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Phytotoxic effects of petroleum hydrocarbons on germination and growth of the native halophyte *Salicornia sinus persica* in oil contaminated soil

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The release of petroleum hydrocarbons (PHCs) into the environment is primarily linked to petroleum industry activities, including drilling, exploration, storage, and related processes. The spillage of PHCs into the environment poses significant threats to ecosystems and can lead to serious risks to human health, the environment, and plants. This research aims to investigate the phytotoxic effect of petroleum sludge on the germination and growth characteristics of *Salicornia sinus-persica*. This study was conducted in a greenhouse using pots to examine the effects of varying concentrations of PHCs on plant growth. Petroleum sludge at concentrations of 0, 0.2, 1, 2, 4, and 8% (w/w) was used to prepare PHC-contaminated soils. In some pots, biochar, and vermicompost were added to them in order to evaluate the effect of soil amendments on plants. The study evaluated several parameters, including seed germination, fresh and dry biomass weight, number of lateral stems, stem and root lengths, and chlorophyll a, b, total chlorophyll, and carotenoids. The results of this study showed that petroleum contamination had negative effects on the growth parameters of *Salicornia sinus persica* and photosynthetic pigments. However, the addition of biochar and vermicompost as soil amendments improved plant growth under contaminated conditions. *Salicornia* dried after 1 month in oil-contaminated soils with a concentration of 8% in all soil treatments, which indicated its high toxicity to the plant.

Keywords Petroleum hydrocarbons, Germination, *Salicornia*, Halophyte plants

Abbreviations

PHCs	Petroleum hydrocarbons
OS	Only soil without amendments
SB	Soil + biochar
SV	Soil + vermicompost
SBV	Soil + biochar + vermicompost
PS	Petroleum sludge
EC	Electrical conductivity
TNV	Total neutralizing value
OC	Organic carbon
Total N	Total nitrogen
Avail P	Available phosphorus
Na	Sodium
Cl	Chlorine

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The rapid expansion of agricultural, municipal activities, and industrial, combined with unprecedented global population growth, has dramatically heightened the demand for petroleum production[1,2]. Worldwide petroleum production is expected to surpass twelve million metric tons annually, reflecting the increasing demand driven by industrial and population growth[3]. The release of petroleum hydrocarbons (PHCs) into the environment, primarily from petroleum industry activities like drilling, transportation, and refining, is a major source of soil contamination in terrestrial ecosystems[2,4].

PHCs encompass diverse organic chemical compounds, consisting of thousands of distinct mixtures and concentrations[5]. These compounds are primarily composed of high levels of hydrogen and carbon, along with lesser amounts of oxygen, sulfur, nitrogen, and trace amounts of metals[2]. PHCs can be categorized into four primary groups: (a) Alkanes (e.g., hexane, octane) are saturated hydrocarbons with straight-chain structures; (b) Cycloalkanes (e.g., methylcyclopentane) are cyclic saturated hydrocarbons; (c) Alkenes (e.g., ethylene) are unsaturated hydrocarbons with double bonds; and d) Arenes (e.g., BTEX and phenanthrene) are hydrocarbons containing benzene rings[6].

The release of PHCs into natural habitats presents a major hazard to ecological systems and could pose significant health risks to humans, along with extensive environmental degradation[7,8]. PHCs primarily comprise hydrogen and carbon, with their soil presence significantly disrupting the carbon-to-nitrogen (C/N) ratio at contaminated sites[9]. This composition often leads to nitrogen deficiency, which disrupts soil productivity and impairs microbial functions. Additionally, the accumulation of biodegradable organic matter in the topsoil can lower oxygen levels, which restricts the movement of oxygen into the deeper layers of the soil[2,10]. Petroleum pollution in soil has profound negative effects on plant growth, which causes severe damage to ecosystems and leads to a reduction in biodiversity[11]. PHCs can accumulate inside plants and destroy organs and tissues, threatening the health of animals and humans throughout the food chain[12]. PHCs can disrupt essential plant functions, such as nutrient uptake, water absorption, and photosynthesis, thereby inhibiting growth, weakening vegetation, and adversely affecting plant survival[10].

Toxicity assays, particularly those focusing on seed germination and early seedling growth, are widely recognized as valuable short-term tests for evaluating the success of remediation techniques in contaminated environments, assessing environmental pollutants, analyzing soil amendments, and determining the phytotoxicity of chemicals[13–17]. These assays provide rapid and quantifiable data on the impact of pollutants such as petroleum hydrocarbons or heavy metals on plant physiology. By monitoring germination rates and early growth metrics, researchers can evaluate the effect of soil amendments (such as biochar or compost) to reduce soil toxicity and plant growth[14,18]. Numerous studies have explored the effects of oil-contaminated soil on seed germination and plant growth across various species, including *Corn*[13], *Vigna radiate*[14], *Festuca pratensis, Lolium perenne* and *Poa pratensis*[19], *Rye*[20], *Myrtaceae*[21], *alfalfa*[22], *Fabaceae*[23], *Grass species*[24], etc. Each plant species shows different sensitivities to contaminated soils, helping researchers identify those more resilient or vulnerable to pollutants. While some studies indicate that crude oil harms plant and seed germination[8,25], others suggest that low oil concentrations may enhance seed germination and promote plant growth[26,27]. Therefore, more studies should be done to clarify the effects of oil pollution on plant growth.

Many oil-contaminated areas, particularly those near coastal regions, may have a high concentration of salt due to their proximity to seawater[28]. Therefore, the use of oil and salt-tolerant plants, as well as native plant species with varied genotypes, proves to be both a practical and effective approach for soil remediation in these areas. These plants ability to adapt and thrive in harsh climatic conditions enhances their suitability for environmental remediation in stressed ecosystems[29]. *Salicornia* is a halophytic plant, meaning it thrives in saline environments, which makes it a candidate for research in oil-contaminated soils[30]. The genus *Salicornia L.* belongs to the *Chenopodiaceae* family and is an annual plant. Two species from this family, *Salicornia persica* and *Salicornia iranica*, are indigenous to Iran, with *S. persica* being a common tetraploid species and *S. iranica* a diploid species. These species have demonstrated a high tolerance to soil salinity[29]. *Salicornia sinus-persica* is an annual plant with a smooth texture and dark green color, which turns orange-reddish on the lower part of its stems. It typically reaches heights between 25 and 60 cm and has a canopy diameter that can reach up to 80 cm[31]. This study aims to assess the phytotoxic effects of oil-contaminated soil on the germination and growth of *Salicornia sinus-persica*.

Material and methods

Materials

Uncontaminated soil was provided from 0 to 20 cm of agricultural lands with no history of oil contamination using a stainless-steel spade to avoid external contamination. Petroleum sludge from a petroleum refinery company (Isfahan–Iran) was used for the preparation of petroleum hydrocarbon-contaminated soils. The petroleum sludge was transported and stored in sealed, leak-proof containers to prevent any leakage or contamination until the preparation of soil treatments. The soil and petroleum sludge were stored in a well-ventilated, temperature-controlled laboratory area at approximately 22–25 °C. The certified seeds of *Salicornia sinus-persica* (Bushehr ecotype) were provided by the Iranian National Salinity Research Center (Yazd, Iran). Vermicompost, biochar, and square plastic pots (polypropylene) were purchased from local producers.

Physical and chemical properties of soil

Three samples of Uncontaminated soil were analyzed and determination of the Physio-chemical properties of the soil including Electrical Conductivity of saturation extract (EC)[32], pH in saturated paste[33], Total Neutralizing Value (TNV) by Calcimeter method[34], Organic Carbon (OC) by Walkley and Black method[35], Total Nitrogen (Total N) by Kjeldahl digestion method[36], and Available Phosphorus (Avail P) by Olsen method[37], Texture by hydrometer method[38], Sodium (Na) by Flame photometer method[39], and Chlorine (Cl) by Titration method[40].

Soil treatments

Soil treatments were promptly prepared after collecting the petroleum sludge, ensuring minimal storage time. Before collecting petroleum sludge, the soil was air-dried for a week and then was sieved using a stainless-steel sieve (mesh width of 2 mm) to remove large particles of stone and sand. Similarly, petroleum sludge, biochar, and vermicompost were sieved[41]. Petroleum hydrocarbons-contaminated soil was prepared by adding different concentrations of petroleum sludge (0, 0.2, 1, 2, 4, 8% (w/w)) to uncontaminated soil (Table 1) and mixed vigorously to obtain a uniform soil mixture. The pots containing treated soils were allowed to stabilize for 2 weeks to achieve a uniform distribution of petroleum hydrocarbons and volatilization processes[42,43]. Biochar and vermicompost were added to the soil at 5% (v/v) to improve soil biological properties and fertility (Table 1)[44]. The final treatments were placed in corresponding pots, with each pot containing 13 kg of the treated soil.

Seed planting and maintenance of plant

Seed planting was conducted in early spring 2023 under greenhouse conditions in Birjand, Iran (32° 52′ N, 59° 13′ E), a region characterized by a hot, arid climate and situated at an altitude of 1470 m above sea level. The humidity level in the greenhouse was maintained at approximately 50%, while the temperature was controlled to range between 15°C at night and 28 °C during the day. The pots received about 12 h of natural sunlight daily. The experiment included three replications, and after filling the pots with treated soil, 300 seeds were evenly distributed on the soil surface and were planted in each pot at a depth of approximately 3 mm. During the test period, irrigation was carried out every 3 days, maintaining soil moisture at field capacity without any leaching from the bottom of the pots. Well water from the greenhouse, with a salinity of 4 dS/m, was used for irrigation.

Determination of germination rate and plant growth

The germination of *Salicornia* seeds was monitored every 48 h for up to two weeks (14 days) after sowing[44]. The seed germination rate (GR) was calculated according to Eq. (1)[45].

$$\mathrm{GR} = \frac{n}{N} \times 100 \tag{1}$$

where n refers to the number of seeds that have germinated, while N indicates the total number of seeds that were planted.

For determination of plant growth indexes, after 6 months of plantation (early Autumn 2023), the plants were carefully removed from the pots, and gently washed with distilled water until the mud was completely removed from the roots. Stem length and root length were measured using a ruler and the number of lateral stems was counted visually. Fresh biomass of shoots and roots of plants were measured separately by a digital balance (AND, HR-200, Japan). For determination of the dry biomass of roots and shoots, the plants were dried in an incubator (70 °C) for 72 h (Froilabo, AP-120, France) until full dryness and then the biomass was measured using a digital balance[46]. Plant pigment content including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid was determined according to the previously developed method[41,47]. Briefly, plant tissues were digested, and then the absorbance (A) at wavelengths of 663, 645, and 470 nm was measured using a spectrophotometer (UV/VIS spectrophotometer T80^{+}, PG Instrument Ltd, England). The concentrations of chlorophyll a, b, total chlorophyll, and carotenoids were determined using Eqs. (2)–(5)[48–51].

$$\text{Chlorophyll a} = (12.25 \times A_{663}) - (2.79 \times A_{645}) \tag{2}$$

$$\text{Chlorophyll b} = (21.50 \times A_{645}) - (5.1 \times A_{663}) \tag{3}$$

$$\text{Total Chlorophyll} = (20.2 \times A_{645}) - (8.02 \times A_{663}) \tag{4}$$

$$\text{Carotenoids} = \frac{(1000 \times A_{470}) - (1.82 \times Chla) - (85.02 \times Chlb)}{198} \tag{5}$$

where Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoids are in mg/g of fresh plant.

Result and discussion

Physical and chemical properties of soil

The physical and chemical properties of the soil sample are indicated in Table 2. As shown in this table, the initial soil is slightly alkaline and has low salinity, which typically supports plant growth without causing adverse

Type of soil treatments	Abbreviation	Petroleum sludge concentration (% w/w)					
		0	0.2	1	2	4	8
Only soil without amendments	OS	*	*	*	*	*	*
Soil + biochar	SB	*	*	*	*	*	*
Soil + vermicompost	SV	*	*	*	*	*	*
Soil + biochar + vermicompost	SBV	*	*	*	*	*	*

Table 1. The types of soil treatments with petroleum sludge and soil amendment.

Physico-chemical properties	Value
EC (dS/m)	0.528 ± 0.1
pH	7.61 ± 0.08
TNV%	8.84 ± 0.1
OC%	0.11 ± 0.04
Total N (mg/kg)	115 ± 3.5
Avail P (mg/kg)	0.123 ± 0.01
Texture	Sandy
Sand	89 ± 1.2
Silt	7 ± 0.8
Clay	4 ± 0.4
Na%	0.042 ± 0.01
Cl (meq/L)	1.60 ± 0.06

Table 2. The results of soil physical and chemical properties.

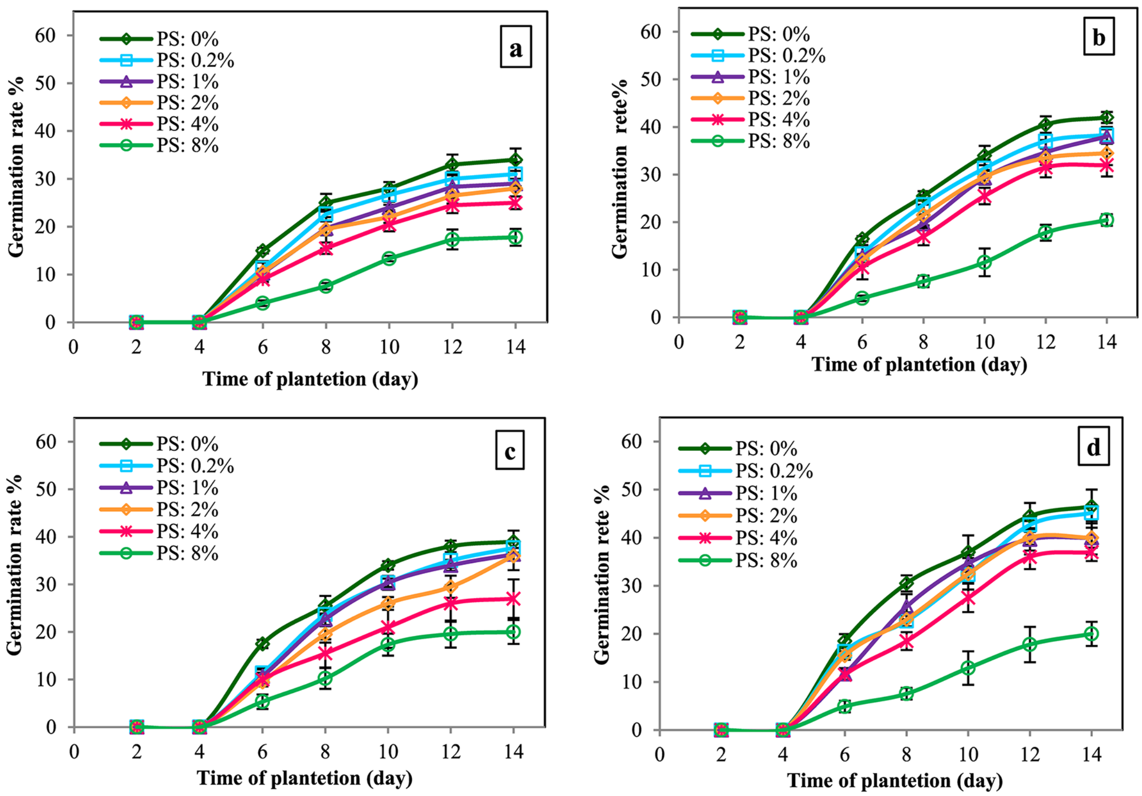

Fig. 1. Effect of different petroleum sludge (PS) concentrations on the germination rate of *Salicornia sinus-persica* in various treatments: (**a**) OS (Only Soil without amendments), (**b**) SB (Soil + Biochar), (**c**) SV (Soil + Vermicompost), and (**d**) SBV (Soil + Biochar + Vermicompost).

effects. Total Neutralizing Value (TNV), Organic Carbon (OC), Total Nitrogen, and Available Phosphorus were 8.84 ± 0.1%, 0.11 ± 0.04%, 0.115 ± 3.5 mg/kg and 0.123 ± 0.01 mg/kg, respectively. These values indicate the low levels of nutrients and the need for soil fertilization. In this study, biochar and vermicompost were used to improve soil quality.

Germination rate

The results related to the effect of petroleum hydrocarbons on the germination of *Salicornia sinus-persica* under different soil treatments are shown in Fig. 1. Seed germination is a critical stage in the plant life cycle, and is highly sensitive to environmental conditions[52]. As it is known, in all treatments (OS, SB, SV, and SBV) the increase in petroleum sludge concentration caused a decrease in the germination rate.

For example, in the OS treatment, the lowest (18%) and highest (34%) germination rates after 14 days were observed at 8% and 0% petroleum sludge in soli, respectively. Additionally, the germination rate decreased in the

SBV treatment, with rates of 47, 45, 40, 40, 37, and 20% at petroleum sludge concentrations of 0, 0.2, 1, 2, 4, and 8%, respectively. It was found that the addition of biochar and vermicompost improved the germination rate. All treatments demonstrated a statistically significant improvement in germination rate when the contaminated soils were amended with biochar and vermicompost. For example, in oil-contaminated soil at a concentration of 2%, the germination rates after 14 days were 28% for OS, 35% for SB, 36% for SV, and 40% for SBV treatments.

Petroleum hydrocarbon contamination significantly alters the physical, chemical, and nutritional properties of soil, leading to disruptions in its fertility and overall quality[53]. These hydrocarbons have been shown to reduce soil porosity and compromise its natural structure. Such contamination impacts soil characteristics, including pH, oxygen availability, ion exchange capacity, moisture retention, and cation saturation. As a result, the presence of petroleum substances in soil adversely affects plant germination and growth[14,54,55]. Adam et al.[56] found that seed germination in diesel-contaminated soil is significantly dependent on plant species, with members of the same plant family exhibiting varying levels of sensitivity to diesel fuel. Akinola et al.[57]) observed a decrease in the germination and growth of *Glycine max* accessions in crude oil-contaminated media. They suggested that this reduction in seed germination may be due to the crude oil damaging the seeds during germination or reducing water uptake and gas exchange. The results of Sharifi et al. study showed that all herbaceous plant species had dose-dependent responses to oil-contaminated soils. Reduction in germination, plant height, and biomass weight occurred for all species compared to their controls. In this study, *Medicago truncatular* showed the highest and *Linum ussitatisimum* the lowest toxic effect including germination, height above ground, and dry weight due to soil oil contamination[58].

Biochar improves soil properties such as pH, water-holding capacity, and nutrient retention, which can positively influence seed germination and plant productivity[18]. Compost improves soil quality, fertility, structure, and moisture retention, which can enhance seed germination and seedling quality. The combined application of biochar and compost can amplify these benefits, offering a synergistic effect on soil health and plant growth[59,60]. Yousaf et al. reported that the addition of biochar and compost to the soil significantly improves the germination rates. Treatment of contaminated soil with compost, restored germination rates to 98.5, 99.6, 96.6, 88.96, and 100% for *alfalfa*, *ryegrass*, *maize*, *white clover*, and *wheat*, respectively. It was also confirmed that plants from the *Poaceae* family, such as *wheat* and *maize*, displayed consistent germination patterns under other soil amendment conditions[41]. Peng et al.[59] stated that the combined application of biochar and sludge compost significantly enhanced the germination potential of oil sunflower seeds in both salt-affected and farmland soils, demonstrating the synergistic benefits of these amendments.

Plant growth

Continuous monitoring of *Salicornia* sprout and growth showed that all the plants in the pots with 8% petroleum sludge after 3–4 week changed their color, eventually turned yellow, wilted, became droopy, and finally died which indicated the high toxicity of this concentration of petroleum sludge for the *Salicornia*. Figure 2 illustrates the effect of petroleum hydrocarbon contamination across different soil treatments on the number of lateral stems of *Salicornia*. A decrease in the number of lateral stems was observed in all soil treatments with increasing concentrations of petroleum sludge in the soil, indicating the negative impact of petroleum hydrocarbon pollution on *Salicornia* growth. For instance, in OS treatment, the average number of lateral stems was 11, 7, 3.5, 2.5, and 1.5 stems for the 0%, 0.2%, 1%, 2%, and 4% of petroleum sludge in the soil, respectively. While, for the SB and SV treatments, the corresponding values were 23, 9.5, 5.5, 4, and 3.5 stems, demonstrating the beneficial effects of biochar and vermicompost on plant growth. Ebrahimi et al. similarly reported that eggplant growth and yield significantly improved with the co-treatment of vermicompost and biochar. Furthermore, the combined use of date palm biochar (DPB), pistachio biochar (PB), and vermicompost was shown to mitigate the water stress damage of plants up to 50%. These findings suggest that these soil amendments can effectively alleviate drought stress, primarily by enhancing soil organic matter, improving nutrient uptake, and increasing soil moisture retention, ultimately boosting plant growth parameters[61]. Numerous studies have demonstrated an

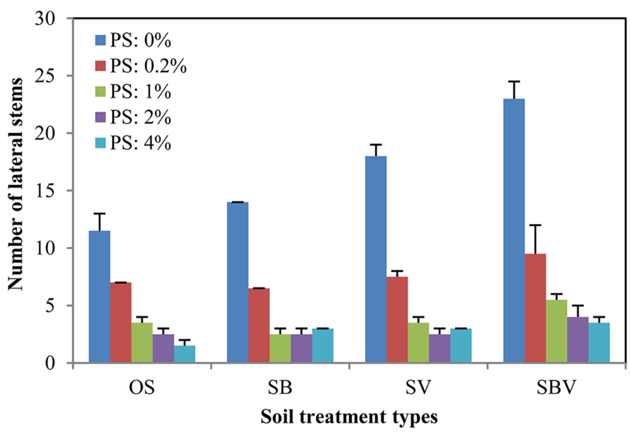

Fig. 2. Effect of petroleum sludge (PS) concentration in various soil treatments on the number of lateral stems of *Salicornia sinus persica*.

increase in the number of leaves, leaf area, root length, and both roots and shoots biomass due to the application of vermicompost and biochar[62–64].

The effect of petroleum sludge concentration on fresh and dry weight of *Salicornia* plant in different treatments is shown in Fig. 3. The results showed that the presence of petroleum hydrocarbon at any level harmed the fresh biomass of *Saliconia* in all treatments. For example, in the OS treatments, with 0% and 4% petroleum sludge levels, the average fresh plant weights were 1.20 and 0.03 g, respectively. Similarly, the average dry plant biomasses were 0.051 and 0.004 g, respectively (Fig. 3). In all soil treatments, the lowest plant growth was observed at the highest petroleum sludge levels (4%), while the highest fresh and dry plant weights were recorded in soil without petroleum sludge contamination (0%). The findings of this study highlighted the detrimental effects of petroleum hydrocarbon contamination on the biomass of Salicornia, likely due to the ability of hydrocarbon molecules to infiltrate plant tissues, causing cell membrane damage and impairing respiration rates[65,66]. Additionally, the decrease in plant growth may be attributed to the disruption of water and nutrient uptake caused by oil pollution, rather than a direct toxic effect[67]. Ekundayo et al. reported that crude oil had a detrimental effect on *maize* growth. Plant growth is significantly lower in crude oil-contaminated soil compared to the uncontaminated control[68]. Ogboghodo et al.[69] reported that crude oil negatively affects the growth and productivity of *Zea* plants, attributing the chlorosis of *Zea* leaves due to nutrient deficiencies in crude oil-contaminated soils. Several studies have similarly noted reduced plant growth and productivity in oil-contaminated soils[70–72].

The results of the present study indicated that among various treatments, the application of biochar and vermicompost significantly increased the average fresh and dry weight of *Salicornia* (Fig. 3). For instance, in soil with 0.2% contamination of petroleum sludge, the average fresh weight of the plant in OS, SB, SV, and SBV treatments were 0.70, 0.75, 1.27, and 1.79 g, respectively. Similarly, the corresponding dry weights were 0.032, 0.034, 0.097, and 0.103 g, respectively. Contaminated soils typically exhibit deficiencies in both macro and micro-nutrients that are crucial for supporting healthy plant growth and facilitating the microbial degradation of contaminants. Furthermore, the presence of petroleum crude oil can hinder nutrient availability for plants by diminishing water accessibility. Consequently, the application of fertilizers can significantly enhance plant growth performance in these contaminated environments. Contaminated soils typically exhibit deficiencies in both macro- and micro-nutrients that are crucial for supporting healthy plant growth and facilitating the microbial degradation of contaminants[73]. The presence of petroleum hydrocarbons in soil can hinder nutrient availability for plants by diminishing water accessibility[69]. As a result, the application of fertilizers can significantly enhance plant growth performance in these contaminated environments[74]. In the context of soil amendment with biochar, studies have shown that biochar can significantly enhance plant growth, primarily by increasing nutrient availability and improving physical soil properties, such as reducing bulk density[75,76]. Additionally, biochar is enriched with active functional groups which improve the chemical properties of the soil, including cation exchange capacity, which enhances plant access to nutrients and ultimately leads to improved plant growth[77]. Chirakkara et al.[78] reported that amending soil with biochar and compost enhanced soil quality, which in turn improved the growth characteristics and biomass production of *sunflower* and *oats*.

In Fig. 4, the effect of petroleum sludge in different soil treatments on the stem length and root length of *Salicornia* is reported. Stem and root lengths, which serve as indicators of plant growth and biomass production, decreased with increasing concentrations of petroleum sludge in all soil treatments. However, the stem and root lengths in treatments containing a mixture of vermicompost and biochar were consistently greater than those in the OS across all petroleum sludge levels.

The average stem length in soil contaminated with 2% petroleum sludge was 1.2 cm in the OS treatment, 1.5 cm in the SB treatment, 1.5 cm in the SV treatment, and 2.1 cm in the SBV treatment. Similarly, the average root length was 0.8, 1.1, 1.1, and 2.0 cm for these treatments, respectively. The results of this study also showed that in the SBV treatment, both the average stem length and root length decreased as the petroleum sludge concentrations increased. For petroleum sludge levels of 0% and 4%, the stem lengths average were 16.8 cm and

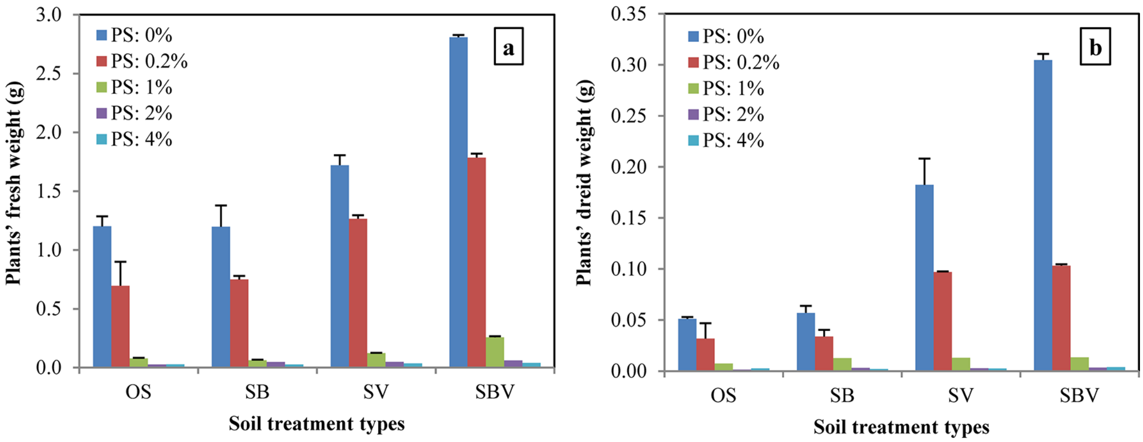

Fig. 3. Effect of petroleum sludge (PS) in various soil treatments on (**a**) fresh plant biomass and (**b**) dried plant biomass of *Salicornia sinus persica.*

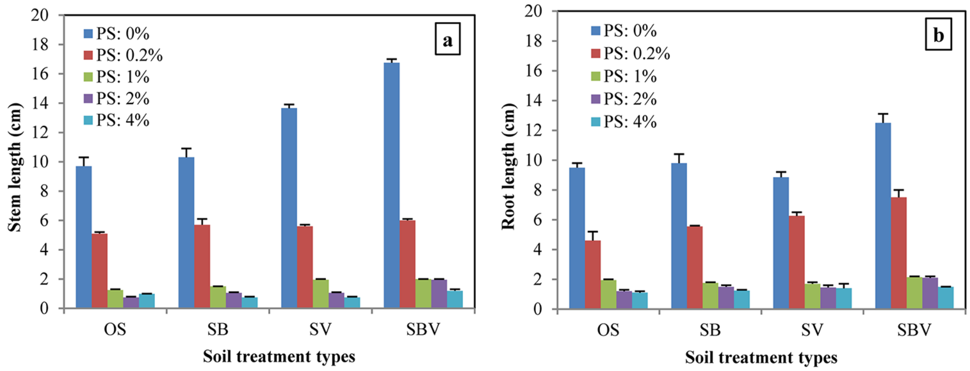

Fig. 4. Effect of petroleum sludge (PS) concentrations in various soil treatments on (**a**) stem length and (**b**) root length of *Salicornia sinus persica.*

12.5 cm, respectively, while the root lengths average were 1.2 cm and 1.5 cm, respectively. As previously noted, petroleum hydrocarbons negatively impact plant growth. Liste and Felgentreu[79] reported a 38.9% reduction in the shoot yield of *rye grown* in contaminated soil by petrol hydrocarbons over a 95-day period, root biomass also showing a 52.6% decrease in their study.

The observed increase in plant growth can be attributed to the additional nutrient supply from vermicompost and the nutrient retention capacity of biochar[80]. Plant roots exhibit strong growth in substrates that contain biochar[75]. By enhancing the physicochemical conditions of the rhizosphere, biochar reduces soil resistance to root growth. Furthermore, it improves soil water permeability, facilitating root penetration and increasing root colonization across larger soil volumes[81]. The increase in stem length is likely attributed to the combined effects of biochar and vermicompost, which enhance mineral availability and promote root growth and nutrient uptake. Similar results were reported by Huang et al.[82] and Hussain et al.[83] who demonstrated that the growth of *tomato* and *basil* in biochar and vermicompost mixtures was higher than the controls.

Content of chlorophyll and carotenoids

The findings of the present study showed that the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in all treatment soil (OS, SB, SV, and SBV) planted by *Salicornia* were lower than in control soils (level of petroleum sludge concentration = 0%) (Fig. 5). For instance, in the SBV treatment by petroleum sludge concentration of 0%, the average levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were 2.24, 0.76, 3.31, and 1.11 mg/g, respectively. However, when petroleum sludge concentration was increased to 4% in SBV treatment, these levels decreased to 1.14, 0.52, 1.82, and 0.78 mg/g, respectively. Baruah et al.[84] highlighted the significant impact of petroleum hydrocarbon contamination on chlorophyll synthesis, which is vital for plant physiological functions and productivity. A decrease in chlorophyll content is the most common effect observed in plants cultivated in petroleum hydrocarbons-contaminated soils. This is due to the interference of high molecular weight aromatic, aliphatic, and organic compounds found in petroleum hydrocarbons, which inhibit the enzymes required for chlorophyll production. Additionally, the alkaline conditions caused by the dissolution of petroleum hydrocarbon compounds in the soil contribute to increased chlorophyll degradation and hinder new leaf formation. Synthesis of carotenoids can also be significantly affected by oil contamination, but research in this area is limited[11].

In the study of Bakina et al. chlorophylls and carotenoids were identified as more sensitive and reliable indicators than oxidative enzymes when assessing the effects of petroleum hydrocarbon pollution. This study demonstrated that *ryegrass* and clover responded differently to petroleum hydrocarbon exposure. Specifically, the levels of photosynthetic pigments in *ryegrass* were significantly reduced, even at low petroleum hydrocarbon concentrations (0.5 L/m^2), and near-complete depletion was observed at higher levels (10 L/m^2). This inhibitory effect affects all plant pigments, including chlorophyll a, chlorophyll b, and carotenoids, reflecting the strong detrimental effect of petroleum hydrocarbon pollution on photosynthetic efficiency[85]. A decrease in chlorophyll content due to increased petroleum hydrocarbon levels in the soil has been reported by Peretiemo-Clarke et al.[86] in the phytochemical effect of petroleum on *Arachis hypogea*. These findings closely correspond with those reported by other researchers, indicating a reduction in the levels of photosynthetic pigments in plants grown in petroleum hydrocarbons-contaminated soils[87–90].

The results of the present study also showed that chlorophyll a and b, total chlorophyll, and carotenoids of the plant increased by using vermicompost and biochar (Fig. 5). For instance, in oil-contaminated soil with a petroleum sludge concentration of 1%, the average chlorophyll levels were 1.31 mg/g for the OS treatment and 1.82 mg/g for the SBV treatment. Likewise, under the same petroleum hydrocarbon pollution conditions and treatments, chlorophyll b content was 0.53 mg/g for the OS treatment and 0.68 mg/g for the SBV treatment. Total chlorophyll levels were 2.03 mg/g for the OS treatment and 2.76 mg/g for the SBV treatment, while carotenoid levels were 0.79 mg/g and 0.97 mg/g, respectively. The use of biochar enhanced chlorophyll a and b levels, primarily due to enhanced absorption of magnesium and nitrogen, which are essential macronutrients for chlorophyll biosynthesis[91]. Similarly, the application of biochar and vermicompost has been found to enhance chlorophyll content, improve leaf photosynthesis, and increase biomass production in various studies[92,93].

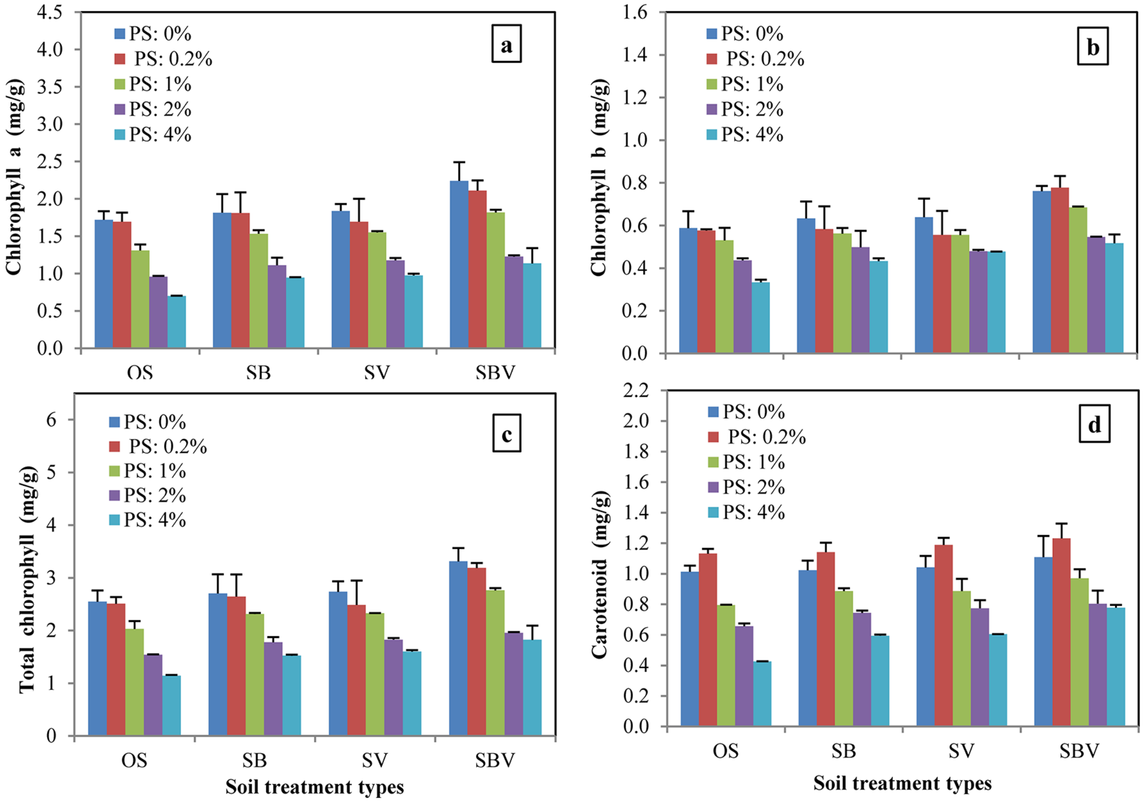

Fig. 5. Effect of petroleum sludge (PS) concentration in various soil treatments on (**a**) Chlorophyll a, (**b**) Chlorophyll b, (**c**) Total Chlorophyll, and (**d**) Carotenoids synthesis in *Salicornia sinus persica.*

Conclusion

This study highlights the effects of petroleum hydrocarbon-contaminated soil on the germination and growth of *Salicornia sinus-persica*. The findings of the present study indicated that petroleum hydrocarbon contamination has significant negative effects on *Salicornia sinus persica* growth parameters such as seed germination, fresh and dry biomass weight, number of lateral stems, stem and root length, chlorophyll a, b, total chlorophyll, carotenoid. However, the addition of biochar and vermicompost as soil amendments improved plant growth under contaminated conditions. The highest germination rate was observed in the SBV treatment. In this treatment, with a petroleum sludge concentration of 0.2%, the germination rate of *Salicornia* was 45%, while in the control group of the same treatment (petroleum sludge concentration = 0%), it was 47%. The experimental results indicated that in all soil treatments (including OS, SB, SV, and SBV) containing 8% petroleum sludge (w/w), the germination rate was greatly reduced, and *Salicornia* plants dried up after one month. At the highest petroleum sludge concentration of 4%, the average fresh biomass in the SBV treatment was 0.04 g (control treatment = 2.81 g), with dry biomass at 0.004 g (control treatment = 0.305 g), root length of 1.5 cm (control treatment = 12.5 cm), and stem length of 1.2 cm (control treatment = 16.8 cm), indicating a significant reduction in plant growth.

Data availability

The data supporting the findings of this study are included in the paper. Any raw data files can be provided by the corresponding author upon reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

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