoxicity/genotoxicity tests in Vicia faba L.: A case study of boron contamination. Environ Sci Pollut Res 23:24327–24336
7. Besalatpour A, Khoshgoftarmanesh AH, Hajabbasi MA, Afyuni M (2008) Germination and growth of selected plants in a petroleum contaminated calcareous soil. Soil Sediment Contam 17:665–676
8. Bhat SA, Cui G, Li F, Vig AP (2019) Biomonitoring of genotoxicity of industrial wastes using plant bioassays. Bioresour Technol Rep 6:207–216
9. Cotelle S, Dhyevre A, Muller S, Chenon P, Manier N, Pandard P, Echairi A, Silvestre J, Guiresse M, Pinelli E, Giorgetti L (2015) Soil genotoxicity assessment-results of an interlaboratory study on the Vicia micronucleaous assay in the context of ISO standardization. Environ Sci Pollut Res 22:988–995

10. Cruz JM, Corroque NA, Montagnoli RN, Lopes PRM, Morales MAM, Bidoia ED (2019) Comparative study of phytotoxicity and genotoxocity of soil contaminated with biodiesel, diesel fuel and petroleum. Ecotoxicol 28:449–456
11. Cui B, Zhang X, Han G, Li K (2016) Antioxidant defense response and growth reaction of Amorpha fruticosa seedlings in petroleum contaminated soil. Water Air Soil Pollut 227:121
12. Ei Hajjouji H, Pinelli E, Guiresse M, Merlina G, Revel JC, Hafidi M (2007) Assessment of the genotoxicity of olive mill waste water (OMWW) with the Vicia faba micronucleus test. Mutat Res/Genet Toxicol Environ Mutagen 634:25–31
13. Fatma F, Verma S, Kamal A, Srivastava A (2018) Monitoring of morphotoxic, cytotoxic and genotoxic potential of mancozeb using Allium assay. Chemosphere 195:864–870
14. Fernandes TC, Mazzeo DEC, Marin-Morales MA (2007) Mechanism of micronuclei formation in polyploidizated cells of Allium cepa exposed to trifuralin herbicide. Pestic Biochem Physiol 88:252–259
15. Fismes J, Perrin-Ganier C, Empereur-Bissonnet P, Morel JL (2002) Soil-to-root transfer and translocation of polycyclic aromatic hydrocarbons by vegetables grown on industrial contaminated soils. J Environ Qual 31:1649–1656
16. Gao Y, Zhu L (2004) Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils. Chemosphere 55:1169–1178
17. Ghalamboran MR, Kordkheli SSH, Bernard F (2020) Enzymatic response and metal ion content in roots of corn and broad beans planted in soil contaminated with gasoline. Int J Environ Sci Technol 17:973–982
18. Hawrot-Paw M, Bakowska T (2014) Growth and development of selected plant species in the phytoremediation of diesel oil contaminated soil. Environ Prot Eng 40:5–13
19. Ighovie ES, Ikechukwu EE (2014) Phytoremediation of crude oil contaminated soil with Axonopus compressus in the Niger Delta region of Nigeria. Nat Res 5(2):59–67
20. Iqbal M (2016) Vicia faba bioassay for environmental toxicity monitoring: A review. Chemosphere 144:785–802
21. Janku M, Luhova L, Petrivalsky M (2019) On the origin and fate of reactive oxygen species in plant cell compartments. Antioxid 8(4):105
22. Jiang X, Chen H, Liao Y, Ye Z, Li M, Klobucar G (2019) Ecotoxicity and genotoxicity of polystyrene microplastics on higher plant Vicia faba. Environ Pollut 250:831–838
23. Kanaya N, Gill BS, Grover IS, Murin A, Osiecka R, Sandhu SS, Andersson HC (1994) Vicia faba chromosomal aberration assay. Mutat Res/Fundam Mol Mech Mutagen 310:231–247
24. Kaur N, Ericson TE, Ball AS, Ryan MH (2017) A review of germination and early growth as a proxy for plant fitness under petrogenic contamination-knowledge gaps and recommendations. Sci Total Environ 603–604:728–744

25. Kayumov AR, Solovyev DA, Bobrov DE, Rizvanov AA (2019) Curent approaches to the evaluation of soil genotoxicity. BioNanoSci 9:758–764
26. Krisch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, Loock KV, Decordier I (2011) The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch Toxicol 85:873–899
27. Liu Y, Gao M, Han W, Liu Z (2017) Evaluation and quantification of genotoxicity of urban waters by using Vicia faba bioassays. Chem Ecol 33:669–683
28. Lorestani B, Kolahchi N, Ghasemi M, Cheraghi M (2014) Changes germination, growth and anatomy Vicia ervilia in response to light crude oil stress. J Chem Health Risks 4:45–52
29. Luo Y, Liang J, Zeng G, Chen M, Mo D, Li G, Zhang D (2018) Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects. Waste Manag 71:109–114
30. Ma J, Shen J, Liu Q, Fang F, Cai H, Guo C (2014) Risk assessment of petroleum-contaminated soil using soil enzyme activities and genotoxicity to Vicia faba. Ecotoxocil 23:665–673
31. Njoku KL, Akinola MO, Oshodin OR (2011) Phytotoxicity assay of crude oil using different accessions of Sorghum bicolor. World Appl Sci J 15:38–46
32. Noori A, Zare Maivan H, Alaie E, Newman LA (2018) Leucanthemum vulgare lam. crude oil phytoremediation. Int J Phytoremediat 20:1292–1299
33. Nouairi I, Jalali K, Essid S, Zribi K, Mhadhbi H (2019) Alleviation of cadmium-induced genotoxicity and cytotoxicity by calcium chloride in faba bean (Vicia faba L. var. minor) roots. Physiol Mol Biol Plant 25:921–931
34. Oyedeji A, Kayode J, Besenyei L, Fullen MA (2015) Germination of seeds of selected leguminous tree species moistened with varying concentrations of crude oil-contaminated soil water extracts. Am J Plant Sci 6:1575–1580
35. Parmar TK, Rawtani D, Agrawal YK (2016) Bioindicators: The natural indicator of environmental pollution. Front Life Sci 9:110–118
36. Pena-Castro JM, Barrera-Figueroa BE, Fernandez-Linares L, Ruiz-Medrano R, Xoconostle-Cazares B (2006) Isolation and identification of up-regulated genes in bermudagrass roots (Cynodon dactylon L.) grown under petroleum hydrocarbon stress. Plant Sci 170:724–731
37. Perez-Hernandez I, Ochoa-Gaona S, Schroeder RA, Rivera-Cruz MC, Geissen V (2013) Tolerance of four tropical tree species to heavy petroleum contamination. Water Air Soil Pollut 224:1–13
38. Radwan SS, Al-Awadhi H, El-Nemr IM (2000) Cropping as a phytoremediation practice for oily desert soil with reference to crop safety as food. Int J Phytoremediat 2:383–396
39. Rao CN, Afzal M, Malallah G, Kurian M, Gulshan S (2007) Hydrocarbon uptake by roots of Vicia faba (Fabaceae). Environ Monit Assess 132:439–443
40. Roberto MM, Jamal CM, Malaspina O, Marin-Morales MA (2016) Antigenotoxicity and antimutagenicity of ethanolic extracts of Brazilian green propolis and its main botanical source determined by the Allium cepa test system. Genet Mol Biol 39:257–269

41. Salazar-Mercado SA, Torres-Leon CA, Rojas-Suarez JP (2019) Cytotoxic evaluation of sodium hypochlorite using Pisum sativum L. as effective bioindicator. Ecotoxicol Environ Saf 173:71–76
42. Shen Z, Som AM, Wang F, Jin F, McMillan O, Al-Tabbaa A (2016) Long-term impact of biochar on the immobilisation of nickel (II) and zinc (II) and the revegetation of a contaminated site. Sci Total Environ 542:771–776
43. Shirdam R, Daryabeygi ZA, Nabi BGR, Mehrdadi N (2009) Removal of total petroleum hydrocarbons (TPHs) from oil-polluted soil in Iran. Iran J Chem Chem Eng 28:105–113
44. Souguir D, El Ferjani E, Ledoigt G, Goupil P (2013) Transcript accumulation of stress-related genes in Vicia faba roots under a short exposure to cadmium. Plant Biosys 149:280–288
45. Thabet AF, Galal OA, El-Samahy MFM, Tuda M (2019) Higher toxicity of nano-scale TiO2 and dose-dependent genotoxicity of nano-scale SiO2 on the cytology and seedling development of broad bean Vicia faba. SN Appl Sci 1:956
46. Tran TH, Gati EM, Eshel A, Winters G (2018) Germination, physiological and biochemical responses of acacia seedlings (Acacia raddiana and Acacia tortilis) to petroleum contaminated soils. Environ Pollut 234:642–655
47. Varjani S, Upasani VN, Pandey A (2020) Bioremediation of oily sludge polluted soil employing a novel strain Pseudomonas aeruginosa and phytotoxicity of petroleum hydrocarbons for seed germination. Sci Total Environ 737:139766
48. Watanabe Y, Kubota Y, Fum S, Kouichi M, Ichikawa S, Kubota M, Yoshida S (2014) Cytokinesis block micronucleus assay in field plants for monitoring radiation-induced genotoxicity of the environment. Mutat Res/Fundam Mol Mech Mutagen 774:41–46
49. Zhu H, Gao Y, Li D (2018) Germination of grass species in soil affected by crude oil contamination. Int J Phytoremediat 20(6):567–573
50. Youssef MS, Elamawi RM (2018) Evaluation of phytotoxicity, cytotoxicity, and genotoxicity of ZnO nanoparticles in Vicia faba. Environ Sci Pollut Res 27:18972–18984

Figures

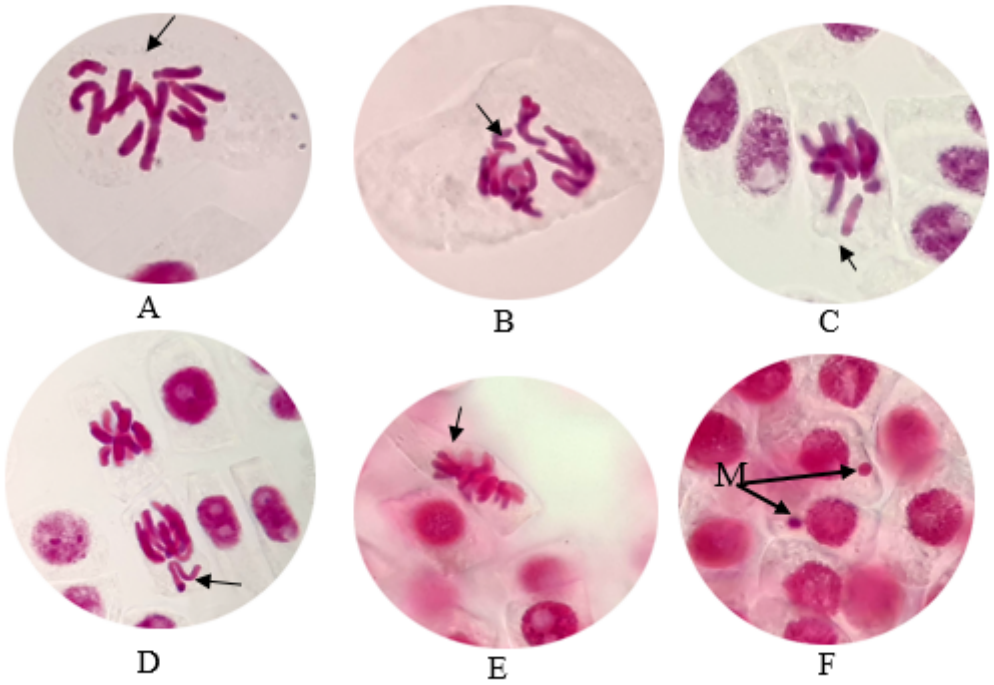

Figure 1

Microscopic images of CA in the seeds exposed to soil contaminated with oil at different concentrations. C-mitosis (A), vagrant chromosome (B), and chromosome break (C) at 1% oil contamination, laggard chromosome at 4% contamination (D), sticky chromosomes (E) and cells with micronuclei (F) at 2% contamination.

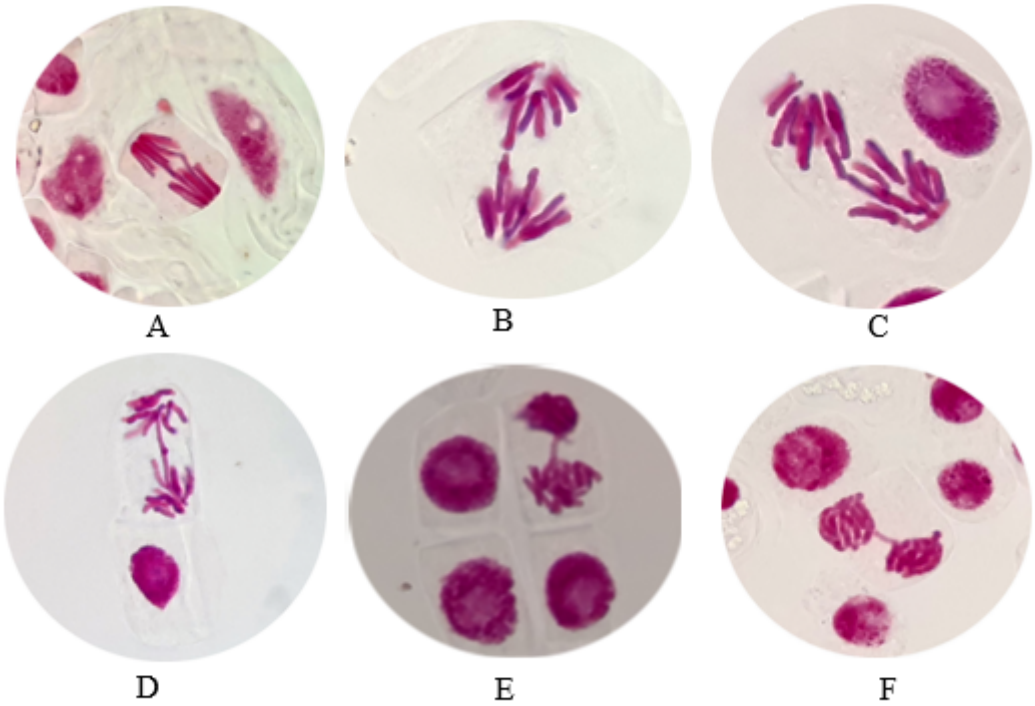

Figure 2

Chromosome bridge in *Vicia faba* root tip cells at control (A) and experimental group (B-F). Chromosome bridge at anaphase alongside diagonal orientation of spindle apparatus at 2% contamination (B, D), the bridge at 4% contamination (C), and the chromosal bridge at early telophase 2% (E) and 4% (F).

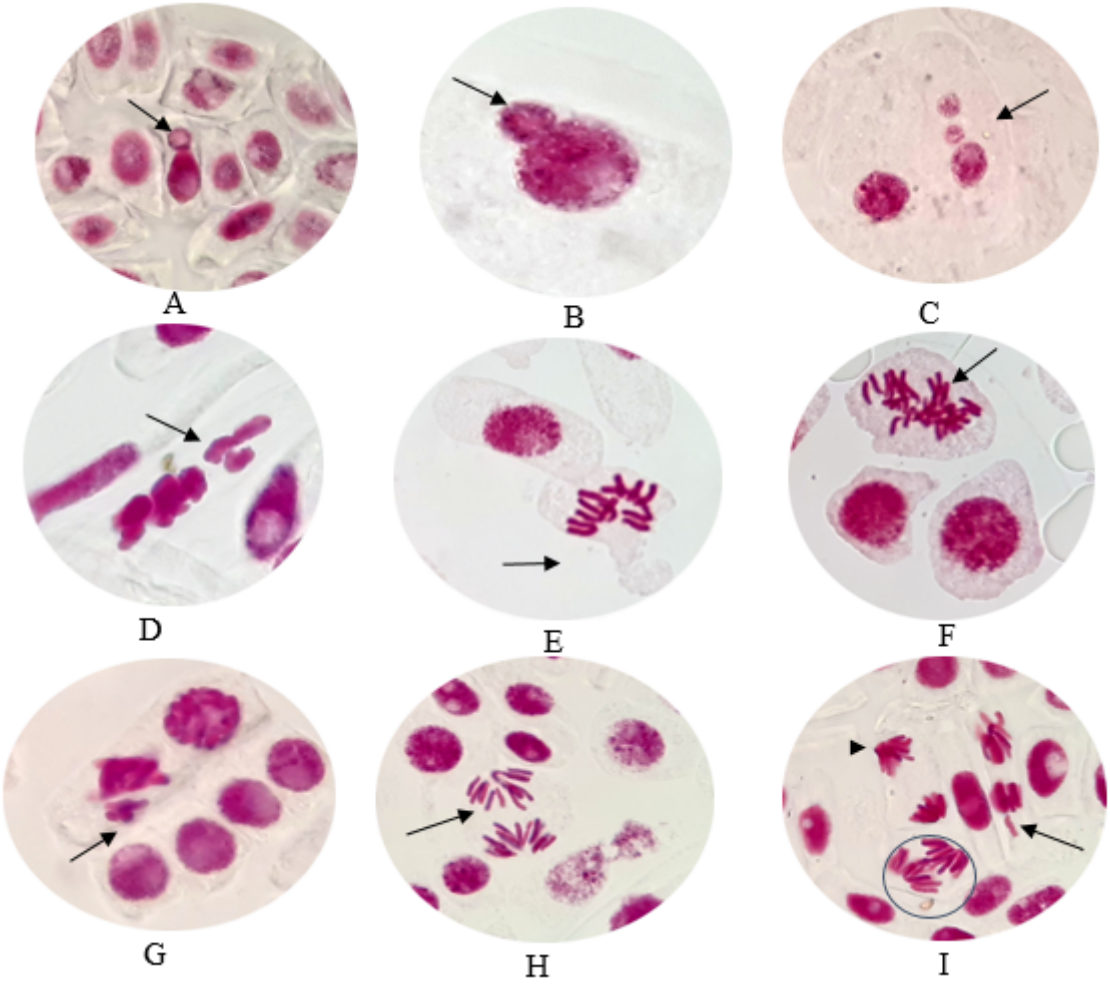

Figure 3

Different kinds of aberrations in the *Vicia faba* root tip cells exposed to the soil contaminated with 4% light crude oil. Nuclear bud (A, B), cells with two large micronuclei (C), disrupted metaphase (D, E), polyploidy (F), micronucleus formation (G), multipolar anaphase (H), and diagonal early telophase (shown by arrowhead), diagonal anaphase (shown by a circle) and chromosome break (shown by an arrow) in a same microscopic slide (I).