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UPTAKE AND PHYTOREMEDIATION OF HEXACHLOROCYCLOHEXANE (HCHS) BY SELECTED PLANT SPECIES FROM CONTAMINATED SOIL

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Abstract

Hexachlorocyclohexanes (HCHs) are persistent organic pollutants that remain widespread in soils of Pakistan despite their global ban. This study assessed the phytoremediation potential of two grass species, *Cynodon dactylon* (Bermuda grass) and *Lolium multiflorum* (rye grass), for the removal of HCHs from contaminated soil. Plants were grown for 60 days in soils spiked with HCH concentrations of 5, 15, and 25 mg kg^{-1}. Soil and plant samples were analyzed using GC-MS. Microbial biomass carbon increased in all treatments, indicating enhanced microbial activity in planted soils. Both species significantly reduced soil HCH concentrations, with *C. dactylon* showing slightly higher removal efficiency at elevated contamination levels. The results demonstrate that both grasses are capable of dissipating and accumulating HCHs from soil. Their rapid growth and low maintenance requirements highlight their suitability for short-term, sustainable phytoremediation of HCH-contaminated soils.

Key words: Phytoremediation; Hexachlorocyclohexane (HCHs); Plant uptake; Pesticide contamination; Soil remediation

Introduction

Environmental contamination is the great destroyer of quality of soil and water, as the urbanization and industrialization are growing so fast (Mukherjee & Bairwa, 2025). Pesticide pollution is not just caused by agricultural activities, the other significant contributors to this pollution are the manufacturing, handling, improper storage, and disposal processes of pesticides and its associated wastes (Kaur *et al.*, 2024). Specifically, organochlorine pesticides (OCPs) have become the universal pollutants of the planet since, with the signing of the Stockholm Convention, most of the OCP production and formulation plants, which are situated in close proximity to cities, have been abandoned without decommissioning (Keswani *et al.*, 2022).

The organochlorinated pesticides are now considered as one of the serious environmental issues (Mrema *et al.*, 2013). Their toxicity and persistence are significant sources of threat to human health, wild animals, and ecosystems resulting in carcinogenesis, immunologic disorders, and reproductive issues (Chauhan *et al.*, 2026). The most extensively investigated OCPs, hexachlorocyclohexanes (HCHs), have also been detected in all the environmental structures including remote areas like the Arctic (Baek *et al.*, 2011). Lindane (γ-HCH), the most toxic isomer, has been extensively used to control agricultural pests. HCHs are harmful not only to humans and animals but also extremely toxic to insects and soil microorganisms (Nayyar *et al.,* 2014). Among the eight known HCH isomers, α-, β-, γ- and δ-HCH are environmentally most significant (Li *et al.*, 2011).

Exposure to HCHs can impair plant germination, primary growth (Pereira *et al.*, 2010), photosynthesis, biomass allocation, and overall physiological functioning (Košková *et al.*, 2022). Due to their toxicity, environmental persistence, and global distribution, HCHs were added to the list of POPs in 2009 (Vijgen *et al.*, 2011). The overgrowth of the agricultural sector has led to irreversible damage to the environment and natural resources due to exposure to chemical contaminants particularly persistent toxic substances (Anjaria & Vaghela, 2024).

In developing South Asian countries, improper management of urban and agricultural wastes contributes substantially to environmental contamination (Islam *et al.*, 2021). Without advanced waste-treatment facilities, solid wastes are often dumped openly (Khan *et al.*, 2012). While residues from obsolete OCP factories and stockpiles have further intensified regional contamination (Kurt-Karakus *et al.*, 2024). The application of prohibited HCH formulations and the lack of the systematic approach to the management of outdated stocks have worsened the

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situation in Pakistan. The pesticide contamination also interferes with the dynamics of microbial activities and enzymes in the soil, posing a negative impact on the productivity of the soil (Devi *et al.*, 2018).

Soil pollutants that persistently affect soil such as Persistent organic pollutants (POPs) have a severe effect on soil microbiota and groundwater concentration affecting the survival and health of plants and soil-dwelling organisms (Ullah *et al.,* 2025). New pollutants, especially POPs, affect basic soil parameters such as pH, thermal stability, and chemical constructions causing a harmful effect on soil fauna (Song *et al.,* 2025). Interestingly, the soil microbial communities have demonstrated the adaptivity to a high level of POPs and this implies their emerging use as significant players in the natural attenuating and bioremediation plans (Alidoosti *et al.*, 2024).

Phytoremediation which refers to the utilization of green plants, the related micro-organisms and modification of soil to eliminate, degrade or immobilize toxic substances is seen as an environmentally friendly and economical remediation method. It is possible to apply biodegradation to contaminated aquatic environments and soils (Nair *et al.,* 2024). Alternative methods of pollution control such as phytoremediation and biosynthesis are innovative bioremediation methods that are environmentally friendly (Lai Huat *et al.,* 2024). Phytoremediation is a process that uses plants to extract, stabilize, or degrade the contaminants by involving the processes of phytoextraction, Phyto stabilization, phytovolatilization and phytodegradation (Chatterjee *et al.*, 2013).

Compared with shrubs and trees commonly used in phytoremediation studies, grass species offer several practical and ecological advantages (Gołda & Korzeniowska, 2016). Grasses typically exhibit rapid germination and growth rates, high aboveground biomass turnover, and extensive fibrous root systems that enhance soil-root contact and contaminant interaction (Rabêlo *et al.*, 2021). Their shallow but dense root networks improve rhizosphere microbial activity, which is critical for the degradation of persistent organic pollutants such as HCHs (Azaizeh *et al.*, 2010). In addition, grasses are easier to establish in contaminated or degraded soils, require shorter growth cycles, and allow repeated harvesting, making them more suitable for large-scale and cost-effective remediation strategies compared to woody plant systems.

Phytoremediation provides a sustainable solution of HCH-contaminated soils by plant absorption, internal metabolism as well as plant microbe interaction (Balazs *et al.*, 2018). Field research indicated that HCHs are capable of being accumulated and transformed in plant tissues, which is supported by carbon and chlorine isotope fractionation, which can affect the accuracy of Phyto screening (Liu *et al.*, 2023). The experimental studies also reveal the isomer-specific uptake, preferential root accumulation, and increased bioaccumulation of δ-HCH (Amirbekov *et al.*, 2024). Furthermore, the increased rhizosphere microbial activity is also a major contributor of β-HCH attenuation (Kaur *et al.*, 2021). Recent literature has underscored that sustained pollutants like HCH should be remedied in an integrated and sustainable manner, and thus phytoremediation should be employed to manage the soil over a long period (Roy *et al.*, 2023). Plants can absorb the contaminants by use of their roots, be able to translocate to shoots and can even be able to convert to less toxic forms (Islam *et al.*, 2024). A number of plant species have demonstrated good outcomes in OCP remediation. As an example, Jatropha was able to remove more than 89 percent of lindane in a glasshouse environment (Kumar *et al.*, 2025), and a sunflower (*Helianthus*) plant exhibited a high phytoextraction capacity of OCPs because of its massive biomass and high uptake (Solhi & Hajabbasi, 2005).

Despite extensive research on phytoremediation of organochlorine pesticides, studies specifically focusing on grass species for hexachlorocyclohexane (HCH) remediation remain limited. Most previous work has emphasized woody plants, hyperaccumulators, or general plant screening for OCP removal, while systematic evaluation of fast-growing grass species under controlled HCH concentration gradients is still scarce. In particular, comparative studies involving *Cynodon dactylon* and *Lolium multiflorum* for HCH uptake, dissipation, and associated effects on soil microbial biomass carbon are rarely reported.

Therefore, the present study is designed to evaluate the phytoremediation potential of two fast-growing grass species, *Cynodon dactylon* and *Lolium multiflorum*, under controlled HCH contamination levels (5, 15, and 25 mg kg^{-1}). This research specifically investigates HCH dissipation in soil, plant growth responses, and associated changes in soil microbial biomass carbon. By integrating plant uptake and soil biochemical responses, this study provides new insights into the effectiveness of grass-based systems for remediation of HCH-contaminated soils under varying pollution loads.

Material and Methods

Study site: The National Agricultural Research Centre (NARC) located in Islamabad; Pakistan was the center where the study was done. It is a subtropical place with alluvial soil at an elevation of 540 meters above sea level, 33.72148 C N and 73.04329 C E. The region has a rainfall of 1,224 mm annually and the temperatures of the area vary between 10°C and 38°C. The NARC growth chambers offer controlled conditions in which experiments on plants can be carried out.

Experimental design: The experiment was conducted to evaluate HCH degradation in soil and to assess the phytoremediation potential of two grass species, *Cynodon dactylon* and *Lolium multiflorum*. Uncontaminated soil was collected from the National Agricultural Research Centre (NARC), air-dried, ground, and sieved through a 2 mm mesh sieve. The soil was artificially spiked with HCH at concentrations of 5, 15, and 25 mg kg^{-1}, while un-spiked soil served as the control treatment. The spiked soils were placed in polythene bags and aged for 15 days to stabilize HCH within the soil matrix. A completely randomized design (CRD) was employed with two experimental factors: plant species and HCH concentration. The plant factor consisted of three levels: unplanted control, *Cynodon dactylon*, and *Lolium multiflorum*. The HCH factor consisted of four concentration levels: 0, 5, 15, and 25 mg kg^{-1}. Each treatment combination was replicated three times, resulting in a total of 36 experimental pots (3 plant treatments × 4 HCH concentrations × 3 replicates). After the aging period, soils were transferred into pots and planted with the selected grass species, while control pots remained unplanted. The experiment was conducted in a controlled growth chamber for 60 days. Analytical results were expressed as mean ± standard deviation (SD) of three replicates.

Sample collection: HCH was purchased, and two plant species with potential for degradation of POPs were selected. Uncontaminated soil samples were collected from field of National Agricultural Research Centre (NARC). The samples were air dried, ground and passed through a 2-mm sieve. The soil samples were spiked with 5, 15 and 25 mg kg^{-1} HCHs. After spiking, the samples were stored in polythene bags and kept for aging for 15 days (Balazs *et al.*, 2018). All the treatments were replicated thrice (Fig. 1).

Physicochemical analysis of soil: Physicochemical analysis of soil samples was carried out before and after the spiking. The pH of the soil was measured by using multi meter of model MM 40 + (APHA, 2005). And electrical conductivity was measured by using Multi-meter (MM 40 + model). Total organic carbon analysis was carried out using the titration method of Walkley (1947). One gram of soil was used to transfer to a conical flask and 10 ml of Normal solution of potassium dichromate with 20 ml concentrated Sulphuric acid was added. The solution was shaken and it was allowed to stand for 30 minutes. 200 ml distilled water and 10 ml orthophosphoric acid was added and then the solution allowed to cool. 10-15 drops of Diphenylamine indicator were added and then the solution was titrated against freshly prepared 0.5M ferrous ammonium sulphate solution, until the color changes from violet blue to green.

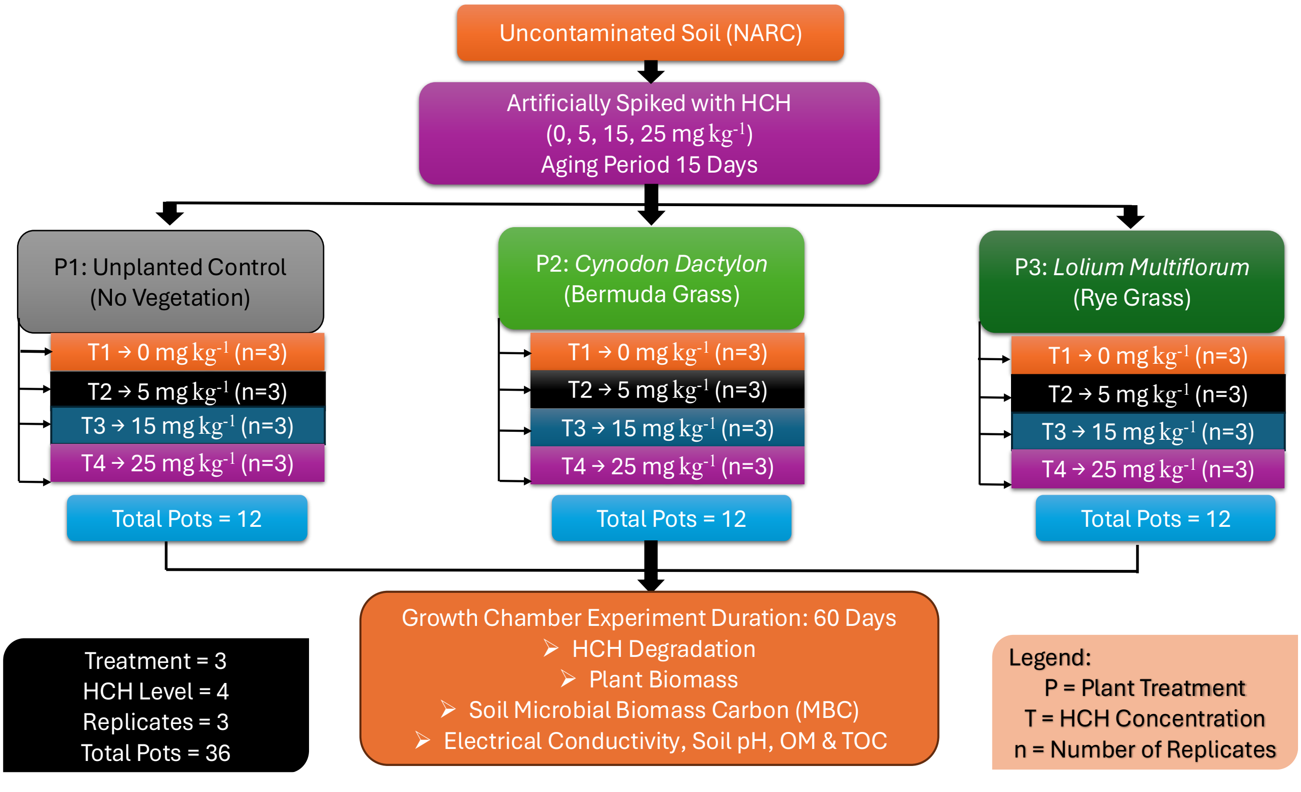

Fig. 1. Schematic representation of the experimental design used to evaluate phytoremediation of HCH-contaminated soil using *Cynodon dactylon* and *Lolium multiflorum* under different HCH concentrations.

$$\% \text{ OOC} = \frac{[\text{Volume of blank} - \text{Volume of sample}] \times 0.3 \times M}{\text{Weight of sample}}$$

$$\% \text{ TOC} = 1.334 \times \% \text{ Oxidizable organic carbon}$$

Similarly, soil organic matter was analyzed by titration method Mohr (1979). Soil Organic Matter is calculted using the following formula;

$$\% \text{ OM} = 1.724 \times \text{Total organic carbon}$$

Microbial biomass carbon was calculated by improved method of rapid microwave [mw] irradiation and extraction method (Tate *et al.*, 1988).

$$\text{MBC} = (\text{MWC}_{ext} - \text{C}_{ext}) \times 2.64$$

Plant growth parameters: After harvesting, the following growth parameters were observed

i. Root and shoot length were measured by means of common meter rod in centimeters (cm).
ii. Electrical balance was used to measure fresh and dry weight in grams (g).

HCHs analysis

HCHs analysis in soil: Soil extraction was done by transferring 5g soil samples in to 100 ml Teflon tubes then mixed with 10 ml of Di chloromethane and then each sample was extracted for 20 minutes in a sonication, then samples were centrifuged at 4000 rpm for 5 minutes and the supernatants were collected. The extract was eluted with 1 and 2ml mix of n-hexane: dichloromethane (v/v

50:50) and the supernatant was extracted for HCHs and dried by sparging with N_2, Solid residues were re dissolved in 1 ml of acetonitrile and run on the GC-MS with ECD detector (Rashid *et al.*, 2010).

HCHs analysis in plant: The plant material was extracted in water bath for 20 min in ethyl acetate. The extracts were passed through silica gel packed column. Elution was concentrated under reduced pressure, dried under nitrogen stream, and then eluent was re-dissolved in 1ml of acetonitrile for analysis by GC- MS (Uduman *et al.*, 2017)

For the analysis of hexachlorocyclohexane samples were run on Gas chromatography, mass spectrometry combined with Electron capture detector GC-MS-ECD (Liu *et al.*, 2015).

Statistical analysis

Statistical analyses were performed using SPSS version 18.0. Exploratory data analysis and multivariate analytical techniques were applied to examine the overall structure and distribution of the dataset. A two-way analysis of variance (ANOVA) was used to evaluate the effects of plant species (*Cynodon dactylon* and *Lolium multiflorum*) and HCH concentration (0, 5, 15, and 25 mg/kg) on soil HCH removal, plant biomass, and soil microbial biomass carbon (MBC). Where significant interactions or main effects were detected, Tukey's HSD post-hoc test was applied to compare treatment means. All statistical tests were performed at a significance level of $p<0.05$.

Result

Physicochemical analysis of soil: Difference was observed in soil parameters before and after the spiking of soil with HCHs. Variations were observed in all the physicochemical properties of the soil at the start and the end of the two months experiment.

Figure 2 represents the variation of soil pH among different treatments. The pH of the soil spiked for two weeks was 7.68, 7.47, 7.43 and 7.30 respectively in all the four treatments (T1, T2, T3, and T4). After planting Rye grass for 60 days pH shows a decreasing trend and reached to 6.77, 7.37, 6.87 and 7.17 among the four treatments. Similarly, the pH of the soil in which Bermuda grass was planted also showed a decreasing trend from 7.68, 7.47, 7.43 and 7.30 to 6.87, 7.17, 7.37 and 7.10 in each of the respective treatment.

Similarly, figure 3 shows the variation in electrical conductivity (EC) of soil among different treatments (T1, T2 T3, and T4) after spiking and then after plantation in the soil. The two types of grasses were planted for 2 months after the spiking of soil with different concentration of HCHs. EC of the soil with different treatments was 250.1 $\mu S\ cm^{-1}$, 249.5 $\mu S\ cm^{-1}$, 249.3 $\mu S\ cm^{-1}$ and 249.1 $\mu S\ cm^{-1}$ respectively. After 60 days of experiment, the soil in which Rye grass was planted showed a significant decrease in EC. The lowest values of EC were 179.0 $\mu S\ cm^{-1}$ and 158.4 $\mu S\ cm^{-1}$shown by T3 and T4, while T1 and T2 showed comparatively higher values but lower than the initial values i.e. 240.5 $\mu S\ cm^{-1}$ and 202.4 $\mu S\ cm^{-1}$. Figure shows the variation in EC among different treatments in which Bermuda grass was planted. The EC of the soil planted by Bermuda grass showed similar variation as of the Rye grass soil. The EC values decreased and finally after 2 months the lowest values were 185.1$\mu S\ cm^{-1}$ and 165.1 $\mu S\ cm^{-1}$, shown by T3 and T4. While maximum values were observed in T1 and T2 (231.2 $\mu S\ cm^{-1}$ and 212.1 $\mu S\ cm^{-1}$). The EC of T1 decreased only slightly because this treatment did not contain HCH.

Soil organic matter among different treatments in soil samples is shown in figure 4. Initially the organic matter in the spiked soil was T1: 1.15%, T2: 1.16%, T3: 1.28% and T4: 0.99%. The spiked soil was then planted with Rye grass and Bermuda grass for 2 months, but it shows a decreasing trend after 2 months of experiment. Figure shows the variations in organic matter of the soil treatments in which Rye grass was planted (0.50%, 0.75%, 0.44% and 0.62%). The organic matter of the soil treatments in which Bermuda grass was planted reached to 0.56%, 0.51%, 0.60% and 0.57%.

Figure 5 represents the total organic carbon concentration among different treatments after spiking in comparison with treatments after pot experiment. Initially the concentration of total organic carbon was 0.67%, 0.67%, 0.74% and 0.57% in the respective treatments. After 2 months of pot experiment, a significant decrease in the TOC content was observed among all the treatments. The variations in the TOC in the soil treatments which are planted with Rye grass for 2 months. A significant decrease in the TOC was observed (0.29%, 0.43%, 0.27% and 0.36%) after two months of pot experiment as compared to the initial concentration. Similarly, the soil in which Bermuda grass was planted also showed a decreasing trend in TOC concentration among all the treatments; 0.33%, 0.30%, 0.35% and 0.33%.

Similarly in figure 6, microbial biomass carbon showed significant variations from the initial values to the final ones. Initially, the MBC of soil spiked for two weeks was T1: 0.85%, T2: 0.83%, T3:0.47%, T4: 0.83%. But after 2 months of pot experiment, the MBC showed an increasing trend in all the treatments and reached to 0.92%, 0.88%, 1.37% and 1.07% in the soil samples which were planted with Rye grass. Similarly, the soil planted with Bermuda grass also showed an increasing trend of MBC from the initial values and reached to 0.88%, 0.88%, 1.73% and 1.95% respectively. Moreover, the treatments T3 and T4 from both the plants showed a relatively higher MBC level.

Plant growth parameters: Initially the root length of Rye grass was 2cm and Bermuda grass had root length of 5cm. With the passage of time experiment progressed, the plants started to grow and biomass also increased in all the treatments. The difference was clear between the plants grown in HCH spiked soil and the control soil. The plants grown in the uncontaminated soil showed faster growth than those grown in the contaminated soil. Moreover, root length showed a slight difference between the planted grown in different treatments. The root length and difference of growth among the treatments of Rye grass. The root length of Rye grass reached from 2cm to 4.00 cm, 3.33 cm, 3.50cm and 3.50cm in the respective treatments. Initially the root length of Bermuda grass was 5cm and reached up to 10.10cm, 9.33cm, 7.67cm and 7.83cm, as shown in Fig. 7.

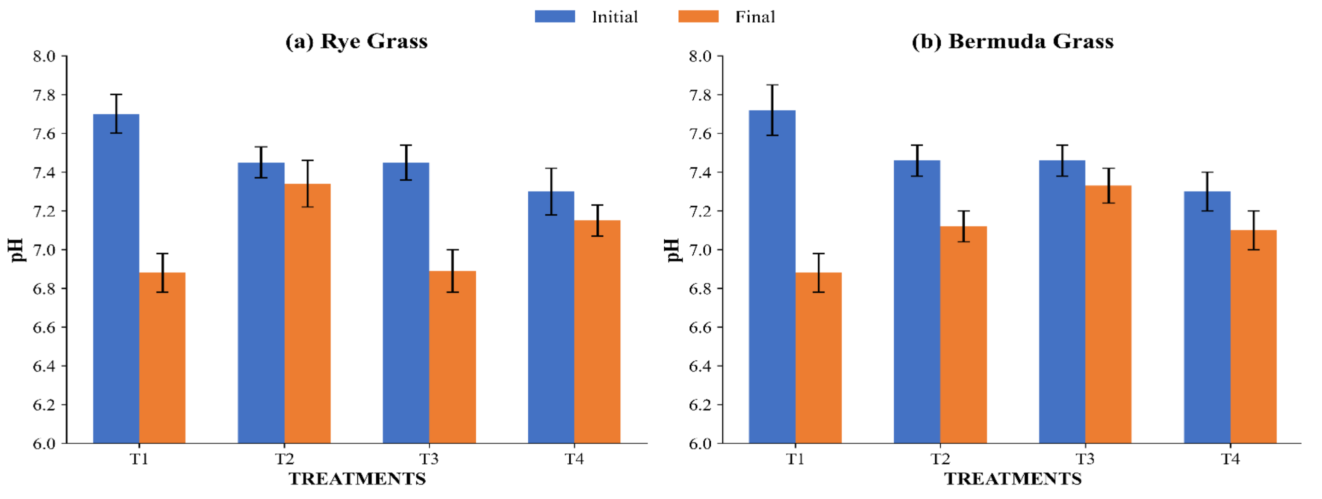

Fig. 2. Variation in soil pH under different HCH contamination levels after two weeks of spiking and two months of plant growth. (a) Rye grass (P2), (b) Bermuda grass (P3). Treatments represent HCH contamination levels as follows: T1 = uncontaminated control soil (0 mg kg^{-1} HCH), T2 = soil spiked with 5 mg kg^{-1} HCH, T3 = soil spiked with 15 mg kg^{-1} HCH, and T4 = soil spiked with 25 mg kg^{-1} HCH. Data are presented as mean ± standard deviation (SD) (n = 3). Error bars indicate SD among three independent replicates.

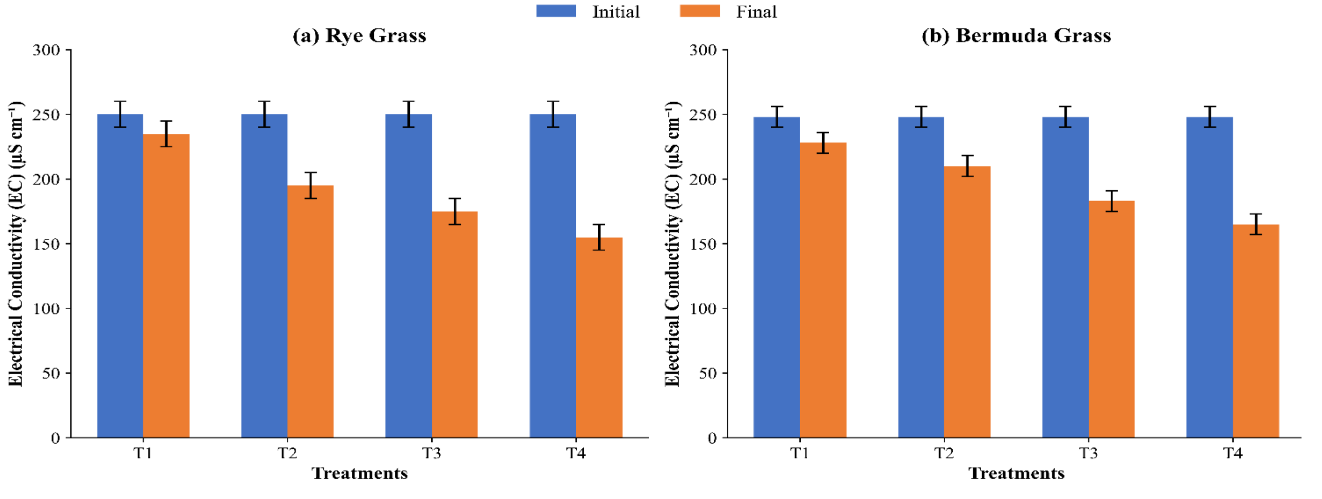

Fig. 3. Variation in soil electrical conductivity (EC) under different HCH contamination levels after two weeks of spiking and two months of plant growth. (a) Rye grass (P2), (b) Bermuda grass (P3). Treatments represent HCH contamination levels as follows: T1 = uncontaminated control soil (0 mg kg^{-1} HCH), T2 = soil spiked with 5 mg kg^{-1} HCH, T3 = soil spiked with 15 mg kg^{-1} HCH, and T4 = soil spiked with 25 mg kg^{-1} HCH. Data are presented as mean ± standard deviation (SD) (n = 3). Error bars indicate SD among three independent replicates.

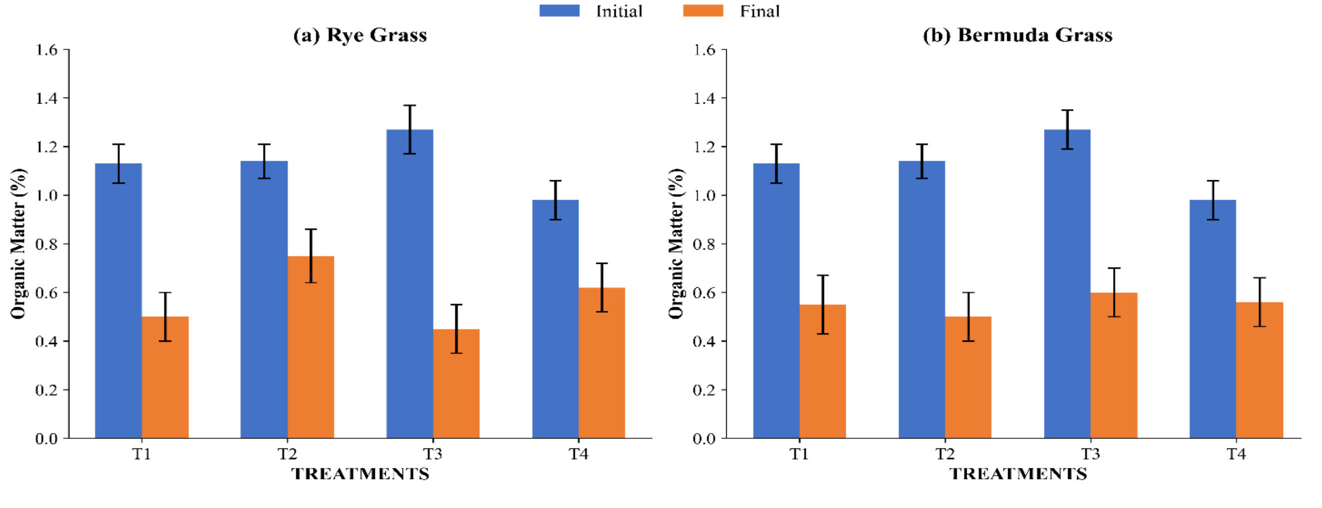

Fig. 4. Variation in soil organic matter content under different HCH contamination levels after two months of plant growth. (a) Rye grass (P2), (b) Bermuda grass (P3). Treatments represent HCH contamination levels as follows: T1 = uncontaminated control soil (0 mg kg^{-1} HCH), T2 = soil spiked with 5 mg kg^{-1} HCH, T3 = soil spiked with 15 mg kg^{-1} HCH, and T4 = soil spiked with 25 mg kg^{-1} HCH. Data are presented as mean ± standard deviation (SD) (n = 3). Error bars indicate SD among three independent replicates.

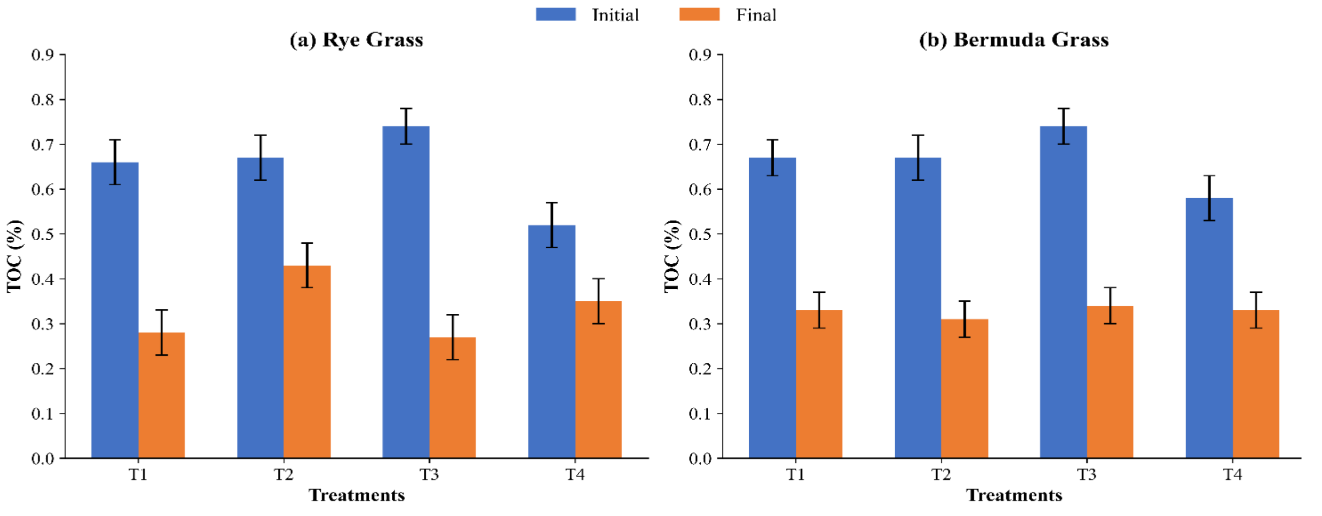

Fig. 5. Variation in total organic carbon content under different HCH contamination levels after two months of plant growth. (a) Rye grass (P2), (b) Bermuda grass (P3). Treatments represent HCH contamination levels as follows: T1 = uncontaminated control soil (0 mg kg^{-1} HCH), T2 = soil spiked with 5 mg kg^{-1} HCH, T3 = soil spiked with 15 mg kg^{-1} HCH, and T4 = soil spiked with 25 mg kg^{-1} HCH. Data are presented as mean ± standard deviation (SD) (n = 3). Error bars indicate SD among three independent replicates.

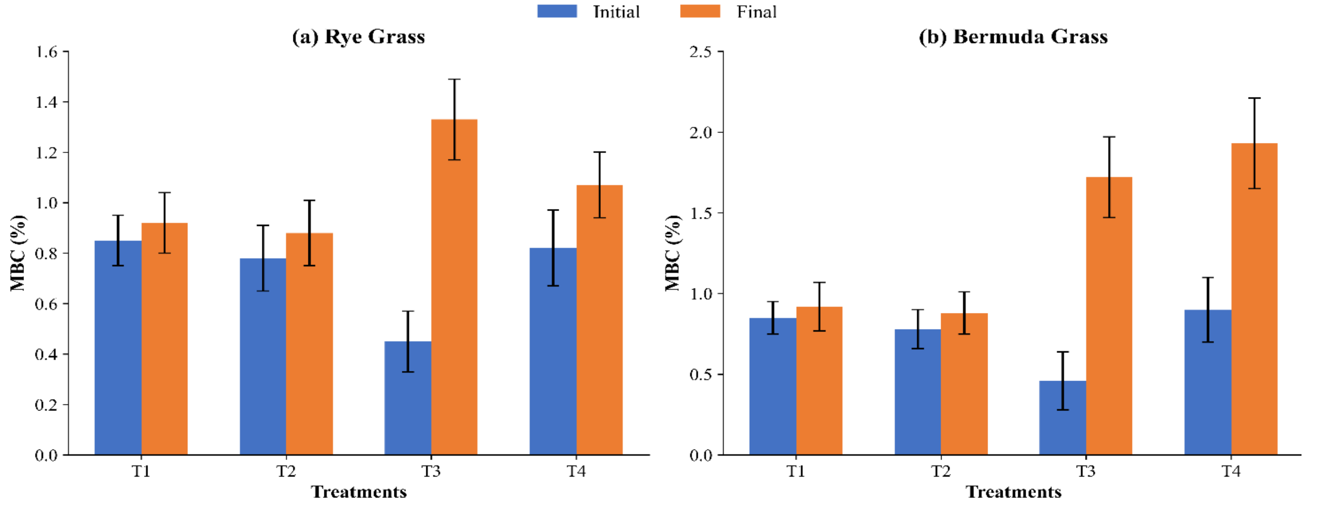

Fig. 6. Microbial biomass carbon content in soil under different HCH treatments after two months of plant growth. (a) Rye grass (P2) and (b) Bermuda grass (P3). T1 represents uncontaminated control soil (0 mg kg^{-1} HCH), whereas T2, T3, and T4 represent soils treated with 5, 15, and 25 mg kg^{-1} HCH, respectively. Values are expressed as mean ± SD (n = 3), and error bars show standard deviation among the replicates.

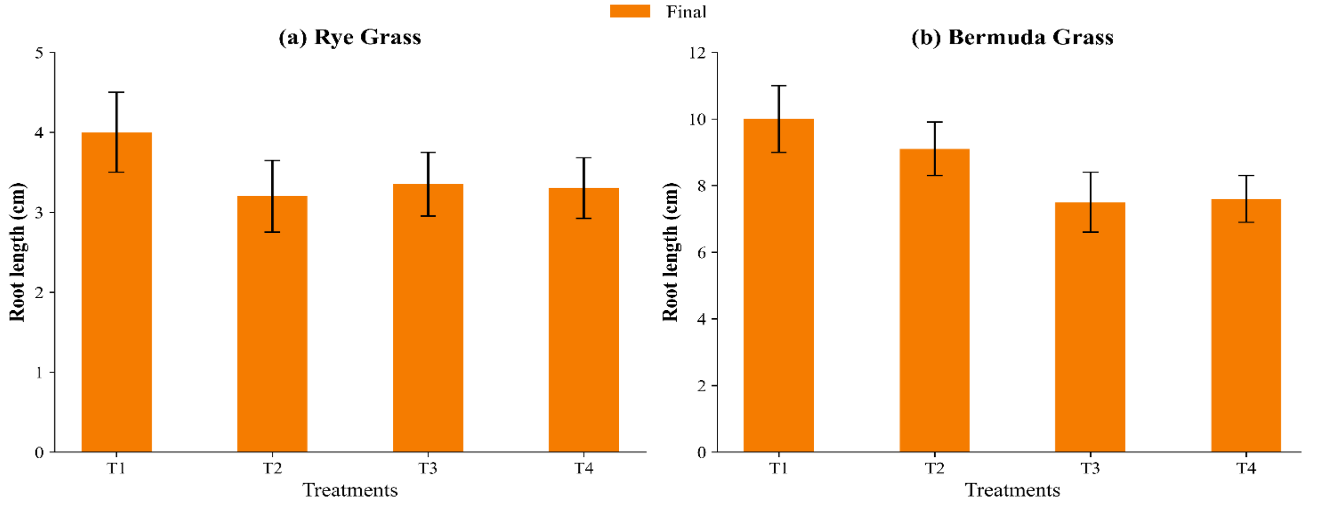

Fig. 7. Root length of plants grown in HCH-contaminated soil after two months of growth. (a) Rye grass (P2) and (b) Bermuda grass (P3). T1 represents uncontaminated control soil (0 mg kg^{-1} HCH), whereas T2, T3, and T4 represent soils treated with 5, 15, and 25 mg kg^{-1} HCH, respectively. Values are expressed as mean ± SD (n = 3), and error bars show standard deviation among the replicates.

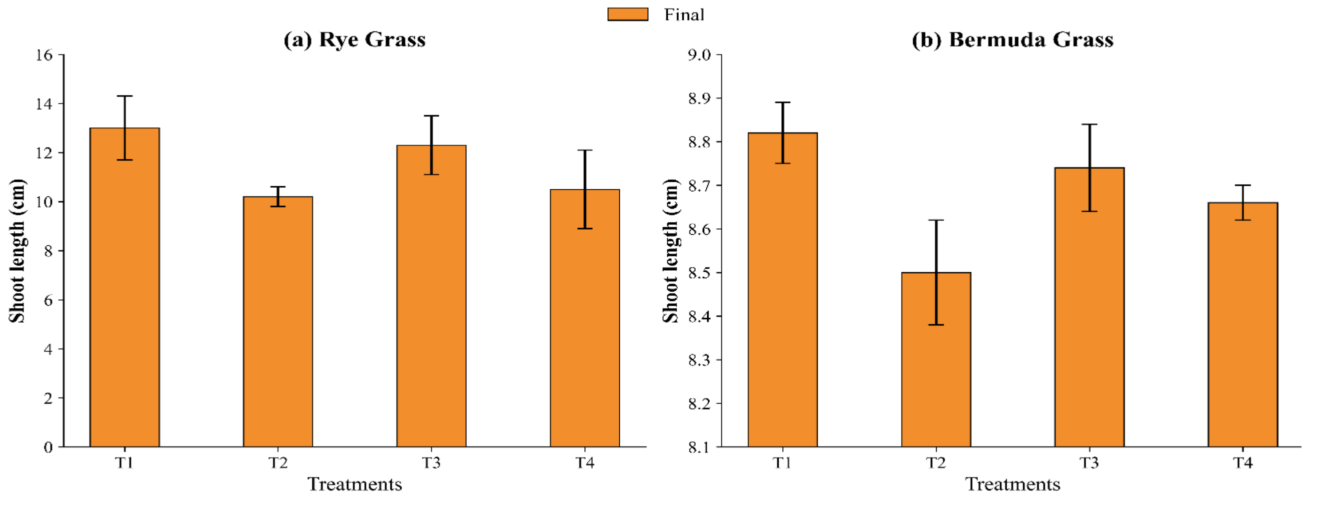

Fig. 8. Shoot length of plants grown in HCH-contaminated soil after two months of growth. (a) Rye grass (P2) and (b) Bermuda grass (P3). T1 represents uncontaminated control soil (0 mg kg^{-1} HCH), whereas T2, T3, and T4 represent soils treated with 5, 15, and 25 mg kg^{-1} HCH, respectively. Values are expressed as mean ± SD (n = 3), and error bars show standard deviation among the replicates.

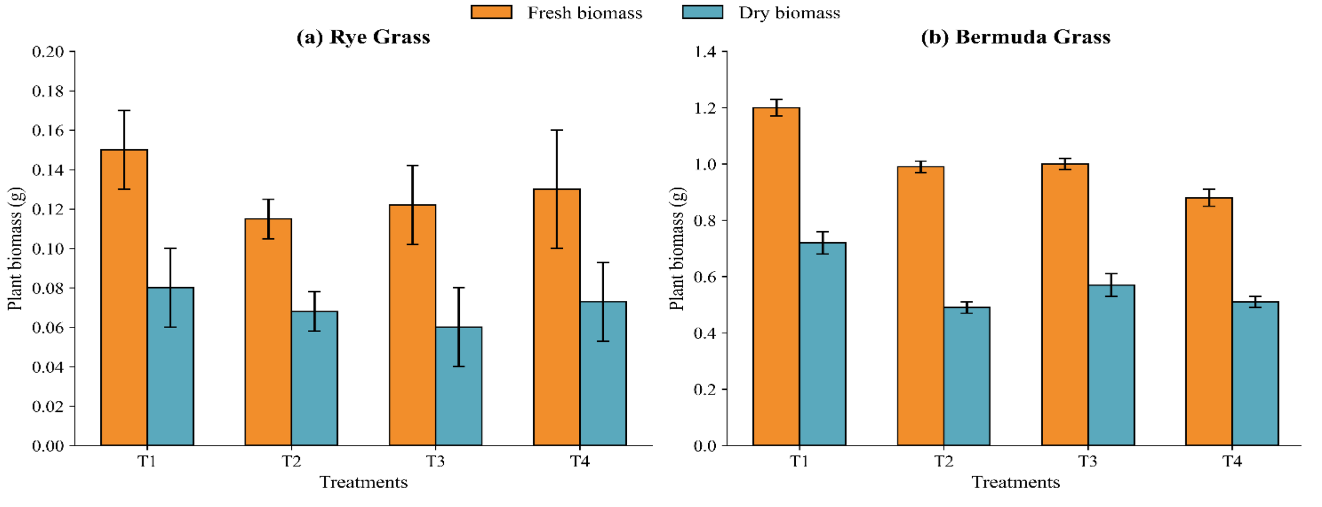

Fig. 9. Plant biomass (fresh and dry weight) of plants grown in HCH-contaminated soil after two months of growth. (a) Rye grass (P2) and (b) Bermuda grass (P3). T1 represents uncontaminated control soil (0 mg kg^{-1} HCH), whereas T2, T3, and T4 represent soils treated with 5, 15, and 25 mg kg^{-1} HCH, respectively. Values are expressed as mean ± SD (n = 3), and error bars indicate standard deviation among the replicates.

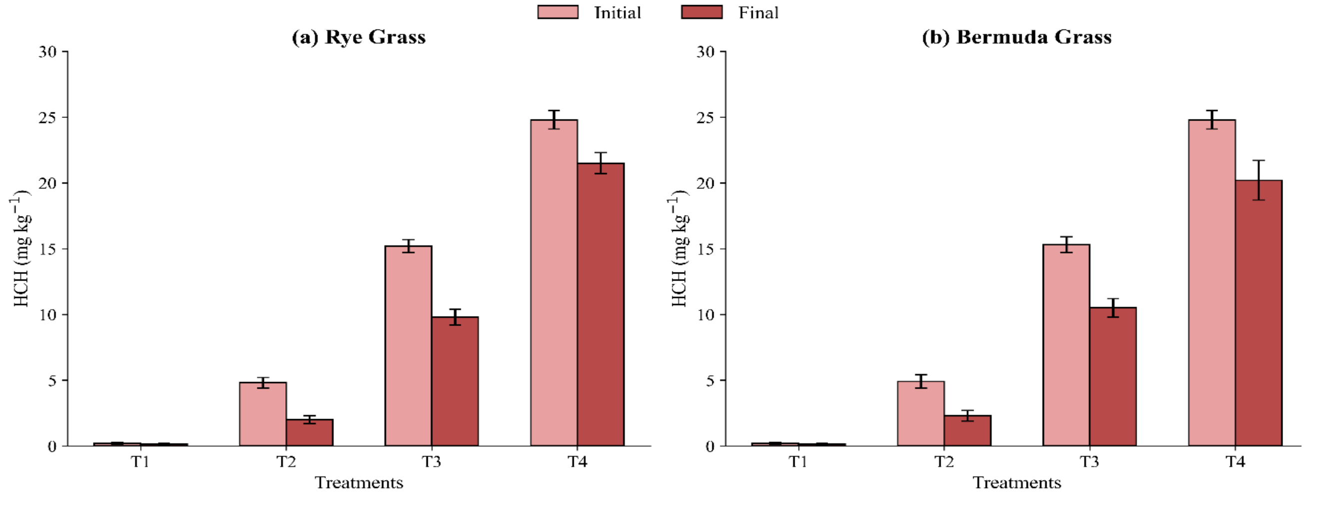

Fig. 10. Residual HCH concentration in soil under different treatments in the presence of (a) Rye grass and (b) Bermuda grass after two months of plant growth. T1 represents uncontaminated control soil (0 mg kg^{-1} HCH), whereas T2, T3, and T4 represent soils treated with 5, 15, and 25 mg kg^{-1} HCH, respectively. Values are expressed as mean ± SD (n = 3), and error bars indicate standard deviation among the replicates.

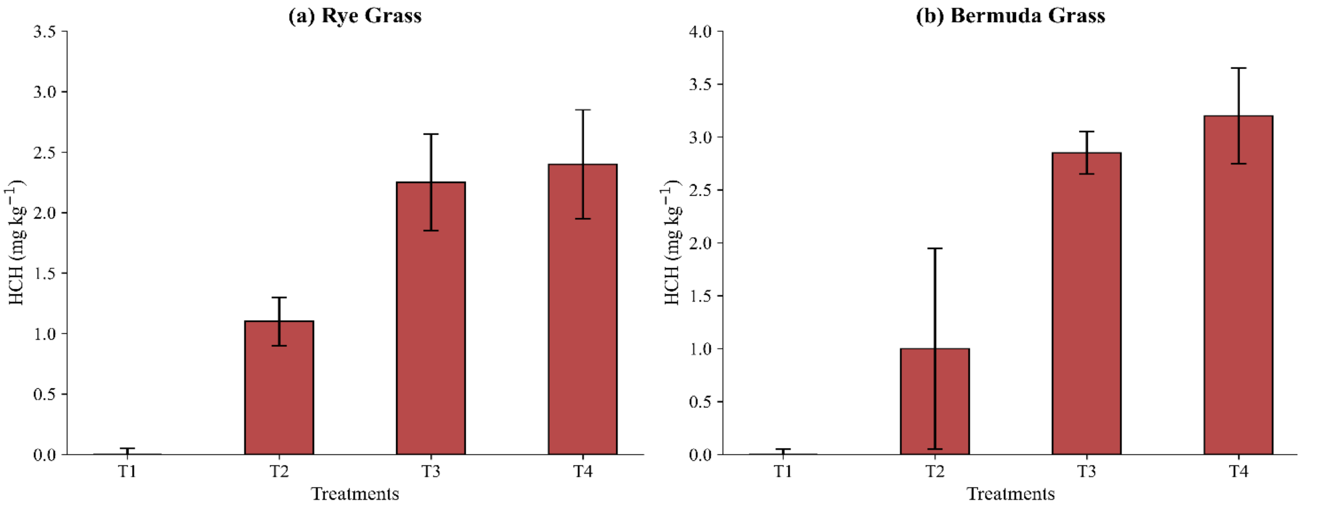

Fig. 11. Concentration of HCHs in plant tissues under different soil treatments. (a) Rye grass (P2) and (b) Bermuda grass (P3). T1 represents uncontaminated control soil (0 mg kg^{-1} HCH), whereas T2, T3, and T4 represent soils treated with 5, 15, and 25 mg kg^{-1} HCH, respectively. Values are expressed as mean ± SD (n = 3), and error bars indicate standard deviation among the replicates.

At the start of the experiment the plants had shoot length of Rye grass was 8 cm and of Bermuda grass was 6 cm. As the experiment progressed, the shoot height increased in all the treatments but the one planted in the control soil has the highest shoot length as compared to the others. Rye grass showed an increase in shoot height from 8cm to 13.11cm, 10.33cm, 12.33cm and 10.50cm in the respective treatments. On the other side, shoot height of Bermuda grass increased from 6cm to 8.81cm, 8.50cm, 8.74cm and 8.67cm, as shown in Fig. 8.

Plant biomass, is the sum total of root and shoot dry weight per pot. The plant biomass varied in different treatments as well of the two plants. It is clear that the total biomass of Rye grass is more in the control treatment (T1: uncontaminated) as compared to the other treatments (contaminated). On the other side, the results are similar and it is evident that the total biomass of Bermuda grass is highest in the control treatment and lowest in the treatment T4 which is spiked with 25 mg kg^{-1} HCH. After oven drying the plants, the plant biomass decreased accordingly, as shown in Fig. 9.

HCHs concentration in soil: Figure 10 shows HCHs concentration in soil after spiking in comparison with HCHs concentration after 60 days of experiment. The difference is very obvious in initial and final HCHs concentration in soil in all the treatments. The concentration of HCHs has decreased significantly in all the treatments. While there is a slight difference in the two sets of samples due to presence of different plants. Figure shows the reduction of HCHs in soil in the presence of Rye grass. Initially, the HCHs concentrations were T1: control, T2: 5 mg kg^{-1}, T3: 15 mg kg^{-1}, and T4: 25 mg kg^{-1}, which finally decreased to 0.00 mg kg^{-1}, 2.01 mg kg^{-1}, 9.97 mg kg^{-1}, and 21.36 mg kg^{-1}, respectively.

Figure shows the reduction of HCHs in soil in the presence of Bermuda grass. The final HCHs concentrations decreased to below detection limit (BDL) in T1, and to 2.56 mg kg^{-1}, 10.38 mg kg^{-1}, and 19.85 mg kg^{-1} in T2, T3, and T4, respectively. The small negative value originally obtained for T1 (-0.03 mg kg^{-1}) was attributed to instrumental baseline correction and analytical noise during GC-MS quantification and therefore was interpreted as effectively zero concentration.

HCHs concentration in plants: Figure 11 shows uptake of HCHs by plants grown in HCHs spiked soil for 60 days. The decrease of HCHs concentration in soil is due to the presence of Rye grass and Bermuda grass. The uptake of HCHs from soil by rye grass is 0.00 mg kg^{-1}, 1.13 mg kg^{-1}, 2.26 mg kg^{-1} and 2.42 mg kg^{-1}, while uptake of HCHs by Bermuda grass is shown in figure, which is 0.00 mg kg^{-1}, 0.98 mg kg^{-1}, 2.89 mg kg^{-1} and 3.15 mg kg^{-1}. There is a slight difference between the uptake of HCHs by Rye grass and Bermuda grass in different treatments. HCHs uptake by Rye grass is more than the other in T2 while, in T3 and T4 HCHs uptake is more by Bermuda grass.

Discussion

Physicochemical parameters: Variations were observed in all the physicochemical properties of the soil at the start and the end of the two months experiment. The pH of the soil after pot experiment has decreased as compared to the spiked soil. This decrease is may be attributed to degradation of hydrocarbons by microorganisms and enzymatic activities of plants (Tang *et al.*, 2009).

Overall electrical conductivity showed a decreasing trend through the course of experiment. The reason behind this decrease is organic matter mineralization. During organic matter mineralization process ions releases and increase the electrical conductivity (Cunha-Santino & Bianchini-Junior, 2004). But, in the present study the plants might have taken up the salts or ions from the soil after mineralization, resulting in a decrease in electrical conductivity of the soil.

But organic matter did not show much difference (from control) after spiking with HCH for two weeks, but it showed a decreasing trend after 60 days of pot experiment. Research studies reported that the mineralization rate of organic matter in soils spiked with pesticides is usually higher compared to the control. This showed that growth and activities of heterotrophic microorganisms are stimulated by biodegradation of pesticides which caused the mineralization of organic matter and biologically transform other plant nutrients in soil, resulted in lowering the concentration of organic matter in soil (Becerra-Castro *et al.*, 2013; Phillips *et al.*, 2005).

In previous studies it was reported that pesticides have a stimulatory effect on mineralization process (Hathout *et al.*, 2022). Studied the effect of metalaxyl (fungicide) which showed a significant decrease in total organic carbon content in soil during 0-30 days of incubation. Similarly, (Kumar *et al.*, 2012) also reported that the mineralization rate of organic C in soils treated with different pesticides (BHC, representing the organochlorine group of insecticide) was higher compared to the control resulted in decrease of organic C in soil.

Similarly, during the course of experiment microbial biomass carbon tend to increase after the two months of experiment. The reason behind this increase is certainly the pesticide (HCH). Pesticides make an interaction with microorganisms of soil and affect their metabolic activities (Arora *et al.*, 2019). Sometimes these pesticides undergo to change the physical, chemical and biological behaviour of soil microorganisms. Microbial biomass carbon is an essential indicator of microbial activities (Ataikiru *et al.*, 2019). Various recent studies revealed that pesticides can have adverse impacts on soil microbial biomass and soil respiration (Shultana *et al.*, 2025). And this increase in respiration indicates the higher growth of microbial population (Izallalen *et al.*, 2008), which leads to increase microbial biomass carbon. The increase in microbial biomass carbon (MBC) observed in this study indicates enhanced microbial proliferation and activity in the rhizosphere, highlighting the central role of soil microorganisms in HCH dissipation. This response is primarily driven by plant-microbe interactions, where root exudates supply readily available carbon substrates that stimulate heterotrophic microbial growth and activate enzymatic functions in the rhizosphere. Such rhizosphere priming effects can significantly intensify microbial metabolism, thereby accelerating the degradation of persistent organic pollutants including HCH. In addition, rhizosphere-associated microbial communities are known to mediate the transformation of chlorinated hydrocarbons through dehydrochlorination, reductive dehalogenation, and co-metabolic pathways. These processes collectively contribute to the breakdown and detoxification of HCH residues in soil. Therefore, the observed increase in MBC reflects an active plant-microbe synergistic system, in which microbial processes play a decisive role in phytoremediation efficiency under HCH-contaminated conditions.

Growth parameters: The root and shoot length of all the plants increased with the passage of time but there is a difference between control and spiked treatments. The plants grown in control samples have relatively higher root and shoot heights than the plants which were grown in the spiked soil. This negative effect of HCH contamination on the growth of Rye grass and Bermuda grass was obvious because of the toxicity of HCHs. The plant biomass varied in different treatments as well of the two plants. Generally, the plant biomass was more in the control samples as compared to the spiked samples. (Dubey *et al.*, 2014) reported that at concentrations 15 and 20 mg kg^{-1} of lindane (γ-HCH), the values of dry biomass, root size, and foliage were declined as compared to their control values. This reduction in these parameters of Spinach can be associated to the noxiousness of the lindane.

The observed growth suppression is consistent with phytotoxic responses reported in other phytoremediation systems, where species such as sunflower and willow generally exhibit higher tolerance due to greater biomass allocation and more developed root systems.

HCHs concentration in soil: In general, HCH concentrations decreased in all treatments. HCH occurs as four major isomers (α-, β-, γ-, and δ-HCH), which differ in environmental persistence and biodegradability. Among these, β-HCH is considered the most persistent due to its low volatility and high stability, whereas γ-HCH (lindane) is more susceptible to microbial degradation (Paolis *et al.*, 2013). Although individual isomers were not quantified in the present study, differential degradation of HCH isomers may have contributed to the overall reduction observed. Future research should investigate isomer-specific behavior to better understand plant uptake mechanisms and degradation pathways.

The reduction in HCH concentration is likely attributable to the presence of plants and their associated rhizosphere processes. Similar findings were reported by Abhilash *et al.*, (2011), who observed substantially greater lindane dissipation in planted soils than in unplanted controls. In the present study, the enhanced dissipation of HCH may also be linked to increased microbial activity in the rhizosphere, as indicated by the higher microbial biomass carbon (MBC) observed during the experiment. Root exudates can stimulate microbial growth and enzymatic activity, thereby promoting the biodegradation of chlorinated pesticides.

Phytoremediation efficiency varies among plant species due to differences in biomass production, root architecture, and rhizosphere interactions. Sunflower (*Helianthus annuus*) and willow (*Salix* spp.) have been reported as effective phytoremediators because of their high biomass and extensive root systems (Amabogha *et al.*, 2023). Likewise, *Jatropha curcas L* has shown potential for remediation of pesticide-contaminated soils owing to its tolerance to toxic compounds and well-developed root system (Álvarez-Mateos *et al.*, 2019). Although the grass species used in this study produced lower biomass than these species, its dense fibrous root system likely enhanced rhizosphere microbial activity and supported HCH dissipation. These findings suggest that grasses can serve as effective and low-maintenance candidates for phytoremediation of HCH-contaminated soils.

HCHs concentration in plants: Plant growth was noticeably reduced in HCH-spiked soils, which is consistent with the known phytotoxic effects of HCHs. Organochlorine pesticides, including HCHs, can disrupt cellular membranes, inhibit enzyme activity, interfere with photosynthetic pathways, and induce oxidative stress, ultimately reducing biomass accumulation. The decrease in soil pH observed in spiked treatments can be attributed to the release of organic acids and CO_2 from plant roots and rhizosphere microbes in response to stress conditions. Similarly, the reduction in electrical conductivity (EC) likely reflects plant uptake of available ions and microbial utilization of soluble nutrients during the detoxification processes.

A small negative concentration value observed in Bermuda grass treatment T1 (-0.03 mg kg^{-1}) is not biologically or environmentally meaningful. This value likely resulted from minor instrumental variability, baseline correction drift, or analytical noise during GC-MS quantification, particularly near the method detection limit. Such small negative values are occasionally produced in chromatographic analyses when the measured signal is lower than the background correction level. Therefore, the value was interpreted as below detection limit (BDL) and effectively considered as zero HCH concentration. This clarification improves the transparency and reliability of the analytical results.

These findings align with previous studies demonstrating rapid dissipation of HCHs in vegetated soils. White (2001) reported enhanced lindane degradation by Spinach compared to non-vegetated soil, while (Becerra-Castro *et al.*, 2013) showed that *Cytisus striatus* with bacterial inoculation effectively degraded HCHs. Similarly, (Roberto Calvelo Pereira *et al.*, 2010) identified plant species capable of tolerating and reducing HCH concentrations despite exhibiting stress symptoms. The results of the present study further support the potential of grass species for HCH phytoremediation, although the extent of accumulation and growth response varied with concentration.

Field-Scale implications of HCH phytoremediation: The results of this study suggest that *Cynodon dactylon* and *Lolium multiflorum* have potential for the phytoremediation of HCH-contaminated soils. Both grass species exhibited tolerance to HCH contamination and contributed to the reduction of HCH concentrations in soil. Their dense root systems may facilitate rhizosphere processes that enhance contaminant dissipation and improve soil quality.

The practical application of these species may be particularly relevant in agricultural areas affected by historical pesticide use. Due to their rapid establishment and extensive root development, these grasses could provide a cost effective and environmentally sustainable approach for the management of contaminated soils. In addition to contaminant removal, vegetation cover may contribute to soil stabilization and reduce the risk of pollutant dispersion.

Conclusion

Remediation of polluted soils with HCHs is much needed in the current situation, as they are banned and included in Stockholm list of POPs. However, phytoremediation has been widely proven for the cleaning of several organic and inorganic pollutants, but only a limited number of plant species have been investigated so far, for the phytoremediation of HCHs in the literature. Therefore, it is much needed to explore more species for the remediation of HCHs. Hence, the present study was designed to explore the phytoremediation potential of two grass species (*Cynodon dactylon* and *Lolium multiflorum*) for the reclamation of HCHs contaminated soils. These species exhibit rapid growth, adaptability, and low maintenance requirements, making them suitable candidates for phytoremediation applications. The results revealed that the selected species can remediate and accumulate the HCHs from polluted soil. Moreover, they can also dissipate the HCHs from soil. Thus, these two species can serve as a better option for phytoremediation of HCHs contaminated soil, but their accumulation and dissipation potential can be raised by appropriate agronomic activities.

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Author's Contribution: Tauhid Khan conceptualized the study, performed data analysis, and wrote the original draft and revisions. Uzma Afridi designed and conducted the laboratory and greenhouse experiments. Saira Kiran assisted with data processing, statistical analysis, interpretation of results, and manuscript editing. Maksud Abbaskhanov acquired funding. Gulfam Mohi-ud-Din contributed to critical review, literature review, and manuscript writing and revisions. Bahadir Kaharov and Satbay Nurjanov acquired funding and assisted with manuscript writing and revisions. Nodir Abdushakhidov contributed to manuscript writing and revisions. Afsan Kiran performed methodological validation, analytical procedures, and manuscript proofreading. Ghulam Haider contributed to manuscript writing and revisions.

References

Abhilash, P.C., S. Srivastava and N. Singh. 2011. Comparative bioremediation potential of four rhizospheric microbial species against lindane. *Chemosphere*, 82(1): 56-63. https://doi.org/10.1016/j.chemosphere.2010.10.009

Alidoosti, F., M. Giyahchi, S. Moien and H. Moghimi. 2024. Unlocking the potential of soil microbial communities for bioremediation of emerging organic contaminants: omics-based approaches. *Microb. Cell Fact.,* 23(1): 210. https://doi.org/10.1186/s12934-024-02485-z

Álvarez-Mateos, P., F.J. Alés-Álvarez and J.F. García-Martín. 2019. Phytoremediation of highly contaminated mining soils by Jatropha curcas L. and production of catalytic carbons from the generated biomass. *J. Environ. Manag.,* 231: 886-895. https://doi.org/10.1016/j.jenvman.2018.10.052

Amabogha, O.N., H. Garelick, H. Jones and D. Purchase. 2023. Combining phytoremediation with bioenergy production: developing a multi-criteria decision matrix for plant species selection. *Environ. Sci. Pollut. Res. Int.,* 30: 40698-40711. https://doi.org/10.1007/s11356-022-24944-z

Amirbekov, A., S. Vrchovecka, J. Riha, I. Petrik, D. Friedecky, O. Novak and A. Sevcu. 2024. Assessing HCH isomer uptake in Alnus glutinosa: implications for phytoremediation and microbial response. *Sci. Rep.,* 14(1): 4187. https://doi.org/10.1038/s41598-024-54235-1

Anjaria, P. and S. Vaghela. 2024. Toxicity of agrochemicals: Impact on environment and human health. *J. Toxicol. Stud.,* 2(1): 250-250.

Arora, S., S. Arora, D. Sahni, M. Sehgal, D. Srivastava and A. Singh. 2019. Pesticides use and its effect on soil bacteria and fungal populations, microbial biomass carbon and enzymatic activity. *Curr. Sci.,* 116(4): 643-649. https://doi.org/10.18520/cs/v116/i4/643-649

Ataikiru, T., G. Okpokwasili and P. Okerentugba. 2019. Assessment of the microbial biomass carbon (MB-C), nitrogen (MB-N) and phosphorus (MB-P) in soil spiked with pesticides (carbofuran and paraquat). *J. Adv. Microbiol. Biotechnol.*, 18(1): 1-12.

Azaizeh, H., P.M. Castro and P. Kidd. 2010. Biodegradation of organic xenobiotic pollutants in the rhizosphere. In *Organic Xenobiotics and Plants: From Mode of Action to Ecophysiology* (pp. 191-215): Springer. https://doi.org/10.1007/978-90-481-9852-8_9

Baek, S.-Y., S.-D. Choi and Y.-S. Chang. 2011. Three-year atmospheric monitoring of organochlorine pesticides and polychlorinated biphenyls in polar regions and the South Pacific. *Environ. Sci. Technol.,* 45(10): 4475-4482. https://doi.org/10.1021/es1042996

Balazs, H.E., C.A. Schmid, I. Feher, D. Podar, P.-M. Szatmari, O. Marincaș and P. Schroeder. 2018. HCH phytoremediation potential of native plant species from a contaminated urban site in Turda, Romania. *J. Environ. Manag.,* 223: 286-296. https://doi.org/10.1016/j.jenvman.2018.06.018

Becerra-Castro, C., P. Kidd, B. Rodríguez-Garrido, C. Monterroso, P. Santos-Ucha and Á. Prieto-Fernández. 2013a. Phytoremediation of hexachlorocyclohexane (HCH)-contaminated soils using Cytisus striatus and bacterial inoculants in soils with distinct organic matter content. *Environ. Pollut.,* 178: 202-210.

Chatterjee, S., A. Mitra, S. Datta and V. Veer. 2013. Phytoremediation protocols: an overview. *Plant-based Remed. Proc.*, 1-18.

Chauhan, R., S. Bhatnagar, S. Sharma, H.O. Yadav and R. Chhabra. 2026. Unraveling Persistent Organic Pollutants: Emerging insights into sources, impacts, and innovative remediation approaches. *J. Integr. Sci. Technol.,* 14(5): 1568-1568.

Cunha-Santino, M. and I. Bianchini-Junior. 2004. Humic substances mineralization: the variation of pH, electrical conductivity and optical density. *Acta Limnol. Bras.,* 16(1): 63-75.

Devi, Y.B., T.T. Meetei and N. Kumari. 2018. Impact of pesticides on soil microbial diversity and enzymes: A review. *Int. J. Curr. Microbiol. Appl. Sci.*, 7(6): 952-958. https://doi.org/10.20546/ijcmas.2018.706.113

Dubey, R.K., V. Tripathi, N. Singh and P.C. Abhilash. 2014. Phytoextraction and dissipation of lindane by *Spinacia oleracea* L. *Ecotoxicol. Environ. Saf.,* 109: 22-26. https://doi.org/10.1016/j.ecoenv.2014.07.036

Gołda, S. and J. Korzeniowska. 2016. Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium. *Environ. Prot. Nat. Resour.,* 27(1): 8-14. https://doi.org/10.1515/oszn-2016-0003

Hathout, A., M. Amer, O. Hussain, A.A. Yassen, A.-T.H. Mossa and M.R. Elgohary. 2022. Estimation of the most widespread pesticides in agricultural soils collected from some Egyptian governorates. *Egypt. J. Chem.,* 65(1): 35-44. https://doi.org/10.21608/ejchem.2021.72087.3591

Islam, A., M.A. Kalam, M.A. Sayeed, S. Shano, M.K. Rahman, S. Islam and M.M. Hassan. 2021. Escalating SARS-CoV-2 circulation in environment and tracking waste management in South Asia. *Environ. Sci. Pollut. Res.,* 28(44): 61951-61968. https://doi.org/10.1007/s11356-021-16396-8

Islam, M.M., N. Saxena and D. Sharma. 2024. Phytoremediation as a green and sustainable prospective method for heavy metal contamination: a review. *RSC Sustain.,* 2(5): 1269-1288. https://doi.org/10.1039/D3SU00440F

Izallalen, M., R. Mahadevan, A. Burgard, B. Postier, R. Didonato Jr, J. Sun and D.R. Lovley. 2008. Geobacter sulfurreducens strain engineered for increased rates of respiration. *Metab. Eng.,* 10(5): 267-275. https://doi.org/10.1016/j.ymben.2008.06.005

Kaur, I., V.K. Gaur, R.K. Regar, A. Roy, P.K. Srivastava, R. Gaur and S.K. Barik. 2021. Plants exert beneficial influence on soil microbiome in a HCH contaminated soil revealing advantage of microbe-assisted plant-based HCH remediation of a dumpsite. *Chemosphere*, 280: 130690. https://doi.org/10.1016/j.chemosphere.2021.130690

Kaur, R., D. Choudhary, S. Bali, S.S. Bandral, V. Singh, M.A. Ahmad and B. Chandrasekaran. 2024. Pesticides: An alarming detrimental to health and environment. *Sci. Total Environ.,* 915: 170113. https://doi.org/10.1016/j.scitotenv.2024.170113

Keswani, C., H. Dilnashin, H. Birla, P. Roy, R.K. Tyagi, D. Singh and S.P. Singh. 2022. Global footprints of organochlorine pesticides: a pan-global survey. *Environ. Geochem. Health,* 44(1): 149-177. https://doi.org/10.1007/s10653-021-00946-7

Khan, A.A., Z. Ahmed and M.A. Siddiqui. 2012. Journal of Environmental and Occupational Science. *J. Environ. Occup. Sci.,* 1(2): 129-131.

Košková, S., P. Štochlová, K. Novotná, A. Amirbekov and P. Hrabák. 2022. Influence of delta-hexachlorocyclohexane (δ-HCH) to Phytophthora× alni resistant Alnus glutinosa genotypes- Evaluation of physiological parameters and remediation potential. *Ecotoxicol. Environ. Saf.,* 247: 114235. https://doi.org/10.1016/j.ecoenv.2022.114235

Kumar, A., A. Nayak, A.K. Shukla, B. Panda, R. Raja, M. Shahid and P. Rath. 2012. Microbial biomass and carbon mineralization in agricultural soils as affected by pesticide addition. *Bull. Environ. Contam. Toxicol.,* 88(4): 538-542. https://doi.org/10.1007/s00128-012-0538-6

Kumar, N., L. Pathak and K. Shah. 2025. Exploring the contribution of non-edible plants in phytoremediation and biofuel production in India. *Environ. Sustain.,* 8(1): 31-44.

Kurt-Karakus, P.B., M. Odabasi, A. Birgul, B. Yaman, E. Gunel, Y. Dumanoglu and L. Jantunen. 2024. Contamination of soil by obsolete pesticide stockpiles: A case study of Derince Province, Turkey. *Arch. Environ. Contam. Toxicol.,* 86(1): 37-47.

Lai Huat, L., Y. Swee Pin, H. Yao, J. Huangpu, S. Shao Feng and T. Swee Sen. 2024. Environmental Bioremediation: Microremediation, Mycoremediation, and Phytoremediation. *Environ. Claims J.*, 36(2): 186-205.

Li, S., D.W. Elliott, S.T. Spear, L. Ma and W.-X. Zhang. 2011. Hexachlorocyclohexanes in the environment: mechanisms of dechlorination. *Crit. Rev. Environ. Sci. Technol.,* 41(19): 1747-1792. https://doi.org/10.1080/10643389.2010.481592

Liu, X., S. Kümmel, S. Trapp and H.H. Richnow. 2023. Uptake and transformation of hexachlorocyclohexane isomers (HCHs) in tree growth rings at a contaminated field site. *Environ. Sci. Technol.,* 57(23): 8776-8784. https://doi.org/10.1021/acs.est.3c01929

Liu, Y., X. Fu, S. Tao, L. Liu, W. Li and B. Meng. 2015. Comparison and analysis of organochlorine pesticides and hexabromobiphenyls in environmental samples by gas chromatography-electron capture detector and gas chromatography-mass spectrometry. J. *Chromatogr. Sci.,* 53(2): 197-203. https://doi.org/10.1093/chromsci/bmu048

Mohr, C. 1979. Determination of soil organic matter by titration. *Vag-Och Vattenbyggaren*, 25(7-8): 19-21.

Mrema, E.J., F.M. Rubino and C. Colosio. 2013. Obsolete pesticides—a threat to environment, biodiversity and human health. In: (Eds.): Simeonov, L.I., F.Z. Macaev and B.G. Simeonova. *Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe*. Springer, Dordrecht, The Netherlands, pp. 1-21.

Mukherjee, S. and H. Bairwa. 2025. Environmental issues arising from urbanization: A study on the ecological consequences of rapid urban growth. *J. Humanities Educ. Dev.,* 7(3): 618268.

Nair, V.A., M.I. Sonali J, P.S. Kumar, C.A.R. Immaculate, R. Mythrayee, K.V. Gayathri and G. Rangasamy. 2024. Nano-phytoremediation approach using Pistia stratiotes: Biosynthesized copper nanoparticles for textile wastewater treatment and toxicity analysis. *Waste and Biomass Valorization*, 15(11): 6465-6477.

Nayyar, N., N. Sangwan, P. Kohli, H. Verma, R. Kumar, V. Negi and R. Lal. 2014. Hexachlorocyclohexane: persistence, toxicity and decontamination. *Rev. Environ. Health,* 29(1-2): 49-52. https://doi.org/10.1515/reveh-2014-0015

Paolis, M.R.D., D. Lippi, E. Guerriero, C.M. Polcaro and E. Donati. 2013. Biodegradation of α-, β-, and γ-Hexachlorocyclohexane by Arthrobacter fluorescens and Arthrobacter giacomelloi. *Appl. Biochem. Biotechnol.*, 170: 514-524. https://doi.org/10.1007/s12010-013-0147-9

Pereira, R.C., C. Monterroso and F. Macías. 2010. Phytotoxicity of hexachlorocyclohexane: Effect on germination and early growth of different plant species. *Chemosphere*, 79(3): 326-333.

Phillips, T.M., A.G. Seech, H. Lee and J.T. Trevors. 2005. Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. *Biodegradation*, 16(4): 363-392. https://doi.org/10.1007/s10532-004-2413-6

Rabêlo, F.H.S., J. Vangronsveld, A.J. Baker, A. van Der Ent and L.R.F. Alleoni. 2021. Are grasses really useful for the phytoremediation of potentially toxic trace elements? A review. *Front. Plant Sci.,* 12: 778275. https://doi.org/10.3389/fpls.2021.778275

Rashid, A., S. Nawaz, H. Barker, I. Ahmad and M. Ashraf. 2010. Development of a simple extraction and clean-up procedure for determination of organochlorine pesticides in soil using gas chromatography-tandem mass spectrometry. *J. Chromatogr. A*, 1217(17): 2933-2939. https://doi.org/10.1016/j.chroma.2010.02.060

Roy, A., P. Vajpayee, S. Srivastava and P.K. Srivastava. 2023. Revelation of bioremediation approaches for hexachlorocyclohexane degradation in soil. *World J. Microbiol. Biotechnol.,* 39(9): 243. https://doi.org/10.1007/s11274-023-03692-3

Shultana, R., K.Z. Ali Tan, M.M. Rana, T.K. Roy, U.A. Naher, M.Z. Ibne Baki and S.A. Shupta. 2025. Exploring the impact of agricultural pesticides on soil microbes: A comprehensive review. *Egypt. J. Soil Sci.,* 65(3).

Solhi, M. and M. Hajabbasi. 2005. Heavy metals extraction potential of sunflower (*Helianthus annuus*) and canola (*Brassica napus*). *Caspian J. Environ. Sci.,* 3(1): 35-42.

Song, J., J. Song, R. Zhang, C. Che, Y. Yuan, W. Tan and J. Zhang. 2025. Study on interaction, feedback, and response between perfluorinated compounds and soil environments. *Emerg. Contam.,* 11(1): 100428.

Tang, J., X. Niu, Q. Sun and R. Wang. 2009. Bioremediation of petroleum polluted soil by combination of rye grass with effective microorganisms. In: *2009 International Conference on Environmental Science and Information Application Technology*.

Tate, K., D. Ross and C. Feltham. 1988. A direct extraction method to estimate soil microbial C: effects of experimental variables and some different calibration procedures. *Soil Biol. Biochem.,* 20(3): 329-335. https://doi.org/10.1016/0038-0717(88)90013-2

Uduman, M., P. Rathinam, Y. Karuru, G. Obili, G. Chakka and A.K. Janakiraman. 2017. GC-MS analysis of ethyl acetate extract of whole plant of Rostellularia diffusa. *Pharmacogn. J.,* 9(1): 70-72. https://doi.org/10.5530/pj.2017.1.13

Ullah, S., B. Hussain, N. Iqbal, M.M. Raza, M. Salam, P. Vasudhevan and S. Pu. 2025. Microbial ecosystem disruption under persistent organic pollutant stress: Consequences for soil biogeochemistry and environmental sustainability-A review. *Environ. Chem. Ecotoxicol.*

Vijgen, J., P. Abhilash, Y.F. Li, R. Lal, M. Forter, J. Torres and A. Schäffer. 2011. Hexachlorocyclohexane (HCH) as new Stockholm Convention POPs a global perspective on the management of Lindane and its waste isomers. *Environ. Sci. Pollut. Res.,* 18(2): 152-162. https://doi.org/10.1007/s11356-010-0417-9

White, J.C. 2001. Plant-facilitated mobilization and translocation of weathered 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene (p,p′-DDE) from an agricultural soil. *Environ. Toxicol. Chem.,* 20. https://doi.org/10.1897/1551-5028(2001)020%3C2047:PFMATO%3E2.0.CO;2