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Comparative Study of the Diesel Fuel Contamination Effects of Different Types of Soils on the Growth and Germination of Four Plant Species

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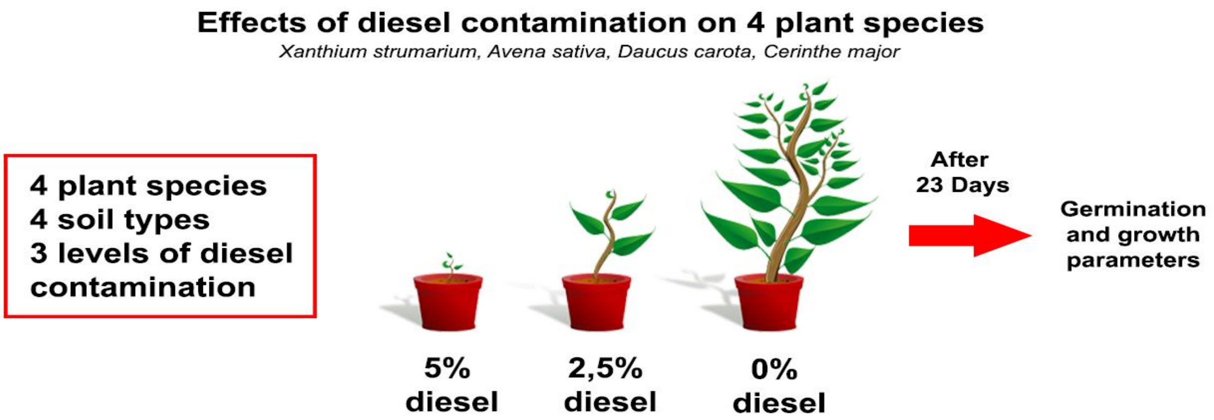
HIGHLIGHTS

- The effects of diesel contamination depend on the species and type of soil.
- *Xanthium strumarium* can tolerate higher levels of diesel contamination than many other species.
- Shoots are more affected by diesel contamination than roots.
- Root length and weight can increase significantly in diesel-contaminated soils.

Abstract: In this work, we studied the effects of different types of soils contaminated with diesel on the germination and early growth of four plant species (*Xanthium strumarium, Avena sativa, Daucus carota, and Cerinthe major*). We used four potting soils that we contaminated with different levels of diesel: 2.5%, 5%, and the control (0%). The results showed that the effect of the contamination depends on the species. Thus, compared with the three other species, *X. strumarium* showed the best results. This species showed good germination rates, with a lower rate of 50%. Its root length and weight were stimulated in the contaminated soils, and its shoot growth was less affected than that of the other species. The results depend also on the soil type. *X. strumarium* showed the best results in the different types of soils; thus, in soil three, at 5% diesel, all species showed a germination rate of 0%, but *X. strumarium* showed a rate of 50%. Our study demonstrated the variation in diesel effects across different plant species and soil types. The four species showed different levels of tolerance that varied with diesel concentrations, soil types, and parameters. We recommend performing phytoremediation tests in soils contaminated with petroleum products using preferably *X. strumarium*, but it would be interesting to test the other species. Additionally, it would be interesting to test the tolerance of this species using seeds extracted from the burs, to investigate the role of the *X. strumarium* burs in its tolerance to diesel contamination.

Keywords: Plant species; diesel; contamination; germination; growth.

GRAPHICAL ABSTRACT



INTRODUCTION

The demand for petroleum products is constantly increasing as the world's population grows. These products are widely used for different purposes. This can lead to various types of contamination, and these products can then represent a serious threat to the environment, including the soil and plant species [1–3]. There is a large variety of petroleum products, and one of those products is diesel fuel, a complex mixture of aliphatic hydrocarbons, aromatic hydrocarbons, and olefinic hydrocarbons that can contaminate the environment during its production and use [4].

After a contamination, and depending on the type and quantity of the petroleum products involved, the different physical-chemical properties of the soil and its stability can be negatively affected [5–7]. When these products contaminate soil and alter its quality, the plants will also be exposed to the pollutants, resulting in many potential negative effects. Soil contamination by diesel fuel is generally toxic to plants, affecting, for example, their seed germination, and consequently their weight, their stem lengths, and their root lengths [8–11]. These effects can vary according to the type of soil, for example, the soil texture can play a role in the tolerance of plant species to contamination [12]. The effects can also vary among the different plant species [13]. For example, the plant species of the Umbelliferae family (Apiaceae) are tolerant of some types of oils, while grasses (Poaceae) are intolerant to them [14]. The investigation of the tolerance of different plant species to petroleum-contaminated soils and assessing their ability to grow and develop in such environments is a very important step in the development of effective phytoremediation techniques [15]. In addition, it is very important to take into account the climatic conditions and characteristics of a site before choosing the plants that will be used for phytoremediation [16].

In this study, we compared the effects of diesel fuel contamination of different soils on four plant species (*Xanthium strumarium*, *Avena sativa*, *Daucus carota*, and *Cerinthe major*). *X. strumarium* is an annual invasive weed from the Asteraceae family. It is widely distributed throughout the world and can cause some negative impacts as an invasive species [17,18]. It has already been studied for its tolerance to diesel fuel contamination by Dib and Sadoudi Ali-Ahmed [19], and the results of this study indicated good perspectives as it seemed to be a tolerant species to diesel contamination. Thus, it is very interesting to compare the behavior of this plant species in contaminated soils to some other species. *A. sativa* is an annual plant species from the Poaceae family, it is cultivated as a cereal crop and also as a fodder crop [20], and it is adapted to different types of soils [21]. *D. carota* is a biennial plant of the Apiaceae family. It can be found in many environments, including, for example, roadsides, waste areas, and fields. It can be an invasive plant in some countries [22]. And finally, *C. major*, an annual plant from the Boraginaceae family, which can be found in fields, meadows, cultivated land, and wetlands [23]. These four plant species have been screened for their ability to grow on different diesel-contaminated soils. The main objective is to compare the effects between the different plant species and determine which of these species can better tolerate diesel contamination in different types of soils and, therefore, be recommended for phytoremediation testing.

MATERIAL AND METHODS

The diesel fuel used in the experiment was obtained from a gas station in the region of Tizi-Ouzou (Algeria) to contaminate different soils. *Xanthium strumarium* mature seeds were collected in the region of Tizi-Ouzou (Algeria) at an altitude of 300 meters. *Avena sativa*, *Daucus carota*, and *Cerinthe major* seeds were purchased respectively from Sativa Rheinau (Germany), Magic Garden Seeds (Germany), and Premier Seeds Direct (United Kingdom). Four different potting soils were used in the experiment. The first potting soil is a universal potting soil composed of peat enriched with finely composted bark and manure (organic matter: 83%, electrical conductivity: 40 mS/m, pH 6.5). The second potting soil consists of compressed coconut fiber waste (organic matter: 80%, electrical conductivity: 25 mS/m, pH 6.5). The third potting soil is a mixture of peat humus, composted manure, and perlite (organic matter: 75%, electrical conductivity: 30 mS/m, pH 5.5). The fourth potting soil is a mixture of peat from high marshes (organic matter: 55%, electrical conductivity: 10 mS/m, pH 6.8). We prepared four soils, mixing each type of the four potting soils with clay soil and sand at a ratio of one-third each, and we added a layer of clay pebbles at the bottom of the plastic pots used for the experiment. Thus, we obtained four soils (soil 1, soil 2, soil 3, and soil 4) corresponding respectively to the potting soil 1, 2, 3, and 4. The prepared soils were then contaminated with different levels of diesel fuel by using the masse/masse method. The control (0 g of diesel in 300 g of soil), 2.5% diesel (by adding 7.5g diesel to 292.5 g of soil), and 5% diesel (by adding 15 g diesel to 285 g of soil). Diesel was mixed with acetone to have a homogeneous distribution in the soil. Acetone was then allowed to evaporate under a fume hood. For each plant species, 10 seeds were sown in each plastic pot, with two repetitions for each level of contamination. The soils were then sprayed with water and watered daily during the experiment. The experiment was conducted in a greenhouse at a temperature of 25 ± 2 °C, a relative humidity between 50 and 70%, and with natural ventilation. The total germination percentage was recorded 23 days after sowing. Shoot and root length and fresh weight were also measured after 23 days. The lengths were measured by a caliper, and the weights by a precision balance of 0.001g precision.

Analysis of variance (ANOVA) and the Newman-Keuls post-hoc test were used to highlight the significant differences ($p < 0.05$) through the different levels of contamination. The tests were conducted under Statistica 10 (©StatSoft).

RESULTS

Germination

We observed high germination rates with *Xanthium strumarium* (Table 1). The lowest rate (50%) was recorded at 5% diesel contamination in soil 3, and we observed a rate of 100% in soil 2 at 0% and 5% diesel contamination. For *Avena sativa*, we observed good germination rates (Table 1), only 3 values were under 50%: soil 3 at 2.5% diesel (20%) and 5% diesel (0%), and soil 4 at 5% diesel (25%). For *Daucus carota* and *Cerinthe major*, generally, the germination rates decreased as the contamination increased (Table 1). The lowest values for these two species were generally observed in soil 3 and soil 4 at 2.5% diesel and 5% diesel. Thus, the most important inhibitions were observed in *D. carota* and *C. major*. In addition, we observed that soil 3 and soil 4 presented the most important germination inhibitions.

Table 1. Germination rates (%) by plant species and soils through the different levels of contamination.

Species	Diesel level (%)	Soil 1	Soil 2	Soil 3	Soil 4
		P-value = 0.464	P-value = 0.464	P-value = 0.035*	P-value = 0.074
Xanthium strumarium	0	66.67 ± 0.00	100.00 ± 0.00	91.67 ± 11.79^{a}	66.67 ± 0.00
	2.5	83.33 ± 0.00	91.67 ± 11.79	91.67 ± 11.79^{a}	91.67 ± 11.79
	5	83.33 ± 23.57	100.00 ± 0.00	50.00 ± 0.00^{b}	83.33 ± 0.00
		P-value = 0.019*	P-value = 0.306	P-value = 0.030*	P-value = 0.137
Avena sativa	0	90.00 ± 0.00^{a}	90.00 ± 14.14	85.00 ± 7.07^{a}	85.00 ± 21.21
	2.5	100.00 ± 0.00^{a}	75.00 ± 7.07	20.00 ± 28.28^{b}	90.00 ± 14.14
	5	75.00 ± 7.07^{b}	90.00 ± 0.00	0.00 ± 0.00^{b}	25.00 ± 35.36
		P-value = 0.012*	P-value = 0.961	P-value = 0.045	P-value = 0.096
Daucus carota	0	60.00 ± 0.00^{a}	60.00 ± 0.00	55.00 ± 21.21^{a}	75.00 ± 7.07
	2.5	00,00 ± 0,00^{b}	60.00 ± 28.28	45.00 ± 7.07^{a}	40.00 ± 14.14
	5	30.00 ± 14.14^{c}	65.00 ± 21.21	00.00 ± 0.00^{b}	25.00 ± 21.21
		P-value = 0.012*	P-value = 0.192	P-value = 0.049*	P-value = 0.015*
Cerinthe major	0	95.00 ± 7.07^{a}	80.00 ± 14.14	55.00 ± 7.07^{a}	85.00 ± 21.21^{a}
	2.5	85.00 ± 7.07^{a}	70.00 ± 0.00	70.00 ± 28.28^{ab}	55.00 ± 7.07^{a}
	5	30.00 ± 14.14^{b}	60.00 ± 0.00	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

*Significant differences ($p < 0.05$). Different letters indicate statistical differences.

Root growth

Tables 2 and 3 show the mean values for root length and root weight for each species and each soil. For *X. strumarium*, we observed high root lengths and weights in the contaminated soils (2.5% or 5% diesel). Low values are generally recorded in the control (0% diesel). A root length of 25.7 cm was observed at 5% in soil 1 and a weight of 4.78 g at 5% in soil 2. Concerning *A. sativa* and *D. carota*, with a few exceptions, the contamination had negative impacts on the root length and weight; the most important values were observed in the control. *C. major* could be situated between the two precedent observations. Thus, with some exceptions, the highest values were observed at the level of 2.5%, and in the control, the highest level of contamination (5%) displayed important inhibitions.

Table 2. Root lengths (cm) by plant species and soils through the different levels of contamination.

Species	Diesel level (%)	Soil 1	Soil 2	Soil 3	Soil 4
		P-value = 0.493	P-value = 0.150	P-value = 0.297	P-value = 0.026*
Xanthium strumarium	0	18.23 ± 5.02	19.42 ± 1.70	15.68 ± 0.22	14.81 ± 1.50[a]
	2.5	18.77 ± 1.82	20.72 ± 1.22	14.67 ± 4.17	22.88 ± 1.87[b]
	5	25.70 ± 9.33	15.25 ± 2.92	19.35 ± 1.44	16.33 ± 1.14[a]
		P-value = 0.031*	P-value = 0.003*	P-value = 0.091	P-value = 0.566
Avena sativa	0	15.98 ± 1.73[a]	19.36 ± 0.04[a]	11.40 ± 1.86	9.75 ± 10.54
	2.5	15.83 ± 0.06[a]	13.90 ± 0.39[b]	3.93 ± 5.55	11.72 ± 1.12
	5	11.33 ± 0.34[b]	10.12 ± 1.33[c]	0.00 ± 0.00	3.92 ± 5.54
		P-value = 0.044*	P-value = 0.024*	P-value = 0.036*	P-value = 0.064
Daucus carota	0	10,87±2,31[a]	13,38±1,08[a]	7,68±1,77[a]	11,98±2,78
	2.5	0,00±0,00[b]	7,40±1,84[b]	4,73±2,05[ab]	15,48±3,84
	5	5,78±3,43[ab]	7,08±0,39[b]	0,00±0,00[b]	2,48±3,51
		P-value = 0.020*	P-value = 0.104	P-value = 0.013*	P-value = 0.042*
Cerinthe major	0	13.67 ± 1.80[a]	7.65 ± 2.64	5.17 ± 0.41[a]	8.60 ± 3.38[a]
	2.5	7.08 ± 0.56[b]	8.66 ± 2.13	8.44 ± 2.04[a]	10.61 ± 2.50[a]
	5	6.60 ± 1.20[b]	13.66 ± 0.55	0.00 ± 0.00[b]	0.00 ± 0.00[b]

*Significant differences (p < 0.05). Different letters indicate statistical differences.

Table 3. Root weight (g) by plant species and soils through the different levels of contamination.

Species	Diesel level (%)	Soil 1	Soil 2	Soil 3	Soil 4
		P-value = 0.110	P-value = 0.512	P-value = 0.067	P-value = 0.003*
Xanthium strumarium	0	2.13 ± 0.14	4.18 ± 0.60	1.98 ± 0.08	1.53 ± 0.08[a]
	2.5	3.31 ± 0.19	4.66 ± 0.53	3.02 ± 0.59	4.20 ± 0.42[b]
	5	3.38 ± 0.73	4.78 ± 0.28	1.74 ± 0.10	1.86 ± 0.16[a]
		P-value = 0.380	P-value = 0.112	P-value = 0.078	P-value = 0.030*
Avena sativa	0	1.59 ± 0.61	3.09 ± 0.28	1.63 ± 0.08	2.26 ± 0.48[a]
	2.5	2.64 ± 1.53	2.25 ± 0.52	0.55 ± 0.78	2.98 ± 0.26[a]
	5	1.10 ± 0.09	1.55 ± 0.61	0.00 ± 0.00	0.46 ± 0.65[b]
		P-value = 0.010*	P-value = 0.421	P-value = 0.043*	P-value = 0.547
Daucus carota	0	0.07 ± 0.01[a]	0.07 ± 0.01	0.13 ± 0.05[a]	0.08 ± 0.01
	2.5	0.00 ± 0.00[b]	0.09 ± 0.01	0.11 ± 0.01[a]	0.06 ± 0.03
	5	0.05 ± 0.01[a]	0.10 ± 0.03	0.00 ± 0.00[b]	0.04 ± 0.05
		P-value = 0.376	P-value = 0.601	P-value = 0.005*	P-value = 0.019*
Cerinthe major	0	0.83 ± 0.52	2.06 ± 0.88	0.17 ± 0.08[a]	1.03 ± 0.04[a]
	2.5	1.49 ± 0.07	2.87 ± 0.93	0.89 ± 0.15[b]	2.27 ± 0.63[b]
	5	1.06 ± 0.46	2.67 ± 0.35	0.00 ± 0.00[a]	0.00 ± 0.00[a]

*Significant differences (p < 0.05). Different letters indicate statistical differences.

Shoot growth

In general, as the contamination increased, the length and weight of the shoots decreased (Tables 4 and 5). In general, for the four plant species used in this experiment, the most important values were recorded in the control (0% diesel contamination), even if there were some exceptions. Thus, the level of contamination of 5% caused the most important inhibitions of shoot growth in all the species and all the soils, except for some observations.

The significant differences (P < 0.05) indicate many differences between the control (0%) and the level of 2.5% or 5%, but we have some exceptions. Thus, in general, the lowest values were recorded at the level of contamination of 5%. However, we observed some differences between the different plant species. Thus, *X. strumarium* seemed to be the most tolerant plant species, for example, in soil 3 at the level of 5% diesel contamination, all the values were null, except for *X. strumarium*, which showed a germination rate of 50%, which was the lowest rate observed for this species. This plant species showed the best performance compared to the three other plant species used in the experiment.

Table 4. Shoot lengths (cm) by plant species and soils through the different levels of contamination.

Species	Diesel level (%)	Soil 1	Soil 2	Soil 3	Soil 4
		P-value = 0.331	P-value = 0.011*	P-value = 0.233	P-value = 0.001*
Xanthium strumarium	0	17.88 ± 0.71	18.89 ± 0.34^{a}	16.31 ± 1.78	17.59 ± 0.27^{a}
	2.5	15.42 ± 0.88	16.27 ± 0.54^{b}	15.07 ± 0.75	15.30 ± 0.59^{b}
	5	17.14 ± 2.14	15.59 ± 0.48^{b}	12.27 ± 2.59	11.40 ± 0.38^{c}
		P-value = 0.020*	P-value = 0.006*	P-value = 0.405	P-value = 0.204
Avena sativa	0	2.16 ± 0.36^{a}	5.72 ± 0.34^{a}	2.53 ± 0.23	3.92 ± 0.15
	2.5	5.77 ± 0.58^{b}	4.49 ± 0.14^{b}	2.10 ± 2.97	5.16 ± 0.25
	5	3.00 ± 0.83^{a}	3.29 ± 0.26^{c}	0.00 ± 0.00	1.76 ± 2.49
		P-value = 0.004*	P-value = 0.225	P-value = 0.040*	P-value = 0.095
Daucus carota	0	1.78 ± 0.11^{a}	2.14 ± 0.37	5.20 ± 1.87^{a}	5.89 ± 2.42
	2.5	0.00 ± 0.00^{b}	1.65 ± 0.16	1.31 ± 0.63^{b}	1.53 ± 0.05
	5	1.68 ± 0.32^{a}	1.71 ± 0.09	0.00 ± 0.00^{b}	0.95 ± 1.34
		P-value = 0.051	P-value = 0.019*	P-value = 0.0006*	P-value = 0.003*
Cerinthe major	0	13.67 ± 1.80	9.44 ± 1.11^{a}	11.00 ± 0.66^{a}	11.67 ± 1.66^{a}
	2.5	4.42 ± 3.20	6.43 ± 0.36^{b}	7.05 ± 0.71^{b}	8.43 ± 0.78^{a}
	5	6.60 ± 1.20	5.36 ± 0.15^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}

*Significant differences ($p < 0.05$). Different letters indicate statistical differences.

Table 5. Shoot weight (g) by plant species and soils through the different levels of contamination.

Species	Diesel level (%)	Soil 1	Soil 2	Soil 3	Soil 4
		P-value = 0.995	P-value = 0.109	P-value = 0.0007*	P-value = 0.001*
Xanthium strumarium	0	6.50 ± 2.55	10.69 ± 1.01	8.61 ± 0.44^{a}	6.22 ± 0.43^{a}
	2.5	6.34 ± 0.45	8.94 ± 1.23	7.07 ± 0.05^{b}	7.62 ± 0.23^{b}
	5	6.44 ± 1.82	6.98 ± 1.24	3.35 ± 0.22^{c}	2.85 ± 0.21^{c}
		P-value = 0.001*	P-value = 0.748	P-value = 0.004*	P-value = 0.045*
Avena sativa	0	5.86 ± 0.06^{a}	2.09 ± 0.29	4.90 ± 0.29^{a}	2.67 ± 0.21^{ab}
	2.5	2.24 ± 0.47^{b}	1.98 ± 0.42	0.58 ± 0.81^{b}	2.50 ± 0.23^{a}
	5	1.35 ± 0.06^{c}	1.80 ± 0.37	0.00 ± 0.00^{b}	0.59 ± 0.83^{b}
		P-value = 0.005*	P-value = 0.562	P-value = 0.028*	P-value = 0.323
Daucus carota	0	0.12 ± 0.01^{a}	0.05 ± 0.00	0.04 ± 0.01^{a}	0.18 ± 0.01
	2.5	0.00 ± 0.00^{b}	0.06 ± 0.01	0.05 ± 0.01^{a}	0.05 ± 0.02
	5	0.10 ± 0.02^{a}	0.07 ± 0.02	0.00 ± 0.00^{b}	0.09 ± 0.13
		P-value = 0.005*	P-value = 0.015*	P-value = 0.010*	P-value = 0.002*
Cerinthe major	0	13.96 ± 1.86^{a}	6.33 ± 0.85^{a}	4.61 ± 0.08^{a}	7.45 ± 0.47^{a}
	2.5	3.95 ± 0.93^{b}	3.46 ± 0.59^{b}	2.62 ± 1.04^{b}	2.89 ± 0.90^{b}
	5	1.55 ± 1.05^{b}	2.43 ± 0.07^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

*Significant differences ($p < 0.05$). Different letters indicate statistical differences.

DISCUSSION

We know that petroleum products can cause serious damage to plant germination and growth. These effects vary depending on the type of soil and plant species, with tolerance levels varying from one species to another [24,25]. This is what we observed in our study: the germination rates, root and shoot lengths, and weights varied between the different species. Thus, in terms of root growth, *Xanthium strumarium* and *Cerinthe major* seemed to be the most tolerant plant species to the contamination. For the shoot length, *X. strumarium* and *Avena sativa* were the least affected, and for the shoot weight, we noticed that *X. strumarium* and *Daucus carota* were less inhibited. Concerning germination, *X. strumarium* was the most tolerant of the four plant species.

D. carota has the potential to tolerate and phytoremediate petroleum hydrocarbons [26]. The fact that we used high levels of diesel fuel contamination could explain the negative effects recorded in our study concerning the shoot and root growth of this species. The remediation potential of some species from the

Boraginaceae family in oil-contaminated soils has been described in a study by Panchenko and coauthors, [27]; moreover, this study did not report the presence of species from the Cerinthe genus. Concerning *A. sativa*, it was reported to have the capacity to tolerate petroleum hydrocarbons [26]. On the other hand, Molina-Barahona and coauthors, [28] have reported the toxic effects of diesel on the weight and the root and stem elongations of *A. sativa*. In this study, the growth of the roots and shoots of this species was generally negatively affected. But we observed that for the parameter shoot length, *A. sativa* is less affected than some other species.

X. strumarium showed good germination rates in the contaminated soils; the highest values for root length and weight were recorded in the contaminated substrates, and the shoot length and weight were less affected than the three other species. Similar results were reported by Dib et Sadoudi Ali-Ahmed [19]. The root growth seemed to be less affected than the shoot growth, especially in *X. strumarium*. When the roots of a plant develop in completely contaminated soil, the roots will grow and search for clean soil [29]; this could explain why the roots of *X. strumarium* were longer in the contaminated soils compared to the control. Thus, *X. strumarium* seemed to be the most tolerant plant species in this experiment, especially at 5% diesel contamination (the highest level of contamination). The seeds of *X. strumarium* are contained in a bur; therefore, it could act as a protection against contamination [19]. Thus, the burs could prevent or reduce the possible damage that can be caused by the contact of the seeds with the contaminant.

The effects of diesel contamination on plant species may also depend on the soil type. Thus, according to the germination and growth results, it seemed that soil 1 and soil 2 were better than soil 3 and soil 4. Soils 1 and 2 are more equilibrated. Soil 3 presents a low electrical conductivity (10 mS/m), which indicates a low amount of nutrients, and soil 4 presents the lowest pH (5.5). *X. strumarium* showed the best adaptation to the different soils. This plant species is able to grow in different soil types [30].

In light of the results obtained, we think that it would be more interesting to study the phytoremediation potential of *X. strumarium* and its potential resistance mechanisms in sites contaminated with petroleum products, and also to study the detoxifying enzymes in *X. strumarium* that make this plant species tolerant to diesel.

CONCLUSION

Our results show that the effects of the contamination depends on the species and on the soil type. *Xanthium strumarium* was the most tolerant plant species compared to the three other species used in this experiment. Moreover, this was also the species that showed the best results in the different types of soils. The three other species were more affected by the contamination, but we noticed some differences between them depending on the different parameters.

We think that *X. strumarium* should be tested primarily for its potential phytoremediation capacity in oil-contaminated soils. But, even if the three other species are less tolerant, we think that they should also be tested in phytoremediation experiments. It would also be very interesting to perform a diesel phytotoxicity experiment on *X. strumarium* by using seeds extracted from the burs to compare the tolerance of this species with and without the burs that contain the seeds and can potentially protect them from contamination.

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