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Research Article

Phytotoxic Effects of Polyethylene Microplastic on the Growth and Yield of Groundnut (*Arachis hypogaea L.*) and Soybean (*Glycine max L.*)

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ABSTRACT
Microplastic contamination of agricultural soils is an emerging environmental concern due to plastic accumulation in terrestrial ecosystems and its potential effects on crop growth, soil quality, food security, and human health. This study investigated the phytotoxic effects of polyethylene (PE) microplastic on germination, growth, and reproductive development of groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* L.). Treatments consisted of 0 mL (control), 6, 9, 12, and 15 mL PE microplastic. PE contamination altered soil physicochemical properties, although effects on electrical conductivity ($p = 0.477$) and water-holding capacity ($p = 0.329$) were not significant. PE significantly affected soybean germination ($p = 0.004$), but not groundnut germination ($p = 0.534$). Groundnut germination was delayed, with 9, 12, and 15 mL treatments recording 13.33%, 13.33%, and 20% germination, respectively, by Day 8, while soybean recorded no germination at 6, 12, and 15 mL and 13.33% at 9 mL. PE concentration significantly affected groundnut plant height, number of leaves, and leaf area, but not branches. In soybean, significant effects were observed for plant height, branches, leaves, and leaf area. Groundnut height declined from 32±9.8 cm in the control to 24±7.6 cm at 6 mL PE. Flowering was significantly affected in groundnut ($p = 0.023$), but not soybean ($p = 0.189$). Chlorophyll content also varied between treatments and crops. Overall, PE phytotoxicity was crop- and concentration-dependent, highlighting potential risks to agricultural productivity and food safety. Improved plastic waste management, reduced use of non-degradable plastics, soil monitoring, and sustainable agricultural practices are recommended.

Keywords: Chlorophyll; Groundnut; Microplastic; Phytotoxicity; Polyethylene; Soybean

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INTRODUCTION

Plastics are synthetic polymers that have become ubiquitous in terrestrial and aquatic environments (Napper *et al*., 2023). Their durability and slow degradation mean that plastic particles, especially microplastics and nanoplastics, accumulate in soils where crops grow (Samani *et al*., 2025). As plastics degrade, they break into smaller fragments which can affect soil structure, water retention, nutrient availability, and interact with chemical pollutants (Dey *et al*., 2024). There is growing concern that such materials may be phytotoxic and harmful to plant growth and development (Fotopoulou *et al*., 2017). Polyethylene (PE) is among the most widely used plastic types employed in packaging, insulation, disposable food ware, and agricultural mulch (Salama & Geyer, 2023). Agricultural soils represent an important sink for microplastics through plastic mulch films, irrigation materials, sewage sludge, compost, wastewater irrigation, plastic-coated fertilizers, agricultural packaging, and atmospheric deposition.

Leguminous crops such as groundnut (*Arachis hypogaea*) and soybeans (*Glycine max*) are important in many parts of the world as sources of food (protein, oil) and income, and improve soil fertility via nitrogen fixation (Nwagboso *et al*., 2024). Any negative effect of plastics in the soil on these crops could have significant agricultural and food security implications. Recent studies have begun to examine the phytotoxic effects of polyethylene microplastics on various crops. Wang *et al*. (2021) found that PE microplastics reduced soybean's seed vigour, root length, shoot length, germination index, and dry weight in a concentration-dependent manner. Halder *et al*. (2025) reported that microplastics in soil reduced nitrogen uptake by groundnut crop caused by damaged root cells and impaired soil nitrogen cycling. While plastic pollution in soil is increasingly documented, the direct toxic effects of specific types of plastics on important legume crops are not well quantified, especially under conditions relevant for agricultural soils in Nigeria or similar environments (Aransiola *et al*., 2023). In Nigeria, peasant farmers who are among the major producers of leguminous crops often use home-produced organic manure which in most cases contains plastic contamination, quantifying phytotoxic risks is important for agricultural management, food security, and environmental health (Ghosh *et al*., 2025).

Groundnut and soybean are economically and nutritionally important leguminous crops. They provide protein and edible oil and contribute to soil fertility through biological nitrogen fixation. Consequently, any reduction in their growth or productivity due to plastic contamination may have implications for agricultural production and food security. Previous work has indicated that polyethylene microplastics can reduce seed vigour and growth parameters in legumes, but comparative information on groundnut and soybean under similar exposure conditions remains limited.

This study therefore aimed to assess the phytotoxic effect of polyethylene on groundnut and soybeans by determining the effects of various amounts of polyethylene on agricultural soil, seed germination, seedling growth, comparing relative phytotoxicity between the two legume species, and assessing health risks for humans consuming contaminated legumes.

MATERIALS AND METHODS

Sample Collection and Identification

Local varieties of Groundnut (*Arachis hypogaea*) and Soybeans (*Glycine max*) were obtained from a reputable supplier at Central Market, Katsina. Samples were authenticated at the herbarium unit of the Biological Sciences Department, Umaru Musa Yar'adua University, Katsina. Voucher numbers UMYUH148 (*Arachis hypogaea*) and UMYUH457 (*Glycine max*) were obtained and deposited.

Experimental Set-up

Experimental set-up was carried out in a screen house at the National Biotechnology Development Agency (NABDA), Katsina following the protocol of (Wang *et al*., 2021) with slight modifications. Sandy loam soil was collected at NABDA Katsina, sun dried and sieved with a mesh of 2 mm. One kilogram of the soil was weighed into clay pots weighing about 800 g. Ten grams of Polyethylene powder was weighed and dissolved in 60 mL of Xylene giving a concentration of 166.7 mg/mL. It was then heated using a heating mantle at 80 watts until it dissolved. A stock solution of the Polyethylene was prepared with 400 mL of distilled water. Different amounts (6 mL, 9 mL, 12 mL and 15 mL) of the Polyethylene were prepared using distilled water. The viability test was performed using the sink or float method by soaking the seeds in distilled water for three hours in a sterile container. The viable seeds sank at the bottom of the container and were used for the sowing. The seeds were sown at the rate of three seeds per pot at a depth of 3 cm into the soil, and three pots per treatment (replicates).

Soil Analysis

Soil analysis was conducted to determine the electrical conductivity and water holding capacity before and after the treatments. Electrical conductivity was determined using the 1:5 soil to water extract method (kargas *et al*., 2022). Twenty grams of prepared soil samples were weighed and placed into a clean flask. Hundred milliliters of distilled water was added to the soil creating a 1:5 ratio. The mixtures were shaken thoroughly for 30 to 60 minutes using a mechanical shaker. The suspensions were filtered using filter paper to obtain a clear soil extract. The electrical conductivity meter was calibrated using a standard potassium chloride solution and readings were recorded at 25°C.

Water holding capacity was determined using the Cylinder Method (Govindasamy *et al*., 2023). Cylindrical plastic rings of 1000 mL with filter papers secured to the bottom were used. Empty cylinders were weighed and soil was carefully packed into the cylinders. The cylinders were weighed again to determine the dry mass of the soil. The cylinders were placed in a shallow tray filled with distilled water and water was allowed to move upward into the soil via

capillary action until the surface of the soil appeared moist. The cylinders were removed from the tray and placed on a layer of absorbent material to allow excess gravitational water to drain away for 24 hours. The soil was oven dried at 30°C for 24 hours. Water holding capacity was expressed as a percentage: (Mass of water/Mass of oven-dry soil) × 100.

Growth Parameters Measured

Plant height (cm), number of branches, number of leaves, leaf area (cm²), number of flowers, and chlorophyll content were measured at two weeks intervals (Pandey *et al.*, 2017). Germination percentage was calculated as: $\frac{Ngs}{Nt} * 100$

Where Ngs is Number of germinated seeds

Nt is total number of seeds sown

Energy Dispersive X-Ray Fluorescence (EDXRF) Analysis

EDXRF analysis was conducted on soil samples to determine elemental composition and distribution. Samples were analyzed using an EDXRF spectrometer to identify and quantify major and trace elements present in the polyethylene-contaminated plant samples.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was performed to identify functional groups and chemical bonds present in the polyethylene samples. Spectra were recorded in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}.

Health Risk Assessment

Health risk assessment was conducted following the U.S. Environmental Protection Agency framework. Exposure assessment calculated the Average Daily Intake (ADI) of toxic elements. The Threshold of Toxicological Concern (TTC) approach (Munro *et al*., 2008) with a reference value of 1.5 µg/day was used as the screening reference. Hazard Quotient (HQ) was calculated as the ratio of estimated exposure to the reference value. An HQ greater than 1 indicates potential non-carcinogenic health concern.

Data Analysis

Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) software, version 25 (IBM incorporation, USA). The data were subjected to descriptive analyses.

RESULT

Effects of polyethylene microplastics on soil properties

Polyethylene treatment altered both electrical conductivity and water-holding capacity of the soil as shown in table 1. Before treatment, the soil had an EC of 0.24 S m^{-1} and a WHC of 34.26%. Following PE treatment, EC declined to 0.13 S m^{-1} at 6 and 9 mL, 0.15 S m^{-1} at 12 mL and 0.01 S m^{-1} at 15 mL, not significant at p = 0.477 . Water-holding capacity also declined under all treatments, ranging from 24.55% at 6 mL to 32.60% at 12 mL, not significant at p = 0.329.

The results indicate that PE contamination modified soil conditions that are important for water availability and ion movement. The mean EC and WHC of the PE-treated soil were 0.105 ± 0.0555 S m^{-1} and 29.2525 ± 2.9504%, respectively.

Effects of polyethylene on seed germination

Groundnut and soybean showed different responses to PE exposure. Groundnut germination was delayed in PE-treated soils as indicated in Table 4. The control achieved 13.33% germination by Day 4 and maintained this value through Day 8. The 6 mL treatment reached 6.67% by Day 6, whereas the 9 and 12 mL treatments reached 13.33% only by Day 8. The 15 mL treatment reached 20% by Day 8. Soybean showed a generally stronger inhibition of germination. The control reached 6.67% germination by Day 6, while the 9 mL treatment reached 13.33% by Day 8. No germination was recorded at the 6, 12 or 15 mL treatments. These findings demonstrate that PE effects on germination were non-linear and species-dependent.

Effects of polyethylene on plant height

Most surviving groundnut plants exposed to PE remained shorter than the corresponding controls as shown in Table 7. Control plants reached final heights of approximately 41.3–41.5 cm in the individual replicates, whereas several treated plants reached final values between 27.7 and 34.3 cm. soybean plants generally attained greater heights than groundnut. Several PE-treated soybean plants maintained substantial growth, with final heights reaching 58.1–64.0 cm in successful treatments. However, several treatments recorded complete establishment failure. The highest final height was observed in the soybean control at 66.5 cm.

Table 1: Effects of polyethylene treatment on soil electrical conductivity and water-holding capacity

Treatment	Electrical conductivity (S m^{-1})	Water-holding capacity (%)
Before treatment	0.24	34.26
PE 6 mL	0.13	24.55
PE 9 mL	0.13	30.47
PE 12 mL	0.15	32.60
PE 15 mL	0.01	29.39

Table 2: Analysis of Variance (ANOVA) for Electrical Conductivity (EC) across Concentration Levels

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Concentration	0.017	4	0.004	1.023	0.477
Error	0.021	5	0.004		
Total	0.038	9			

Table 3: Analysis of Variance (ANOVA) for Water Holding Capacity (WHC) across Concentration Levels

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Concentration	40.806	4	10.202	1.500	0.329
Error	33.998	5	6.800		
Total	74.804	9			

Table 4: Final germination percentage of number of seeds that germinated across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	2.00 ± 0.00	0.67 ± 0.58
6	0.67 ± 0.58	0.00 ± 0.00
9	0.67 ± 1.15	1.33 ± 0.58
12	0.67 ± 1.15	0.00 ± 0.00
15	1.00 ± 1.73	0.00 ± 0.00

Table 5: Analysis of Variance (ANOVA) for Number of Seeds Germinated for PE (G) across Concentration Levels

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Concentration	4.000	4	1.000	0.833	0.534
Error	12.000	10	1.200		
Total	16.000	14			

Table 6: Analysis of Variance (ANOVA) for Number of Seeds Germinated for PE (S) across Concentration Levels

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Concentration	4.267	4	1.067	8.000	0.004*
Error	1.333	10	0.133		
Total	5.600	14			

Table 7: Plant Height (cm) across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	41.43 ± 0.12	42.27 ± 36.73
6	30.93 ± 2.96	19.37 ± 33.55
9	20.07 ± 17.48	60.37 ± 1.77
12	21.10 ± 18.40	20.50 ± 35.50
15	21.47 ± 18.70	21.33 ± 36.95

Table 8: Two-Way ANOVA for Plant Height of PE (G)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	4,171.574	5	834.315	6.781	0.000*
Concentration	4,110.292	4	1,027.573	8.352	0.000*
Duration × Concentration	277.798	20	13.890	0.113	1.000
Error	7,381.793	60	123.030		
Total	15,941.457	89			

Table 9: Two-Way ANOVA for Plant Height of PE (S)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	8,624.517	5	1,724.903	3.087	0.015*
Concentration	10,934.330	4	2,733.583	4.893	0.002*
Duration × Concentration	2,091.618	20	104.581	0.187	1.000
Error	33,522.867	60	558.714		
Total	55,173.332	89			

Effects on number of branches

Groundnut branch number increased throughout the experimental period in surviving plants as indicated in Table 10. Controls generally produced higher final branch numbers than treated plants, although the 15 mL treatment in one replicate reached 30 branches compared with 33 branches in its corresponding control.

In contrast**,** Soybeans exhibits a highly irregular and variable pattern**.** The control shows moderate branching **(**14.33 ± 12.06**),** but a sharp decline is observed at 6 mL (5.33 ± 9.24)**.** A notable peak occurs at 9 mL (17.67 ± 0.58), representing the highest and most consistent branching response for PE(S), as indicated by the very low standard deviation. However, this increase is not sustained, as branching decreases again at 12 mL and 15 mL (6.33 ± 10.97)**.** The generally high standard deviations (except at 9 mL) indicate substantial variability**,** suggesting that PE(S) response is inconsistent and highly sensitive to concentration changes**.**

Effect of polyethylene microplastic on number of leaves

A similar pattern was observed for leaf production as shown in Table 13. Groundnut controls reached between 55 and 69 leaves at the final observation. Some treated plants nevertheless showed substantial leaf development, particularly at 9–15 mL. Soybean controls also generally showed strong vegetative development. Surviving treated soybean plants produced up to 45 leaves, whereas the highest-performing control reached 48 leaves.

Effects on leaf area

Untreated groundnut plants generally had larger final leaf areas than PE-treated plants as shown in Table 16. The highest groundnut control leaf area was 28.98 cm^2, compared with 25.80 cm^2 among the treated plants. Soybean exhibited substantially larger leaf areas than groundnut. Some PE-treated soybean plants performed close to the control, with the 15 mL treatment reaching 80.04 cm^2 compared with 84.49 cm^2 in the highest-performing control.

Table 10: Number of Branches across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	25.00 ± 6.93	14.33 ± 12.06
6	19.67 ± 4.04	5.33 ± 9.24
9	21.67 ± 4.04	17.67 ± 0.58
12	16.00 ± 14.42	6.33 ± 10.97
15	16.00 ± 15.10	6.33 ± 10.97

Table 11: Two-Way ANOVA for Number of Branches of PE (G)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	2,622.856	5	524.571	8.933	0.000*
Concentration	519.489	4	129.872	2.212	0.078
Duration × Concentration	156.644	20	7.832	0.133	1.000
Error	3,523.333	60	58.722		
Total	6,822.322	89			

Table 12: Two-Way ANOVA for Number of Branches of PE (S)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	937.333	5	187.467	4.996	0.001*
Concentration	723.489	4	180.872	4.820	0.002*
Duration × Concentration	225.444	20	11.272	0.300	0.998
Error	2,251.333	60	37.522		
Total	4,137.600	89			

Table 13: Number of leaves across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	60.33 ± 7.57	30.00 ± 24.49
6	49.00 ± 5.29	11.00 ± 19.05
9	37.33 ± 5.03	39.67 ± 4.16
12	37.67 ± 26.03	15.00 ± 25.98
15	40.33 ± 34.66	15.00 ± 25.98

Table 14: Two-Way ANOVA for Number of Leaves of PE (G)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	10,805.789	5	2,161.158	6.411	0.000*
Concentration	4,300.889	4	1,075.222	3.190	0.019*
Duration × Concentration	266.711	20	13.336	0.040	1.000
Error	20,225.333	60	337.089		
Total	35,598.722	89			

Table 15: Two-Way ANOVA for Number of Leaves of PE (S)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	3,513.556	5	702.711	3.143	0.014*
Concentration	4,726.489	4	1,181.622	5.285	0.001*
Duration × Concentration	742.444	20	37.122	0.166	1.000
Error	13,414.667	60	223.578		
Total	22,397.156	89			

Table 16: Area of leaves (cm^2) across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	26.56 ± 2.09	51.68 ± 59.70
6	17.87 ± 1.42	18.55 ± 32.13
9	20.14 ± 3.89	68.57 ± 1.96
12	13.59 ± 11.64	24.34 ± 42.17
15	21.08 ± 1.32	26.68 ± 46.35

Table 17: Two-Way ANOVA for Leaf Area of PE (G)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	1,866.252	5	373.250	7.447	0.000*
Concentration	921.597	4	230.399	4.597	0.003*
Duration × Concentration	154.108	20	7.705	0.154	1.000
Error	3,007.126	60	50.119		
Total	5,949.083	89			

Table 18: Two-Way ANOVA for Leaf Area of PE (S)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	7,735.841	5	1,547.168	1.775	0.132
Concentration	19,923.323	4	4,980.831	5.714	0.001*
Duration × Concentration	2,241.056	20	112.053	0.129	1.000
Error	52,305.302	60	871.755		
Total	82,205.522	89			

Effect on number of flowers

For Groundnut the control recorded 4.33 ± 2.08 flowers, and although there is a slight reduction at 6 mL (2.33 ± 0.58), the number of flowers increases and stabilizes at 9 mL, 12 mL, and 15 mL (approximately 3.00 flowers) as shown in Table 19. This suggests that groundnut maintains moderate flowering across treatments, indicating a less pronounced inhibitory effect. The low standard deviations at some treatments, particularly at 9 mL and 15 mL, indicate consistent responses among replicates.

In contrast, PE(S) exhibits a more variable and treatment-dependent pattern. The control shows relatively high flowering (6.67 ± 5.03), although with

considerable variability. A sharp decline occurs at 6 mL (1.67 ± 2.89), indicating a strong inhibitory effect at this treatment. However, a noticeable increase is observed at 9 mL (4.67 ± 1.70), suggesting that moderate treatment may enhance flowering in PE(S). This improvement is not sustained, as flowering decreases again at 12 mL and 15 mL, accompanied by relatively high variability. This indicates that PE(S) response is inconsistent and sensitive to treatment changes.

Effect on chlorophyll content

For Groundnut, a generally lower and more stable chlorophyll content is observed across all treatments as shown in Table 22. The control recorded 0.5142 ± 0.0651, after which chlorophyll content decreased at 6 mL (0.3851 ± 0.0204). Across 9 mL, 12 mL, and 15 mL, values remain relatively close (0.3943 ± 0.0242, 0.4030 ± 0.0524, and 0.3863 ± 0.0495, respectively), indicating that PE(G) maintains a consistently reduced chlorophyll level under treatment. The relatively small standard deviations suggest more uniform responses among replicates.

In contrast, Soybeans exhibits a more variable and treatment-dependent pattern. The control shows moderate chlorophyll content (0.4701 ± 0.0978), followed by a sharp decline at 6 mL (0.3301 ± 0.0000), indicating a strong inhibitory effect at this treatment. An increase is observed at 9 mL (0.3931 ± 0.1019) and 12 mL (0.3980 ± 0.0000), with the highest value recorded at 15 mL (0.4810 ± 0.0000). However, the presence of zero standard deviation at some treatments indicates limited observations, and therefore these values should be interpreted with caution.

Energy Dispersive X-Ray Fluorescence (EDXRF) Analyses

The EDXRF analysis identified a range of major, minor and trace elements in both the polyethylene-treated groundnut (PEG) and soybean (PES) samples. The analysis measured the concentration (%) of each element together with its corresponding peak intensity in counts per second per milliampere (cps/mA).

Calcium (Ca) was the dominant element in all samples, with concentrations ranging from approximately 1.95% to 2.89% in PEG samples and 2.37% to 3.43% in PES samples**.** Calcium also produced the highest peak intensities, reaching 18,707 cps/mA in PES iii**,** indicating its strong abundance among the detected elements. Potassium (K) was the second most abundant major element, ranging from 0.51% to 0.69% in PEG and 0.53% to 0.71% in PES**.**

Other relatively abundant elements included silicon (Si), phosphorus (P), sulfur (S), iron (Fe), aluminium (Al), chlorine (Cl), magnesium (Mg) and sodium (Na)**.** Phosphorus concentrations ranged from 0.19433–0.24598% in PEG samples**,** whereas PES samples generally recorded higher concentrations, ranging from 0.26169–0.32230%**.** Similarly, sulfur was consistently detected in both groups, with concentrations of approximately 0.12–0.18%**.** Iron was also consistently present, generally ranging from 0.09–0.19% across the samples.

Several elements occurred at relatively low or trace concentrations. These included nickel (Ni), copper (Cu), zinc (Zn), tantalum (Ta), tungsten (W), manganese (Mn), bromine (Br), rubidium (Rb), strontium (Sr), niobium (Nb), lead (Pb) and bismuth (Bi)**.** Among these, lead was detected in all samples, although its concentration varied considerably. PEG samples recorded Pb concentrations of approximately 0.119–0.155%**,** while PES samples ranged from 0.045–0.148%**.** Nickel was detected at low concentrations in both crop groups, with some variation among treatments.

Some elements, including silver (Ag), chromium (Cr), arsenic (As) and, in most cases, vanadium (V)**,** were reported as having zero concentrations. However, some entries showed non-zero peak values despite a reported concentration of zero, indicating that detectable spectral signals were recorded without a quantified elemental concentration.

Comparative interpretation

A comparison of the two crop groups suggests that the PES samples generally contained higher concentrations of calcium and phosphorus than the PEG samples. PES iii had the highest calcium concentration of 3.4336%, whereas the highest value among PEG samples was 2.8867% in PEG i. PES iii also recorded the highest phosphorus concentration **(**0.32230%**)**. In contrast, PEG samples generally showed comparatively higher chlorine concentrations than PES samples.

Table 19: Number of flowers across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	4.33 ± 2.08	6.67 ± 5.03
6	2.33 ± 0.58	1.67 ± 2.89
9	3.00 ± 0.00	4.67 ± 1.70
12	3.00 ± 2.65	1.67 ± 2.89
15	3.00 ± 0.00	2.00 ± 3.46

Table 20: Two-Way ANOVA for Number of Flowers of PE (G)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	47.511	2	23.756	6.723	0.004*
Concentration	46.756	4	11.689	3.308	0.023*
Duration × Concentration	22.044	8	2.756	0.780	0.624
Error	106.000	30	3.533		
Total	222.311	44			

Table 21: Two-Way ANOVA for Number of Flowers of PE (S)

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p-value)
Duration	507.911	2	253.956	5.852	0.007*
Concentration	285.689	4	71.422	1.646	0.189
Duration × Concentration	206.311	8	25.789	0.594	0.775
Error	1302.000	30	43.400		
Total	2301.911	44			

Table 22: Chlorophyll Content across treatments (Mean ± Standard Deviation)

Treatments (mL)	PE (G) (Mean ± SD)	PE (S) (Mean ± SD)
0	0.5142 ± 0.0651	0.4701 ± 0.0978
6	0.3851 ± 0.0204	0.3301 ± 0.0000
9	0.3943 ± 0.0242	0.3931 ± 0.1019
12	0.4030 ± 0.0524	0.3980 ± 0.0000
15	0.3863 ± 0.0495	0.4810 ± 0.0000

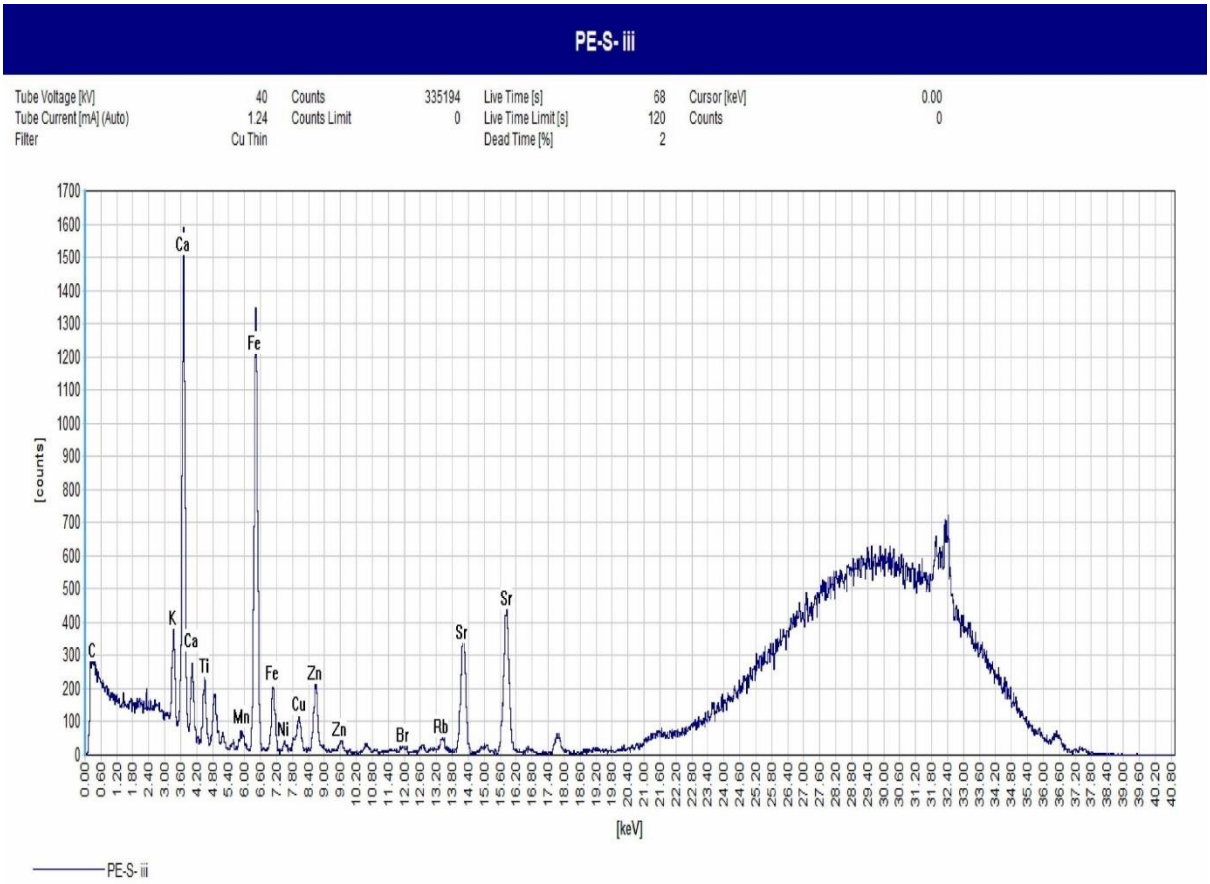

Figure 1: Showing EDXRF analysis of Polyethylene soybean sample iii

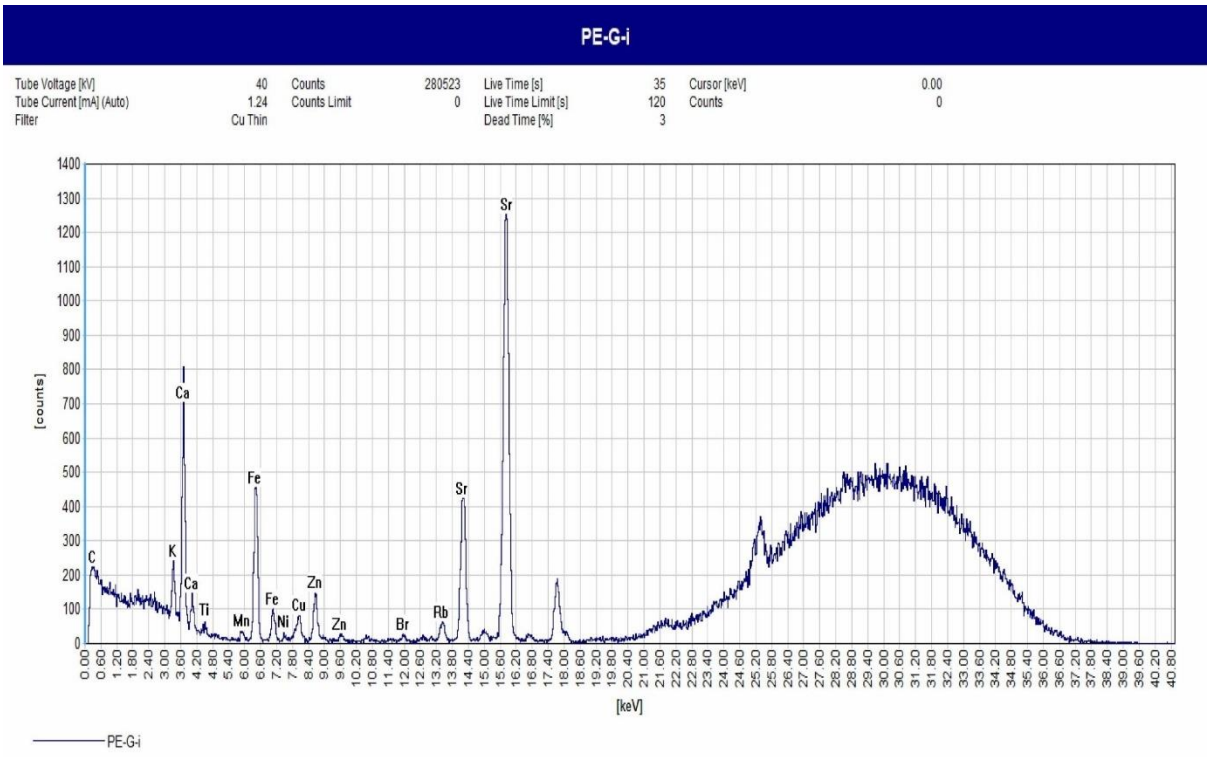

Figure 2: Showing EDXRF analysis of Polyethylene groundnut sample i

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSES

Stacked FTIR spectra of PEG Samples

The stacked FTIR spectra of PEG samples are presented in Figure 3 to facilitate comparison of the principal absorption bands across the four replicates. Vertical dashed lines indicate the major spectral features identified at 3272.60 cm^{-1}, 2914.78 cm^{-1}, 2847.69 cm^{-1}, 1602.76 cm^{-1}, 1408.93 cm^{-1}, 1371.66 cm^{-1}, 1312.02 cm^{-1}, 1237.48 cm^{-1}, and 1006.38 cm^{-1}. The close alignment of these peaks across all samples demonstrates excellent reproducibility and indicates that the molecular structure remains consistent among the replicates.

The broad O–H stretching band at 3272.60 cm^{-1} is evident in all spectra and confirms the presence of hydroxyl functionalities. The absorption bands near 2914.78 cm^{-1} and 2847.69 cm^{-1} are associated with methylene C–H stretching vibrations and represent the hydrocarbon backbone of the polymer. The bands observed within the fingerprint region arise from CH_2 deformation modes and C–O–C stretching vibrations characteristic of PEG. The strong absorption around 1006.38 cm^{-1} is particularly significant because it reflects the ether linkage that forms the repeating unit of polyethylene glycol.

The absence of additional major absorption bands and the consistency of peak positions suggest that no substantial chemical degradation, contamination, or structural modification occurred among the PEG samples. Therefore, the stacked spectra provide further evidence of the chemical stability and homogeneity of the polymer.

Stacked FTIR Spectra of PES Samples

The stacked FTIR spectra of the PES samples are shown in Figure 4, where the major absorption bands have been highlighted using vertical dashed lines. The identified peaks occured at approximately 3287.51 cm^{-1}, 2884.96 cm^{-1}, 2832.78 cm^{-1}, 1596.30 cm^{-1}, and 1021.29 cm^{-1}. The close alignment of these peaks across the four samples demonstrates a high level of reproducibility and confirms the structural similarity of the PES replicates.

The broad absorption feature at 3287.51 cm^{-1} reflects O–H stretching vibrations arising from hydroxyl groups and intermolecular hydrogen bonding. The pair of bands observed near 2884.96 cm^{-1} and 2832.78 cm^{-1} are associated with methylene C–H stretching vibrations and provide evidence of the aliphatic framework of the polymer. The absorption around 1596.30 cm^{-1} corresponds to skeletal vibrations of the polymer chain and may indicate aromatic ring contributions or deformation modes within the structure.

The strong absorption at 1021.29 cm^{-1} represents one of the most prominent features of the spectrum and is attributed to C–O stretching or ether-related vibrations. The consistency of this band across all replicates suggests that the oxygen-containing functional groups are uniformLy distributed throughout the samples. The absence of additional major bands or significant peak shifts further supports the chemical homogeneity and stability of the PES material.

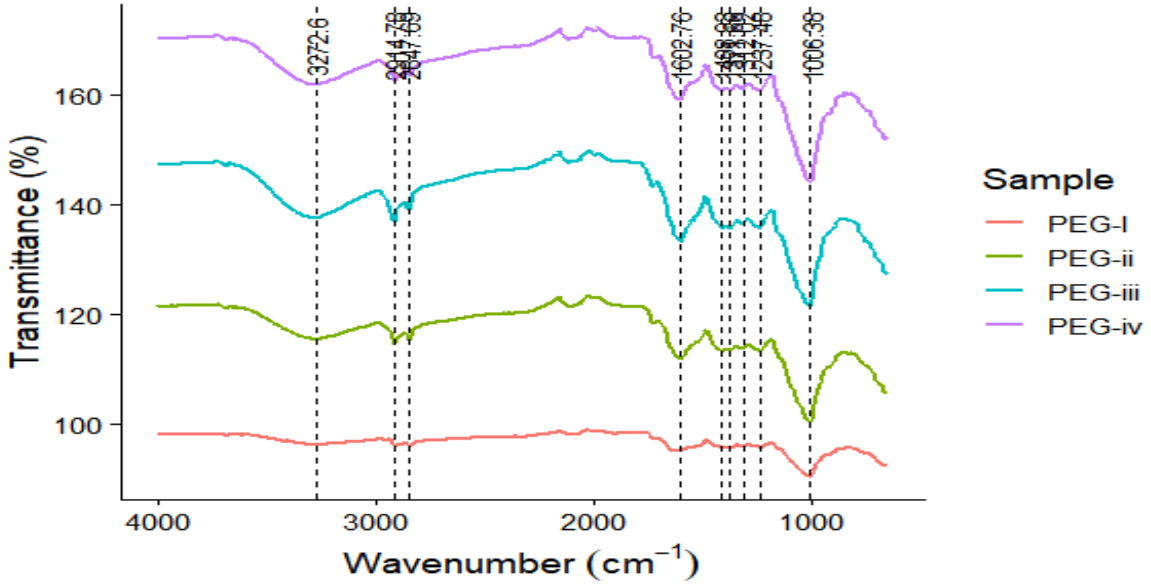

Figure 3: Stacked FTIR Spectra of PEG Samples showing Major Absorption Bands

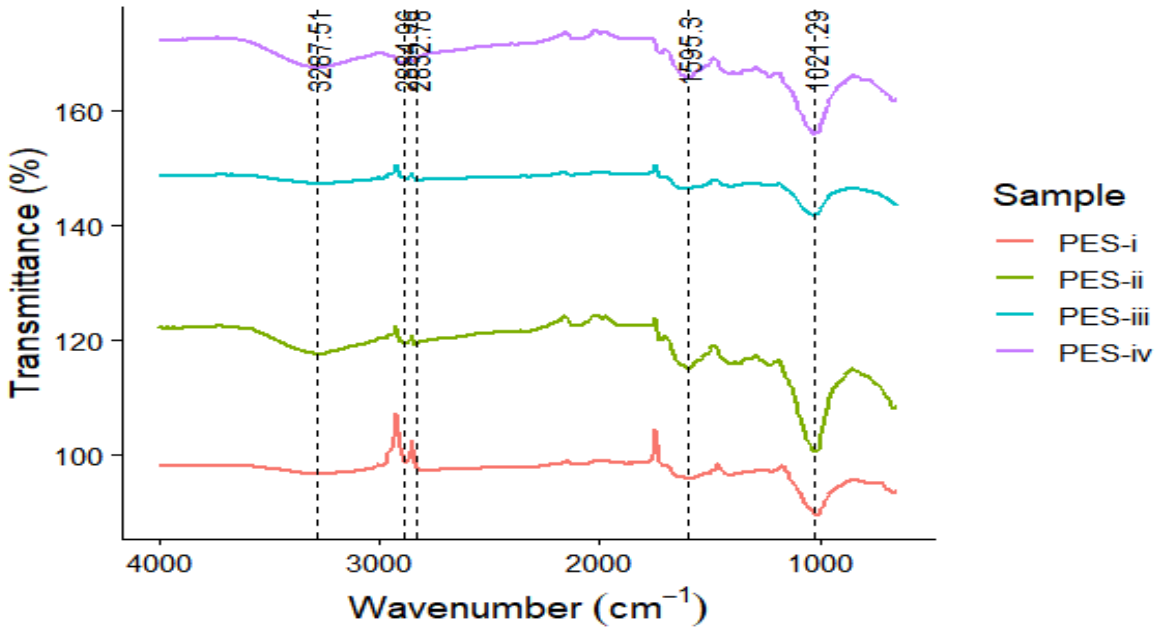

Figure 4: Stacked FTIR Spectra of PES Samples Showing Major Absorption Bands

Exposure and health-risk considerations

Exposure assessment

The exposure assessment as shown in table 23 shows the average daily intake (ADI) of nickel (Ni), phosphorus (P), and chlorine (Cl) associated with the contaminated groundnut and soybean samples. For groundnut, the ADI of nickel ranged from 0.17550 to 0.41530 g/L/kg body weight/day, with the highest value recorded in PE (G) iii (0.41530) and the lowest in PE (G) iv (0.17550). Phosphorus had comparatively higher ADI values, ranging from 3.78543 to 4.79154, while chlorine ranged from 1.88171 to 2.45440. These results indicate that the magnitude of exposure varied considerably among the different groundnut samples. For soybean, nickel intake ranged from 0.17159 to 0.38876, phosphorus from 6.08467 to 7.49394, and chlorine from 1.03864 to 1.47739. The highest phosphorus exposure occurred in PE (S) iii (7.49394), whereas the highest nickel exposure occurred in PE (S) i (0.38876). In contrast, chlorine exposure was highest in PE (S) ii (1.47739).

A comparison between the two legumes shows that phosphorus generally had the highest estimated intake, particularly in soybean samples. This suggests that phosphorus contributed substantially to the overall dietary exposure represented in the assessment. However, exposure alone does not establish the existence of a health risk; the estimated exposure must be compared with an appropriate toxicological reference value. This is the purpose of the subsequent toxicological and risk-characterization stages.

Toxicological data

The toxicological assessment as shown in table 24 uses a reference value of 1.5 µg/day**,** identified as the threshold of regulation associated with the Threshold of Toxicological Concern (TTC) approach, (Munro *et al*. 2008). The TTC approach provides a screening framework for substances for which complete chemical-specific toxicity information may not be available. Munro *et al.* (2008) explain that the original FDA threshold of regulation for packaging migrants was 1.5 µg/person/day, although modern TTC approaches use different thresholds according to chemical structural classes. Therefore, the 1.5 µg/day value used in the present assessment should be interpreted as a screening/reference value**,** rather than as a universal toxicity threshold for every contaminant. The toxicological table demonstrates that the calculated concentrations varied among elements and samples. For groundnut PE (G) i, for example, the calculated concentrations were 0.1154 for Ni, 2.0797 for P, and 1.2600 for Cl, whereas PE (G) iii had 0.2132 for Ni, 2.3130 for P, and 1.0800 for Cl**.** For soybean, phosphorus again produced the highest calculated concentrations, reaching 3.2230 in PE (S) iii**,** while nickel and chlorine remained substantially lower.

The results therefore suggest that phosphorus was the major contributor to the estimated toxicological exposure in both legumes. Nevertheless,

interpretation should account for the fact that phosphorus is an essential nutrient and its presence or concentration cannot automatically be equated with toxicological risk. Risk depends on the chemical form, dose, duration of exposure, and suitability of the reference dose used.

Hazard quotient

The hazard quotient results in table 25 provide the clearest indication of the potential non-carcinogenic health concern associated with consumption of the contaminated legumes. The HQ is generally calculated by comparing estimated exposure with a corresponding reference dose. According to the U.S. Environmental Protection Agency, an HQ of ≤1 generally indicates that exposure is not expected to pose a significant non-carcinogenic hazard, whereas an HQ greater than 1 indicates that exposure exceeds the reference value and that a potential for adverse effects exists. Importantly, HQ is not a probability of disease or toxicity.

For groundnut, the nickel HQ values were relatively low, ranging from 0.0601 to 0.1420, with the highest value observed in PE (G) iii. Chlorine also produced HQ values below one, ranging from 0.6440 to 0.8400. These values indicate that the estimated exposures to nickel and chlorine were below the reference level used in the assessment. In contrast, phosphorus produced HQ values above one in all four groundnut samples, ranging from 1.0932 in PE (G) iv to 1.5420 in PE (G) iii. The highest groundnut phosphorus HQ therefore occurred in PE (G) iii. This indicates that phosphorus exposure exceeded the selected reference value in these samples and, according to the screening criterion used, represents a potential non-carcinogenic health concern. A similar pattern was observed for soybean. Nickel HQ values were all below one, ranging from 0.0492 to 0.1115, while chlorine values ranged from 0.2978 to 0.4236, also remaining below one. However, phosphorus again produced HQ values above one in every soybean sample, ranging from 1.7446 to 2.1487. The highest phosphorus HQ was recorded in PE (S) iii (2.1487), followed by PE (S) i (1.9447), PE (S) iv (1.7912), and PE (S) ii (1.7446).

The soybean results are particularly noteworthy because all phosphorus HQ values were higher than those recorded for groundnut. This suggests that, based on the assumptions and reference values used in the assessment, consumption of the contaminated soybean samples could result in a greater potential phosphorus-related non-carcinogenic concern than consumption of the corresponding groundnut samples.

Table 23: Exposure assessment of the toxic elements in the contaminated legumes

Groundnut Sample	Element	ADI (g/L/kg bw/day)	Soybeans Sample	Element	ADI (g/L/kg bw/day)
PE (G) i	Ni	0.22479	PE (S) i	Ni	0.38876
	P	4.05113		P	6.78245
	Cl	2.45440		Cl	1.03864
PE (G) ii	Ni	0.20492	PE (S) ii	Ni	0.27180
	P	3.78543		P	6.08467
	Cl	1.88171		Cl	1.47739
PE (G) iii	Ni	0.41530	PE (S) iii	Ni	0.17159
	P	4.50558		P	7.49394
	Cl	2.10377		Cl	1.41392
PE (G) iv	Ni	0.17550	PE (S) iv	Ni	0.35691
	P	4.79154		P	6.24720
	Cl	2.18559		Cl	1.43973

Table 24: Toxicological data showing Average Daily Intake, Reference Dose and Concentrations of toxic elements from EDXRF

Sample	Element	Average Daily Intake (g/L/kg bw/day)	Reference Dose (RfD) (µg/day)	Concentration (g/L)
PE (G) i	Ni	0.22479	1.5	0.1154
	P	4.05113	1.5	2.0797
	Cl	2.45440	1.5	1.2600
PE (G) ii	Ni	0.20492	1.5	0.1052
	P	3.78543	1.5	1.9433
	Cl	1.88171	1.5	0.9660
PE (G) iii	Ni	0.41530	1.5	0.2132
	P	4.50558	1.5	2.3130
	Cl	2.10377	1.5	1.0800
PE (G) iv	Ni	0.17550	1.5	0.0901
	P	4.79154	1.5	2.4598
	Cl	2.18559	1.5	1.1220
PE (S) i	Ni	0.38876	1.5	0.1672
	P	6.78245	1.5	2.9170
	Cl	1.03864	1.5	0.4467
PE (S) ii	Ni	0.27180	1.5	0.1169
	P	6.08467	1.5	2.6169
	Cl	1.47739	1.5	0.6354
PE (S) iii	Ni	0.17159	1.5	0.0738
	P	7.49394	1.5	3.2230
	Cl	1.41392	1.5	0.6081
PE (S) iv	Ni	0.35691	1.5	0.1535
	P	6.24720	1.5	2.6868
	Cl	1.43973	1.5	0.6192

Table 25: Risk characterization of the toxic elements in the contaminated legumes Hazard Quotient (HQ)

Groundnut Sample	Element	Hazard Quotient (HQ)	Soybeans Sample	Element	Hazard Quotient (HQ)
PE (G) i	Ni	0.0769	PE (S) i	Ni	0.1115
	P	1.3865		P	1.9447
	Cl	0.8400		Cl	0.2978
PE (G) ii	Ni	0.0701	PE (S) ii	Ni	0.0779
	P	1.2955		P	1.7446
	Cl	0.6440		Cl	0.4236
PE (G) iii	Ni	0.1420	PE (S) iii	Ni	0.0492
	P	1.5420		P	2.1487
	Cl	0.7200		Cl	0.4054
PE (G) iv	Ni	0.0601	PE (S) iv	Ni	0.1023
	P	1.0932		P	1.7912
	Cl	0.7480		Cl	0.4128

An HQ greater than 1 indicates a potential health risk.

DISCUSSION

The present study demonstrates that polyethylene (PE) contamination can influence soil properties and plant performance, although the magnitude of the response differed among treatments and between groundnut and soybean. The reduction in electrical conductivity observed after PE treatment suggests that the presence of polyethylene altered the soil environment and may have influenced the movement, retention, or availability of soluble ions. Similarly, the decline in water-holding capacity indicates that PE contamination modified the physical

arrangement of soil particles and pore spaces. Previous studies have shown that microplastics can alter soil bulk density, porosity, aggregation, hydraulic properties and other physical characteristics, with potential consequences for nutrient and water dynamics (de Souza Machado *et al*., 2018; Rillig *et al.*, 2019).

Changes in soil water relations are particularly important during seed germination because successful emergence depends on adequate water uptake while maintaining sufficient oxygen availability. The delayed and reduced germination observed in several PE treatments may therefore be associated with PE-induced modification of the soil microenvironment. Previous studies have similarly reported that microplastics can affect seed germination and early plant development by altering soil physical conditions, water availability, nutrient dynamics and root-zone processes (Bosker *et al*., 2019; de Souza Machado *et al*., 2019).

The responses of groundnut were variable. Although several PE treatments delayed germination and reduced subsequent growth, some treatments supported substantial plant development. For example, certain treated groundnut plants showed considerable increases in height, branching and leaf production. This variability indicates that the response to PE was not strictly proportional to treatment volume. Such non-linear responses have also been reported in studies of microplastic–plant interactions, where the effects of plastics depend not only on concentration but also on particle characteristics, polymer type, soil conditions and plant species (Rillig *et al.*, 2019; de Souza Machado *et al*., 2019).

Soybean displayed a different pattern. Germination was completely inhibited at several treatment levels, but plants that established successfully were capable of substantial vegetative growth. Some soybean treatments approached control values for plant height and leaf area, while physiological responses such as chlorophyll content occasionally exceeded those of the control. These findings suggest that soybean may possess a degree of tolerance or physiological plasticity under some PE exposure conditions. The differences between groundnut and soybean demonstrate the importance of species-specific responses in evaluating phytotoxicity. Plant sensitivity to microplastics may depend on seed characteristics, root development, physiological tolerance and interactions between the polymer and the soil environment (Bosker *et al*., 2019; de Souza Machado *et al*., 2019). The present findings therefore support the view that microplastic phytotoxicity cannot be predicted solely from polymer quantity.

The reduction in groundnut chlorophyll content and flower production further suggests that the effects of PE may extend beyond early germination and vegetative growth. Reduced leaf development and chlorophyll concentration could limit photosynthetic capacity and consequently reduce biomass accumulation, while reduced flowering may ultimately affect reproductive success and crop yield. Microplastic exposure has been associated with altered plant growth and physiological functioning, including changes in photosynthesis, nutrient acquisition and biomass production (Pflugmacher *et al.*, 2021; de Souza Machado *et al*., 2019). Although direct yield data should be statistically analysed before strong conclusions are made, the observed effects indicate a potential risk to crop productivity.

The health-risk component of the study raises an important additional concern. Microplastics and associated contaminants may enter agricultural food systems through contaminated soils and potentially contribute to dietary exposure. Microplastics can also act as carriers of chemical additives and environmental contaminants, although the extent to which this contributes to human health risks under realistic dietary exposure conditions remains uncertain (Wright & Kelly, 2017; Campanale *et al*., 2020). Nevertheless, the present results should be interpreted cautiously because exposure estimates alone do not establish a causal human health effect. Further studies should directly quantify microplastic accumulation in edible plant tissues and combine measured concentrations with validated toxicological reference values and dietary exposure models to provide a more reliable assessment of potential health risks (Wright & Kelly, 2017; Campanale *et al*., 2020).

CONCLUSION

This study demonstrates that polyethylene microplastic contamination can alter soil properties and influence the germination, growth and physiological performance of groundnut and soybean. Polyethylene treatment reduced soil electrical conductivity and water-holding capacity relative to untreated soil, indicating modification of important physicochemical conditions. Both legume species exhibited treatment-dependent responses. Groundnut showed delayed germination and generally reduced vegetative growth under PE exposure, although some treatments supported substantial development. Soybean exhibited strong

germination inhibition at several treatment levels, but surviving plants demonstrated considerable growth and, in some cases, physiological responses comparable with or greater than those of the control. The results therefore indicate that the phytotoxicity of polyethylene microplastics is both treatment- and species-dependent. Groundnut appeared to show more consistent reductions in vegetative and physiological parameters, whereas soybean displayed greater variability and possible tolerance after successful establishment. The increasing contamination of agricultural soils with persistent plastic particles may pose risks to soil functioning, legume productivity and food safety. Further research using clearly defined PE particle sizes and environmentally relevant concentrations, rigorous statistical analysis, field experiments and direct measurement of microplastics in edible tissues is required to establish the agricultural and human-health significance of these findings.

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