

Effects of Pentachlorophenol on Soil Microbes-Alfalfa (*Medicago sativa*) Interaction

Devasena Mahesh

dv248789@dal.ca

Dalhousie University <https://orcid.org/0009-0006-2037-006X>

Dr Chijioke Emenike

Dalhousie University

Research Article

Keywords: Pentachlorophenol (PCP), Soil microbial activity, Alfalfa (*Medicago sativa*), wood preservative, phytoremediation

Posted Date: October 13th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7829924/v1>

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Additional Declarations: The authors declare no competing interests.

Abstract

Pentachlorophenol (PCP) is a legacy wood preservative that, despite its regulatory phase-out, remains present in treated structures and soils due to its long service life and persistence. This study evaluates the effects of PCP on soil microbial communities and alfalfa (*Medicago sativa*) growth under greenhouse conditions. Two concentrations of PCP (1.58% and 2.7%) were applied to soil, with and without alfalfa cultivation, to assess changes in microbial colony counts and plant development over 60 days. Results revealed a concentration-dependent decline in microbial populations and plant biomass, with the 2.7% PCP treatment causing severe reductions. However, soils planted with alfalfa exhibited improved microbial recovery, especially at the lower PCP concentration, indicating a rhizosphere-mediated resilience. These findings suggest that alfalfa may enhance microbial resilience through rhizosphere interactions, offering potential benefits for managing historically contaminated sites. Given that PCP degrades over time, these insights contribute to understanding plant–microbe dynamics in aged preservative-treated environments without overstating long-term risks.

1. Introduction

Pentachlorophenol (PCP) is a chlorinated aromatic compound that served for decades as a heavy-duty wood preservative. First introduced in the 1930s, PCP gained prominence in the post-war period due to its effectiveness against rot and wood-boring pests (Worthing & Walker, 1987). By the 1970s, it had largely supplanted creosote as the preservative of choice for industrial wood products (Brudermann, 2004). Consequently, PCP-treated wood became a mainstay of critical infrastructure – from telephone poles and railway ties to pilings and outdoor timber structures – valued for dramatically extending service life in wet or soil-contact conditions (Wood preservation Canada, 2012).

Wood has long been an essential structural material. Its biological origin (Sheng & Fukuju, 2007), high strength-to-weight ratio (Youngs, 2009), and minimal processing requirements (Isaac Akpan et al., 2021) make it a versatile and economically beneficial material. Despite these advantages, wood's susceptibility to various forms of deterioration, both abiotic and biotic, limits its durability and widespread use. Abiotic factors such as sunlight, temperature, and weathering contribute to wood's degradation, but it is the biotic agents like bacteria, fungi, and insects (termites, beetles, and marine borers) that present significant challenges to wood preservation (Sundararaj, 2022).

Wood preservatives such as PCP have been used for decades to extend the durability and service life of wood, especially in environments prone to fungal decay and insect damage (Schultz et al., 2007). Like most effective preservatives, PCP relies on a certain degree of toxicity to perform its protective function. Over time, interest has grown in understanding how residual compounds from wood treatments interact with surrounding ecosystems, particularly soils and plants. For instance, studies have examined the response of aquatic plants like *Lemna minor* to varying PCP concentrations, noting changes in growth and enzyme activity under high experimental doses (Song & Huang, 2005). In occupational contexts, some research has explored associations between long-term exposure to PCP-treated wood and

potential health outcomes (Guyton et al., 2016), though such findings are influenced by factors like dose, duration, and exposure conditions. As of October 4, 2023, Canada moved to discontinue new uses of PCP, aligning with broader international policies such as the Stockholm Convention, which has prompted restrictions in regions like the European Union (European Commission, 2019; Health Canada, 2023). Despite these regulatory shifts, PCP-treated wood remains in long-term service, and its role in infrastructure durability continues to be recognized. Continued research into its environmental fate and plant–soil interactions will help inform responsible land management.

Soil microorganisms are among the first biotic components to respond to xenobiotic compounds like PCP. Numerous studies have shown that even low concentrations of PCP can inhibit microbial enzymatic activities, reduce biomass, and disrupt essential soil functions such as nutrient cycling (Chaudri et al., 2000). While these impacts on microbial health are well documented, less is known about how PCP affects microbial distribution pattern particularly in the presence of plant root systems that could influence microbial recovery. Plants play a pivotal role in shaping the soil microbial community through root exudates and rhizosphere interactions, and certain species may help buffer the negative effects of contaminants on soil life.

Alfalfa (*Medicago sativa*), a deep-rooted perennial forage crop, has demonstrated potential in phytoremediation due to its extensive root system, nitrogen-fixing capability, and association with beneficial rhizobacteria (Hechmi et al., 2014). Its root exudates not only enhance microbial colonization but also stimulate microbial degradation of pollutants.

This study aims to assess the impact of PCP on soil microbial distribution and alfalfa growth under controlled greenhouse conditions. By evaluating microbial colony counts and plant growth parameters before and after planting, this study investigates the impact of PCP on soil microbial function and the potential of alfalfa to support microbial recovery and plant productivity in contaminated soils.

2. Methodology

2.1 Site Description and Soil Treatment

The experiment was conducted at Dalhousie University's Agricultural Campus greenhouse, located in Truro, Nova Scotia. Surface soil (0–20 cm) was collected from a demonstration garden on campus. The soil was air-dried and sieved to remove debris and homogenize the texture. The technical grade PCP (86% pure) required for the preparation of PCP treating solution was obtained from KMG Chemical (Texas, United States). Two concentrations of PCP (1.58% and 2.7%) were prepared by dissolving PCP in a wood preservative carrier oil (WCO) consisting of 70% diesel and 30% biodiesel. Control (no contamination) and WCO only treatments were also included. Each treatment was replicated three times.

The amount of PCP to be used is calculated by using the following formula:

Density of The WCO (g/mL) = Weight of the WCO taken/volume of WCO taken

Amount of PCP required = Density of WCO × concentration in %/0.86

According to the formula, 15.41 g of PCP was used to prepare 1.58% PCP solution, while 26.35 g of PCP was used for a 2.7% PCP solution. To simulate contamination, 2 kg of soil was thoroughly mixed with 100 mL of the corresponding PCP solution and air dried for 48 hours. After drying, the soil was placed in plastic pots (17 cm diameter x 17 cm width x 17.5 cm height). To maintain moisture and replicate environmental conditions, the soils were watered every three days and kept at 20–25°C throughout the experiment. In the preliminary test, when treated with more than 3% PCP, 100% mortality of alfalfa was observed. Hence, concentrations below 3% were used for the experiment.

2.2 Plant Cultivation and Experimental Design

Alfalfa seeds (Multi-leaf Common No. 1) sourced from Interlake Forage Seeds, Manitoba, were used as the test plant. The seeds were inoculated with Nitragin Gold, a commercial rhizobia inoculant designed to enhance nitrogen fixation. Seeds were directly sown at a rate of 0.5 g per pot. Treatments were arranged in a completely randomized design (CRD) with three replicates each. Plants were fertilized biweekly using a balanced NPK fertilizer and observed for 60 days. Three replicates of each treatment were prepared in a completely randomized manner. To observe the difference in microbial colony count between planted and unplanted soil, one set of pots with different contamination levels were left unplanted and the other set was planted with alfalfa. The initial experimental design was adapted from Hechmi et al. (2014), who conducted a similar study with alfalfa and three other plant species, with subsequent modifications made to suit the specific objectives of this research.

2.3 Microbial Population Count Procedure

To assess the microbial response to PCP contamination and alfalfa planting, soil samples were collected from both planted and unplanted pots at two critical time points: the second week and the eighth week. Approximately 10 g of soil was aseptically collected from each pot and homogenized in 90 mL of sterile distilled water to produce a 1:10 dilution. Samples were subjected to serial dilution and plated on three different types of agar media: nutrient agar, potato dextrose agar, and plate count agar. Nutrient agar is chosen for its broad-spectrum support of bacterial and fungal growth (Clark & Pazdernik, 2012), while potato dextrose agar is particularly conducive to fungal proliferation due to the rich nutrient content derived from potatoes (Westphal et al., 2021). Plate count agar, on the other hand, is specifically designed for enumerating bacteria in diverse environmental samples (Massa et al., 1998).

Each medium was prepared following the manufacturer's instructions, sterilized by autoclaving, and poured into sterile petri dishes in a laminar flow hood to avoid contamination. Once solidified, plates were labeled and used immediately. Plates were incubated in a controlled growth chamber at 37°C for the duration of 2 days for bacterial enumeration and up to 7 days for fungal growth, depending on the medium and colony development rates. After incubation, colony-forming units (CFUs) were manually counted using a digital colony counter.

2.4 Plant Growth and Biomass Measurements

Plant height, number of trifoliate leaves, leaf length, and leaf width were measured biweekly at 2, 4, 6, and 8 weeks after sowing to monitor plant growth progression. Plant height was measured from the soil surface to the apex of the main stem using a standardized ruler. The number of trifoliate leaves was counted manually for each plant, considering fully developed leaves. Leaf length and width were measured using vernier calipers, focusing on the most recently matured trifoliate leaf to ensure consistency across measurements.

At the end of the 60-day experimental period, all alfalfa plants were carefully uprooted and thoroughly washed to remove any adhering soil particles. The plants were then separated into aboveground (shoots) and belowground (roots) components (Hechmi et al., 2014). Fresh weights of both parts were recorded immediately using a digital weighing balance. Subsequently, the samples were oven-dried at 50°C for 24 hours to determine dry biomass. These measures allow assessment of growth performance and biomass allocation under different soil treatment conditions.

2.5 Statistical Analysis

The data analysis for this study involved performing one-way Analysis of Variance (ANOVA) to assess the effects of different treatments on plant growth parameters, including plant height, number of trifoliate leaves, leaf length and width, fresh and dry weight and population counts using GraphPad Prism 10. A significance level of $P < 0.05$ was used to determine whether significant differences existed among the treatment groups. Following significant ANOVA results, pairwise comparisons were conducted using Tukey's Honest Significant Difference (HSD) test to identify which treatments differed significantly. The graphs included in this study were made with the help of GraphPad Prism 10. All data were checked for normality and homogeneity of variance before analysis.

3. Results

3.1 Microbial Colony Counts

Colony counts declined with increasing PCP concentrations across all conditions, as illustrated in Fig. 1. Unplanted soils (A and B) showed a marked decrease in microbial populations over time, particularly in the 2.7% PCP treatment, indicating severe microbial inhibition. In contrast, planted soils (C and D) maintained higher colony counts by week 8, especially in the 1.58% PCP treatment, where there was clear evidence of microbial recovery. These results suggest that alfalfa planting contributes to microbial resilience in PCP-contaminated soils, likely through root exudates and rhizosphere interactions that promote microbial regrowth.

3.2 Plant Growth Trends

Significant reductions in plant height, number of trifoliolate leaves, and leaf dimensions were observed with increasing PCP concentrations, as shown in Fig. 2. While the control and WCO (carrier oil) treatments supported normal alfalfa growth, PCP treatments particularly at the 2.7% concentration resulted in visibly stunted growth, chlorosis, and reduced leaf formation. Statistical analysis confirmed that all measured growth parameters were significantly affected by PCP exposure, highlighting the compound's phytotoxic effects on alfalfa.

3.3 Biomass Accumulation

The data shows a consistent decline in biomass with increasing PCP concentration, as illustrated in Fig. 3. Control and WCO (carrier oil) treatments yielded the highest biomass across all measured categories, indicating minimal phytotoxic impact. In contrast, the 2.7% PCP treatment significantly reduced both shoot and root biomass, with the most pronounced effect observed in belowground dry weight (D). These results confirm the strong inhibitory effect of PCP on plant biomass production particularly on root development and further highlight that the carrier oil alone did not significantly impact alfalfa growth.

4. Discussion

PCP has been shown to influence soil microbial activity and biochemical functions, particularly at higher concentrations. In contaminated soils, PCP has been shown to suppress overall microbial metabolic activity, evidenced by 92.59% decrease in colony counts especially at 2.7% concentration compared to the control at the end of 8 weeks. Though not measured in this study, key soil enzymes involved in nutrient cycling are especially sensitive to PCP. For instance, dehydrogenase activity, a broad indicator of microbial oxidative metabolism, is sharply reduced under PCP exposure (Scelza et al., 2008). Enzymes of the nitrogen cycle, such as urease and protease, are also inhibited, reflecting PCP's disruptive impact on soil nitrogen transformations (Siczek et al., 2020). In a controlled field study, Siczek et al. (2020) observed that PCP amendments led to a strong decline in soil DNA yields and a narrowed substrate utilization profile of the indigenous microbes, indicating losses in microbial abundance and functional diversity. These changes convey that PCP can deteriorate the soil's biological capacity by reducing the variety of carbon sources microbes can metabolize and by selecting against sensitive strains. Such alterations can lead to cascading effects on soil processes. PCP has been observed to affect soil microbiota by influencing microbial respiration, degradation activity, and other biologically driven dissipation processes, with some studies noting stronger effects compared to other chlorophenols (Chaudri et al., 2000). These interactions may lead to shifts in soil microbial function, including reduced enzymatic activity, which can alter nutrient cycling and potentially affect plant–soil dynamics. While the degree of impact depends on factors such as concentration, exposure duration, and soil properties, understanding these effects remains important for evaluating the long-term behavior of PCP in soil environments where treated materials are still in use.

Prolonged or repeated PCP contamination can select for more tolerant microbial communities and encourage adaptation, especially in the presence of plants. Over time, PCP-degrading microorganisms

may emerge in contaminated soils, partially restoring microbial functions. In agricultural soil spiked with PCP, a certain Ascomycete fungus (*Byssoschlamys fulva*) proliferated in the treated samples; this fungus was found to tolerate PCP up to 25 mg/L and could degrade about 20% of the PCP in just over a week (Scelza et al., 2008). The appearance of such indigenous degraders suggests a natural attenuation mechanism: the microbial community's composition shifts to include PCP-resistant and PCP-metabolizing species. This finding implies that, given sufficient time (on the order of weeks to months), microbial communities can adapt to PCP stress by enriching organisms capable of withstanding or transforming the pollutant.

Plant presence can markedly influence microbial response and resilience in PCP-impacted soils. Roots and the surrounding rhizosphere often mitigate pollutant stress by stimulating microbial activity and providing refugia for degraders. Rhizosphere microorganisms benefit from root exudates (sources of carbon, energy, and growth factors) which can offset some toxic effects and even induce metabolic degradation of pollutants. In the case of PCP, studies have shown that the root–soil interface is a hotspot of PCP dissipation. He et al. (2005) demonstrated a pronounced “rhizosphere effect” with perennial ryegrass: in soils spiked with PCP, the highest degradation rates occurred within a few millimeters of the root surface, where PCP concentrations plummeted and residual levels were lowest. In planted soil, total PCP fell dramatically near roots (e.g. to < 1% of initial levels at 3 mm distance in one treatment), whereas unplanted soil showed much less reduction. Enhanced microbial activity in the rhizosphere likely drives this effect – the same study noted that microbial biomass, as well as enzymes like phosphatase and urease, were elevated in the root zone relative to bulk soil, correlating with faster PCP breakdown. Essentially, root exudates and root-associated microbes create a microenvironment that can accelerate the biotransformation of PCP.

Another study by Hayat et al. (2011) investigated the dissipation behavior of PCP in the rhizosphere of rice (*Oryza sativa* L.) roots. Using a specially designed rhizobox, they found that the maximum dissipation of PCP in planted soil occurred at a 3-mm distance from the root zone. This area also exhibited rapid changes in concentrations of sulfate, chloride, nitrate, and ammonium, indicating active biogeochemical processes. In contrast, unplanted soil showed no significant variation in PCP concentration with distance from the root zone. After 45 days, a significantly higher concentration of PCP was degraded in planted soil compared to unplanted soil, highlighting the role of the rhizosphere in enhancing PCP dissipation.

Moreover, certain plants can reduce the bioavailability of PCP to microbes by modifying soil properties, effectively shielding the microbial community. Adsorption of PCP to root-zone soil and organic matter can lower the freely available (toxic) fraction. In a rhizotron study with ryegrass, over 96% of PCP introduced was removed from solution mainly via strong adsorption to the andisol soil and subsequent rhizosphere degradation (Rubilar, 2013). In that case, because much of the PCP became soil-bound, the toxic impact on total microbial biomass was minimal – neither microbial biomass (quantified by DNA) nor β -glucosidase activity (carbon cycle enzyme) showed significant decline even at 250 mg/kg PCP. Only the dehydrogenase activity was inhibited at high PCP, reflecting that microbial metabolic intensity

was affected, even though biomass remained. Such results suggest a degree of microbial resilience stemming from reduced contaminant bioavailability in the root zone. The rhizosphere's physical, chemical, and biological characteristics collectively contribute to microbial community resilience in PCP-contaminated soils: root-driven changes (pH, exudation of organic acids, added organic carbon, etc.) can immobilize or transform PCP, while root-associated microbial consortia carry out its degradation.

PCP not only affects microbes but also directly impairs plant growth, with legumes and other sensitive plants. Root growth is particularly sensitive – PCP exposure leads to shorter, sparser roots, likely due to the compound's interference with cell division and elongation in root meristems. This is in line with the results presented by Hechmi et al. (2014) which assessed mixed and single cropping of rapeseed, alfalfa, white clover, and ryegrass on PCP degradation in contaminated soil. Leguminous plants suffer additional harm under PCP contamination due to disruptions in their nitrogen-fixing symbiosis. PCP has been shown to strongly inhibit the chemical signaling between legumes and rhizobia bacteria that is necessary for nodule formation. Specifically, PCP interferes with flavonoid-induced expression of rhizobial *nod* genes – one study found that PCP could suppress luteolin-induced nod gene activation by about 90% (Fox et al., 2004). This blockage fails nodulation. Without nodules, biological nitrogen fixation is lost, leaving the plant nitrogen starved.

Certain hardy legume plants such as alfalfa have shown remarkable capacity to establish and even thrive in moderately contaminated soils, making them valuable agents for phytoremediation. Alfalfa is a deep-rooted perennial legume with traits like activating its antioxidant defense system that enables it to tolerate pollutants and assist in soil restoration (Dai et al., 2015). One key mechanism is through its root exudates, which are rich in organic compounds (sugars, amino acids, organic acids, etc.) that can stimulate soil microbial activity (Mehmannavaz et al., 2002). These exudates serve as carbon sources and growth substrates for soil microorganisms, including those capable of degrading organic pollutants. In a two-year field trial on a polychlorinated biphenyls (PCB) contaminated site, planting alfalfa (along with other species) led to the detection of known xenobiotic-degrading bacterial groups in the root zone that were otherwise scarce in unvegetated soil (Tu et al., 2011). Notably, the alfalfa-planted plots became enriched in certain PCB-degrading bacteria (e.g. members of *Chloroflexi*), (Tu et al., 2011). This explains our finding on alfalfa sustaining the microbial population.

In addition to enhancing microbial communities, alfalfa contributes to phytoremediation through its capacity to uptake and tolerate certain contaminants. A four-year field study at Quebec reported that alfalfa can endure soils co-contaminated with PCP and heavy metals/metalloids, maintaining growth where many plants would not survive (Yanitch et al., 2020). Its deep, extensive root system can explore a large volume of soil, diluting the exposure per root biomass and accessing pockets of less contaminated soil for essential nutrients. Furthermore, alfalfa is found to sequester some pollutants into its tissues, which is a form of phyto stabilization or phytoextraction. In the same study, alfalfa (alongside willow and grasses) was able to uptake trace elements from chromated-copper-arsenate (CCA) wood preservatives in the soil and even accumulate dioxins and furans (Yanitch et al., 2020). This ability to tolerate and

compartmentalize toxic compounds, despite some growth reduction, demonstrates alfalfa's capacity to reduce contaminant load in the soil matrix.

The findings of this study reinforce the dual role of alfalfa in mitigating the adverse effects of PCP on soil microbial health while also demonstrating resilience in moderately contaminated environments. Although high concentrations of PCP (2.7%) significantly impaired plant growth and microbial activity, the ability of alfalfa to sustain microbial populations and accumulate biomass in the 1.58% treatment highlights its potential as a phytoremediation candidate. The observed rhizosphere effect, characterized by enhanced microbial recovery in planted soils, suggests that plant–microbe interactions are crucial for promoting soil resilience in chemically stressed conditions. These results not only validate alfalfa's utility in remediation efforts but also point to the importance of integrating biological strategies into the management of contaminated soils.

5. Conclusion

This study demonstrates that PCP contamination significantly hampers soil microbial activity and alfalfa growth, with severity increasing with concentration. However, alfalfa cultivation enhanced microbial recovery, particularly in the 1.58% PCP treatment. These findings highlight the dual role of alfalfa as both a soil health promoter and a potential phytoremediator in PCP-affected environments. Future research should explore long-term field trials, interactions with other soil amendments, and the scalability of alfalfa-based remediation strategies in contaminated landscapes.

Declarations

6. Author contributions

D.M. conceived the study, conducted the experiments, collected and analyzed the data, and prepared the manuscript. C.U.E. supervised the research, provided guidance on experimental design and data interpretation, and reviewed and revised the manuscript. All authors have read and approved the final version of the manuscript.

7. Acknowledgements

The authors acknowledge Koushika Kumaresan for her guidance on experimental methodology and chemical preparation.

8. Funding declaration

This research was supported by a Dalhousie University Research Grant (No. 39364) and Mitacs Accelerate award (Application Ref.IT33255).

9. Declaration

The authors declare no conflict of interest.

9.1 Ethics declaration

Ethics declaration not applicable.

9.2 Consent to Participate

Consent to participate not applicable.

9.3 Consent to Publish declarations

Consent to publish declarations not applicable.

10. Data availability statement

The authors declare that the data supporting the findings of this study are available within the paper. Raw data files are available from the corresponding author on reasonable request.

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Figures

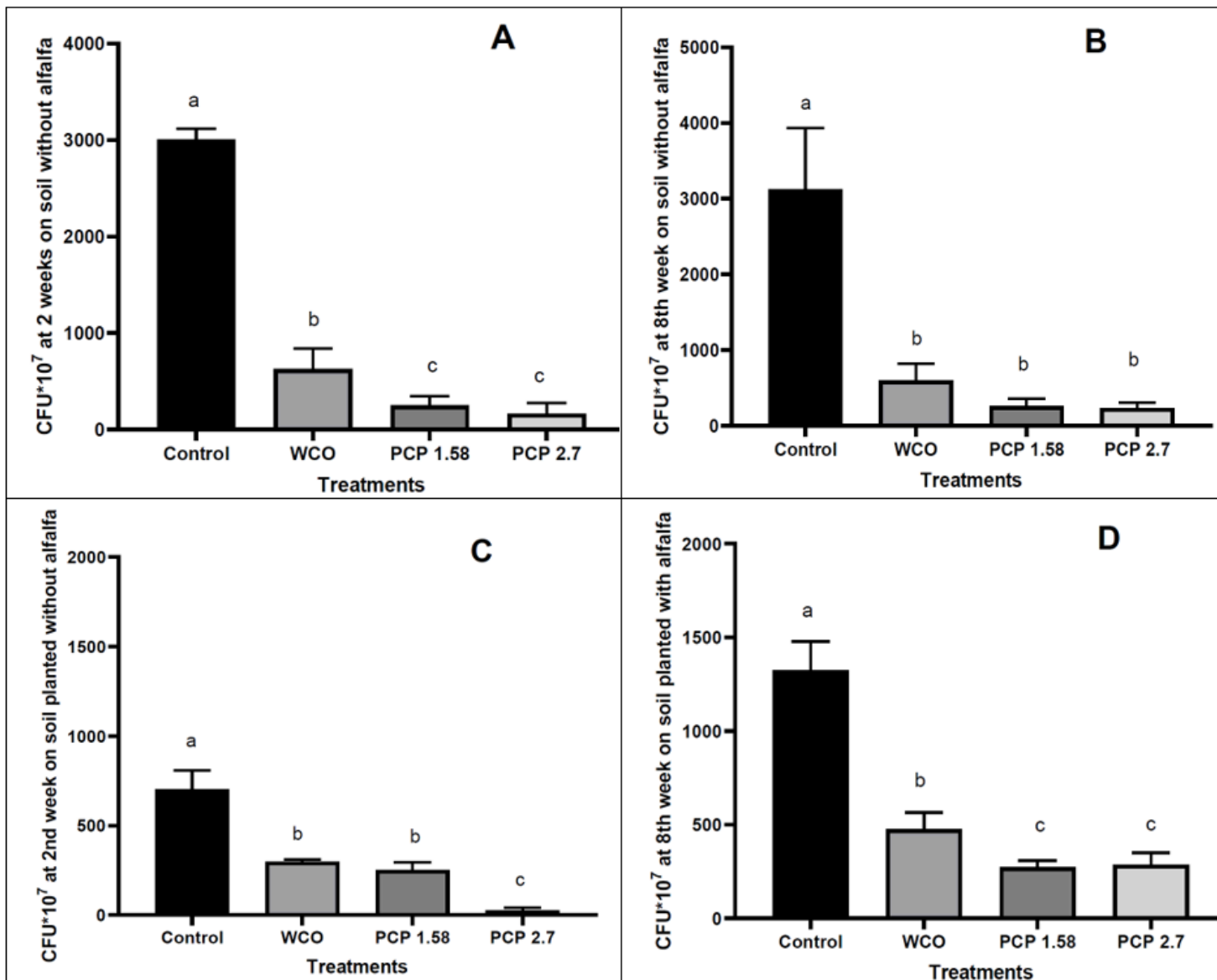


Figure 1

Bar chart representing microbial colony counts in soil under different treatments at two time points. A: Unplanted soils – Week 2 B: Unplanted soils – Week 8 C: Planted (alfalfa) soils – Week 2 D: Planted (alfalfa) soils – Week 8. Bars indicate the standard error of the mean. Mean values that do not share a letter are significantly different

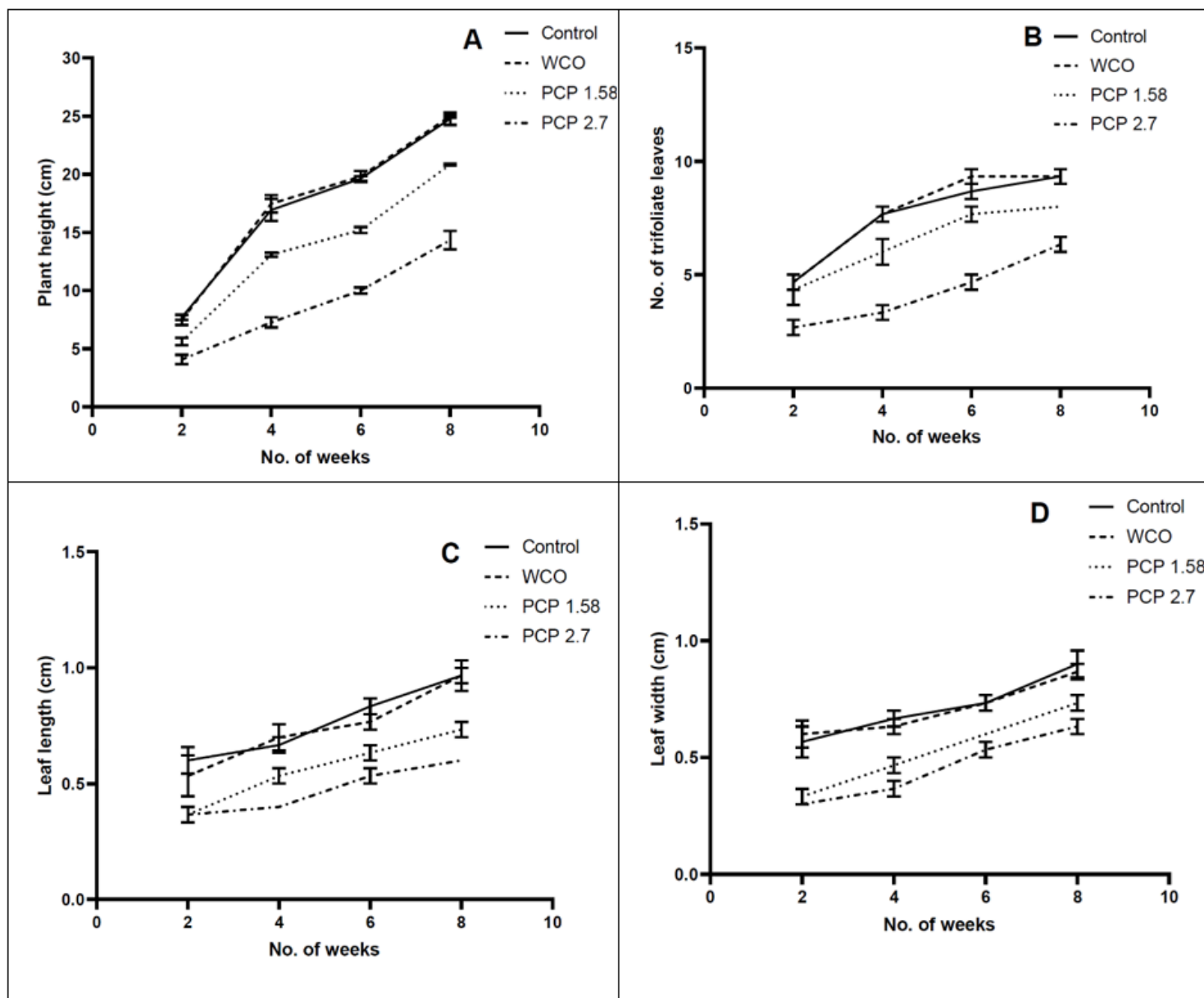


Figure 2

Line plot represents plant growth parameters of alfalfa under different soil treatments over an 8-week period. A: Plant height (cm) B: Number of trifoliolate leaves C: Leaf length (cm) D: Leaf width (cm). Bars indicate the standard error of the mean. Mean values that do not share a letter are significantly different

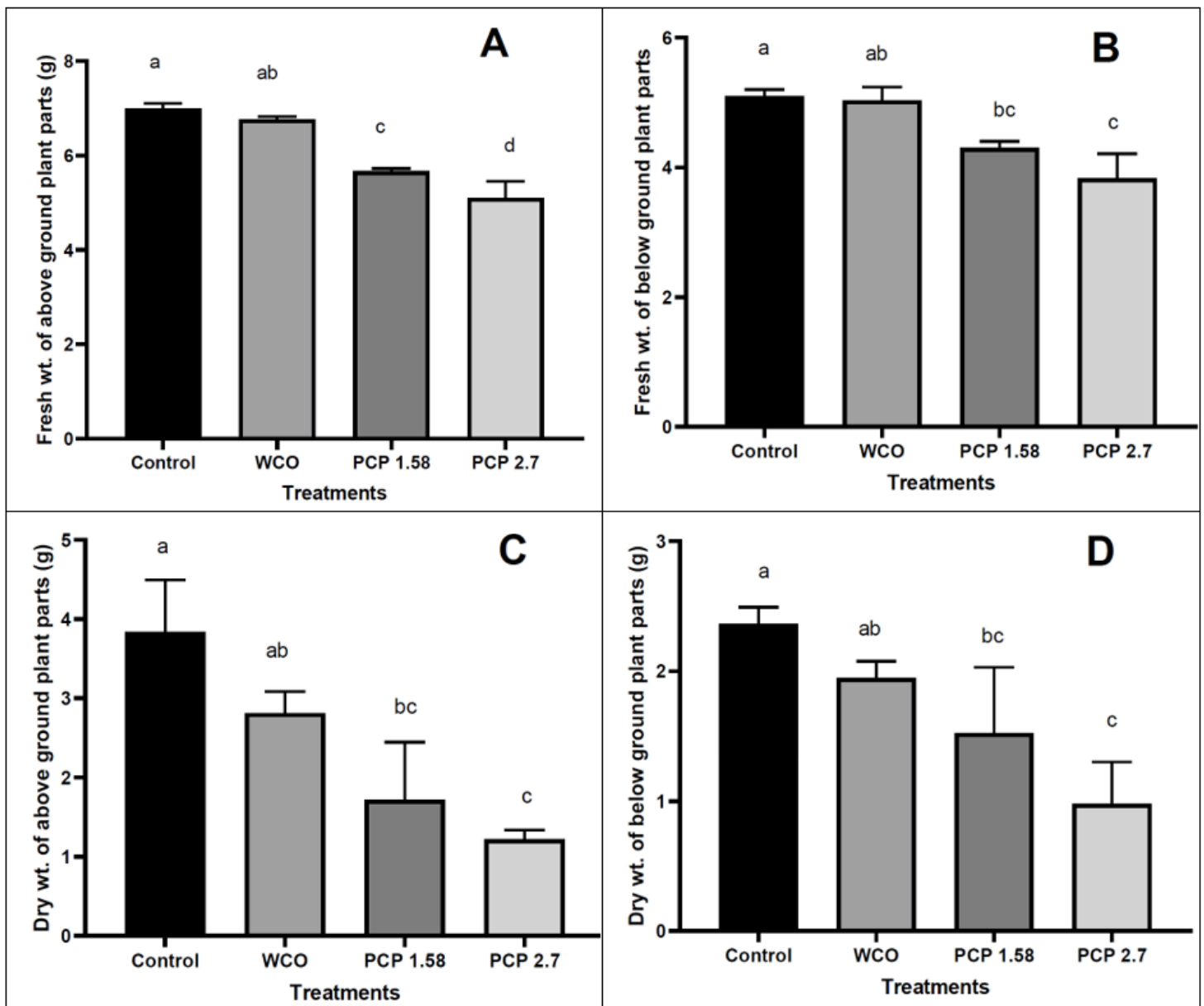


Figure 3

Bar chart represents biomass accumulation of alfalfa under different soil treatments after 60 days of growth. A: Aboveground fresh weight B: Aboveground dry weight C: Belowground fresh weight D: Belowground dry weight. Bars indicate the standard error of the mean. Mean values that do not share a letter are significantly different