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Interactive effect of biochar and compost with Poaceae and Fabaceae plants on remediation of total petroleum hydrocarbons in crude oil contaminated soil

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Abstract

The current study was dedicated to finding the effect of soil amendments (biochar and compost) on plants belonging to Poaceae and Fabaceae families. Plants selected for the phytoremediation experiment included wheat (*Triticum aestivum*), maize (*Zea mays*), white clover (*Trifolium repens*), alfalfa (*Medicago sativa*), and ryegrass (*Lolium multiflorum*). The physiological and microbial parameters of plants and soil were affected negatively by the 4% TPHs soil contamination. The studied physiological parameters were fresh and dried biomass, root and shoot length, and chlorophyll content. Microbial parameters included root and shoot endophytic count. Soil parameters included rhizospheric CFUs and residual TPHs. Biochar with wheat, maize, and ryegrass (Fabaceae family) and compost with white clover and alfalfa (Poaceae family) improved plant growth parameters and showed better phytoremediation of TPHs. Among different plants, the highest TPH removal (68.5%) was demonstrated by ryegrass with compost, followed by white clover with biochar (68%). Without any soil amendment, ryegrass and alfalfa showed 59.55 and 35.21% degradation of TPHs, respectively. Biochar and compost alone removed 27.24% and 6.01% TPHs, respectively. The interactive effect of soil amendment and plant type was also noted for studied parameters and TPHs degradation.

Keywords: Phytoremediation, TPHs, Poaceae plants, Fabaceae plants, Biochar, Compost

1. Introduction

Among organic contaminants, the most widespread class is petroleum hydrocarbons (Khan et al., 2016ab). Several processes, including accidental leakage during extraction, spillage during transportation, release, processing, and waste generation with industrial activities, contribute to soil contamination with petro-chemical, more specifically hydrocarbons (Yu et al., 2019). For the last few years, the exposition of these ticklish contaminants, which occurred due to contaminated soil, has raised harmful health risks and environmental impacts. The threats associated with petro-chemicals include toxicity, mutagenicity, carcinogenicity, and food chain bioaccumulation. These impacts are ominously threatening humans and the environment (Hussain et al., 2018; Khan et al., 2019). Apart from social and sanitary, the economic aspect is another concern for the contaminated sites, as impacted soil shows the reduction in productivity and financial depreciation (Khan et al., 2020). Proper treatment is required to prevent the migration of contaminants into the environmental matrices and powerful menace. If left untreated, soil aging occurs, reducing the bioavailability of petroleum hydrocarbon (Gong et al., 2015).

It is well established that petroleum hydrocarbons are degraded in the soil. There is almost negligible volatilization from the plant. Hence, it should not concern setting up phytoremediation, specifically rhizo-degradation of fresh or aged soils (Ingrid et al., 2016; Wu et al., 2019). Therefore, it will be beneficial to improve the degradation of petrochemical pollutants within the rhizosphere. The plants produce root exudates containing catabolic enzymes. These exudates are necessary for the remediation of harmful compounds. These exudates provide favorable conditions for the rhizospheric microbial populations involved in the treatment process (Alagić et al., 2015). Therefore, the selection of plant species is crucial and a limiting factor in phytotechnology, as application, advancement, and widespread acceptance is dependent on it (Abdullah et al., 2020).

44 The grass species belonging to Fabaceae and Poaceae family have been reported to perform better
45 TPHs phytoremediation in the hydrocarbon-impacted soils (Hussain et al., 2018). Multiple
46 adaptations predict phytoremediation efficiency. The plants' symbiotic association with N-fixing
47 bacteria and arbuscular mycorrhizal fungi influence phytoremediation activities (Arshad et al.,
48 2017). Phosphorus utilization from sources unavailable to other plant species can also help the
49 plant prevent the stress of nutrient deprivation (Hussain et al., 2018). Further, the release of root-
50 derived exudation, like flavonoids and phenolics, and enzymes that promote the associated
51 microbial communities, improve plants' remediation capacities (Mushtaq et al., 2020).
52 Another approach adopted to improve the degradation process of petroleum hydrocarbon in the
53 soil is soil amendments (Chen et al., 2016). Among many soil amendments used, organic
54 amendments such as compost and biochar have gained considerable interest in the last few years.
55 They have shown multiple benefits. They are also known to influence microbial abundance, soil
56 conditioning properties and help in the improvement of physicochemical characteristics (Mushtaq
57 et al., 2020). The biochar removes contaminants through multiple methods, including cation
58 exchange, complexation, electrostatic interaction, and precipitation (Arshad et al., 2017). Compost
59 is known to have high organic content that improves soil enzymatic and microbial activities. Under
60 aerobic conditions, the organic matter (from plants and animals) is decomposed into compost.
61 (Iqbal et al., 2019).
62 Though several plant species and several soil amendments have been successfully applied for the
63 phytoremediation of petroleum hydrocarbon contaminated soils, improving the efficiency and
64 optimizing the conditions of phytoremediation is one of the major concerns. The hydrocarbon
65 phytoremediation efficiency relies significantly on soil physicochemical properties and microbial
66 population (Hoang et al., 2021; Khan et al., 2018). Grasses provide ideal conditions for

67 phytoremediation due to the fibrous root system (Hussain et al., 2018). Their elevated root surface
68 area, which takes part in the mineralization of the organics like TPHs. Even though efforts were
69 made to identify the best plant capable of TPHs phytoremediation, there was lack of research work
70 that compares different grass plants and organic amendments for the TPHs removal. Further, the
71 domain of interaction effect of plants and the organic amendment is not reported to its statistically
72 significant depth. Based on the above-discussed literature, the main objective of this study was to
73 assess the effect of biochar and compost as organic amendments with different grass plants (from
74 Poaceae and Fabaceae family) on hydrocarbon removal and bacterial community in aged
75 contaminated soil.

76

77 ## 2. Materials and Methods

78 ### 2.1. Soil collection, preparation, and characterization

79 TPHs contaminated soil was collected from the remediation site of an oil refinery located in
80 Rawalpindi, Pakistan. In contrast, clean soil was collected from the agricultural site located in
81 Quaid-i-Azam University, Islamabad. Both clean and contaminated soils were sieved from 2 mm
82 mesh to obtain uniform texture. Sieved soil was used for quantification of soil pH, electrical
83 conductivity (EC), moisture content, texture, nitrates, phosphate, potassium, total organic carbon,
84 and initial total petroleum hydrocarbons (TPHs) according to the method described by Hussain et
85 al., (2018).

86

87 ### 2.2. Selection of plant species and soil amendments

88 Plants belonging to Poaceae and Fabaceae families were selected for the phytoremediation
89 experiment (plants details are presented in Table 1). These included wheat (*Triticum aestivum*),

90 maize (*Zea mays*), white clover (*Trifolium repens*), alfalfa (*Medicago sativa*), and ryegrass (*Lolium*
91 *multiflorum*). Seeds of different plant species were collected from the Seed Preservation
92 Laboratory and the National Gene Bank department of the National Agricultural Research Center
93 (NARC), Islamabad.

94 Organic amendments (biochar and compost) were collected from the Austrian Institute of
95 Technology (AIT) and Horticulture Department of NARC, Islamabad. Biochar used was prepared
96 from woodchip feedstock have following characteristics; pH (in $CaCl_2$) 8.9, ash content 15.2%,
97 EC 1.6 mS cm^{-1}, SSA 26.4 m_2 g^{-1}, CEC 93.0 $cmol_c$ kg^{-1}, 1.23% N, 0.63% P, 2.08% K and 46.24%
98 organic carbon (Kloss et al., 2012). Compost used in this study was prepared from green waste
99 material and had the following characteristics; pH 7.8, NPK 1.19, 0.309, and 3.3%, respectively,
100 total Zn, Cu, Fe and Mn 158, 37, 290, and 229 mg kg^{-1}, respectively and a total organic carbon of
101 18% (Mushtaq et al., 2020).

102

103 **2.3. TPHs bioremediation pot experiment**

104 **2.3.1. Seed sterilization**

105 For the pot experiment, seed surface sterilization of each selected plant was done earlier than
106 sowing in the pot. *L. multiflorum* seeds were surface sterilized by soaking them in 5% sodium
107 hypochlorite solution (2 min), followed by dipping in 70% ethanol (2 min), as was done by Yousaf
108 et al. (2010). Seeds of *M. sativa* and *T. repens* were sterilized by soaking them in 10% hydrogen
109 peroxide solution (10 min) (Muratova et al., 2012). The seeds of *L. multiflorum*, *M. Sativa,* and *T.*
110 *repens*, after these steps, were rinsed three times with autoclaved distilled water (ADW). For *T.*
111 *aestivum*, seeds were dipped in 1% sodium hypochlorite + 0.05% Tween 20 (15 min), followed by
112 thorough rinsing with ADW (Perata et al. 1992), while seeds of *Z. mays* were sterilized by dipping

113 the seeds in 0.1% $HgCl_2$ (Li et al. 1997). After respective sterilization, *T. aestivum* and *Z. Mays*
114 seeds were washed thrice with ADW and, after final washing, were soaked for 2 hours in ADW.
115

116 **2.3.2. Pot experiment**

117 For the pot experiment, the total weight of soil and soil + soil organic amendment was maintained
118 to 4 kg per pot. Organic amendments were added into each pot based on a percentage ratio of
119 weight to volume (W: V), where W is the weight of soil, and V is the volume of an amendment
120 added). As the density (kg L^{-1}) of biochar and compost were 0.37 and 0.75, receptivity, the volume
121 used for biochar was added 5%, while compost was added 10% (W: V), in each pot. The percentage
122 of amendment selected was based on the rationale of the experiment conducted previously by
123 Hussain et al., (2018) and Benhabylès et al., (2020). The following treatment plan was adopted for
124 the experimentation with each plant; clean soil, contaminated soil (CS), CS + biochar (BC), CS +
125 compost (CM). Controls (without plants) of each of the treatments were also maintained to
126 compare each plant's vegetative treatments. The experiment was conducted in triplicates. Surface
127 sterilized seeds of *M. sativa*, *L. multiflorum*, *T. aestivum,* and *T. repens* were sown at 50 seeds per
128 pot, while for *Z. mays,* 15 seeds per pot were sown within each of the respective treatments. For
129 each of the individual treatments, weaker seedlings were removed after germination, leaving
130 behind 35 healthy seedlings per pot for each plant species, except for *Z. mays*, for which ten healthy
131 seedlings per pot were left. A pot experiment was conducted in the greenhouse of Quaid-i-Azam
132 University, Islamabad, Pakistan. Day to night duration of 16:8 hr was maintained, with temperature
133 25–30 °C. Pots were watered periodically with distilled water. Further, to reduce/eliminate the
134 edge growth effect, pots were repositioned throughout the experiment, and plants were harvested
135 after 62 days for further analysis.

136

137 **2.3.3. Soil and plant analysis**

138 **2.3.3.1. Plant parameters**

139 Percentage germination, root, and shoot length of the plant were noted as done by Arshad et al.
140 (2017). Seed germination of candidate plants were monitored every 24 hours until three weeks.
141 After that, the seed germination was reported on a percentage basis. Plant biomass was estimated
142 according to the protocol adopted by Raza et al. (2019). Total chlorophyll was calculated by adding
143 the chlorophyll a and b, calculated by acetone chlorophyll extraction, followed by noting
144 absorbance with a spectrophotometer at 645 and 663 nm, respectively (Raza et al. 2019). The
145 following equations were used for the estimation of photosynthetic pigments,

146 $$Chlorophyll\ a = (12.25 \times A_{663}) - (2.79 \times A_{645})$$

147 $$Chlorophyll\ b = (21.50 \times A_{645}) - (5.1 \times A_{663})$$

148 $$Carotinoids = {(1000 \times A_{470}) - (1.82 \times Chl_a) - (85.02 \times Chl_b} / _{198}$$

149 Total endophytic bacterial count (for root and shoot interior), capable of tolerating crude oil, was
150 estimated. Briefly, plant material was washed and surface sterilized with 70% ethanol (root: 5 min,
151 shoot: 3 min), then treated with mercuric chloride 0.02% (root: 5 min, shoot: 2 min) followed by
152 washings thrice with ADW. Sterilization efficacy was assured, as done by Coombs and Franco
153 (2003). Homogenized material in 0.9% saline (produced with 5 gm surface-sterilized plant part)
154 was agitated for 1 hour at 30 °C. After stabilizing solid material, the supernatant was spread on
155 nutrient agar containing cycloheximide (100 mg l^{-1}), amended with 2% crude oil, incubated at
156 30°C for 48 hours. Endophytic abundance was noted in CFU $g\text{-}^{1}$ of air-dried soil.

157

158 **2.3.3.2. Soil parameters**

159 After plant harvesting, TPHs were determined using the method defined by Hussain et al. (2018).
160 The quantification of TPH (C10-C40) was performed using the Gas Chromatograph model
161 Hewlett Packard 5890 (series II) coupled with a flame ionization detector (FID). The whole unit
162 of GC-FID and was also equipped with HP automatic liquid injector 7673. The instrument operated
163 in SIM mode, particularly as ion monitoring mode. An already baked column (30 m×0.25 mm)
164 glazed with 0.25 µm 5% phenyl and 95% methyl polysiloxane as the stationary phase for
165 hydrocarbons analysis. The temperature for both detector and injector was set at 300 °C and 280
166 °C, respectively. Helium as carrier gas throughout this procedure was used, and the quantification
167 of TPH via MS ChemStation was performed. The GC oven was programmed according to the
168 following program, 60 °C for 60 seconds, 20 °C min-1 up to the level of 340 °C, and lastly kept at
169 340 °C for 10 min. The results of TPHs concentrations (75 days) were presented in percentages
170 (%). All the calculated hydrocarbons concentrations were expressed on a dry matter basis. The
171 microbial abundance of crude oil tolerating rhizospheric bacteria was estimated (2016a). Briefly,
172 serial dilutions (100 µl of 10^{-1} to 10^{-5} dilutions) of separated soil samples were made and
173 subsequently spread on nutrient agar media having 2% crude oil. Then, plates were incubated at
174 30 °C for 24 hrs, and colonies were counted.
175
176 **2.4. Statistical analysis and model of a factorial experiment**
177 Statistical analysis was performed on the R software environment for statistical computing. In the
178 current investigation, the treatment combinations were the varying levels of investigating factors.
179 We are using two factors;
180 (i) Factor one is the soil amendment type.
181 (ii) Factor two is a plant type.

182 The complete set of treatments is applied at random to the experimental unit in 'r' replicates. The
183 collection of remedies to investigate are used in experimental units. Let us consider the model;

184 $$f(x_1, x_2) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2$$

185 Where the treatment factors display a factorial structure. For a simple factorial structure, we
186 consider two factors, x_1 (soil amendment type) and x_2 (plant type) say, with 'a' and 'b' levels each,
187 respectively. There are "a×b" treatments. As discussed above, the two factors were soil amendment
188 type and plant type run in "r" replicates. We consider this structure if 'Y_{ijk}' is the observation at i^{th}
189 level of factor soil amendment type to j^{th} level of factor plant type in the kth replicate. The model
190 for data can be expressed as;

191 $$Y_{ijk} = \mu + \eta_i + \gamma_j + (\eta\gamma)_{ij} + \varepsilon_{ijk}$$

192 Where μ represents the overall mean effect, η_i is the effect of ith level of soil amendment type 'i'
193 and γ_j effect of j^h level of plant type 'j'. $(\eta\gamma)_{ij}$ are interaction effects among plant type 'i' and soil
194 amendment type 'j'. The random error is ε_{ijk} represents experimental error. By using this model,
195 we are testing the hypothesis;

196 i) Whether the factors, soil amendment type, and plant type significantly affect their varying
197 levels?
198 ii) What are the significant interactions of applied factors against studied parameters?
199 iii) Comparisons among families of plants.

200 For the third hypothesis, i.e., comparisons among families of plants, we test the following contrast
201 in plant parameters ANOVA (hypothesis at α= 1% level of significance is rejected). In the
202 remaining two experiments, this test is meaningless as factors are not independent. This contrast
203 can be tested. Similarly, if factors would have been independent. For the test, our null hypothesis
204 is;

205 a) H_0: Poaceae and Fabaceae are similar. Or

206 b) H_0: $2\mu_{wheat} + 2\mu_{maize} + 2\mu_{Rye\ grass} - 3\mu_{White\ clover} - 3\mu_{Alfalfa} = 0$

207 It is possible to test the above hypothesis from the analysis of variance (ANOVA) table. From the
208 set of different independent contrasts, each of which, the sums of squares of linear contrasts can
209 be easily computed as the sum of $squares_{contrast}= (n(\sum c_j x_j)^2)/(\sum c_j^2)$, with a single degree of freedom,
210 where *n* is the equal number of observations for all samples. In general, we need to compute the
211 F-ratio, $F_{(contrast)}$= (Sum of $squares_{contrast}$)/ MSE, and check its significance comparing with $F_{\alpha(1,}$
212 $_{error)}$. Further, the correlation between TPHs degradation and rhizospheric bacterial CFUs was
213 quantified using Pearson's correlation.

214 With the help of principal component analyses (PCA), the impact of BC and CM application with
215 the plants was made. Using PCA, the implications of conditioners (BC and CM) application in
216 noted with the help of a scattered plot. The PCA scatterplot visualizes the data on two dimensions,
217 principal components (PC) 1 and 2. The parameters g with green color in the scattered plot
218 represent the parameters contributing to higher loading values in PC1. In comparison, the
219 parameters with yellow color have higher loading values in the PC2. Extraction and representation
220 of dimensions were done based on the Eigenvalue greater than or equal to 1.

221

222 **3. Results**

223 **3.1. Characterization of contaminated and clean soil**

224 Details of physicochemical parameters are presented in Table 2. Soil collected from the oil refinery
225 showed 4% TPHs contamination, and agriculture site soil was free from TPHs contamination. Both
226 soils were used for further experimentation.

227

228 **3.2. Performance of plants in different soil conditions**

229 **3.2.1. Plant physiological parameters**

230 Germination rates (%) of studied plants with different soil conditions are shown in Figure 1.
231 Statistically significant reduction in percentage germination rates for the seeds of alfalfa and white
232 clover were noted cultivated in TPHs contaminated soil, 15.1 and 24.5%, respectively, compared
233 with the quality of clean soil. Ryegrass, maize, and wheat seeds were unaffected. Though there
234 was a minimal reduction in percentage germination, it was statistically non-significant. The
235 addition of biochar and compost improved the germination rates. In general, all plants showed
236 statistically improved germination when contaminated soil was amended with compost.
237 Germination rates of 98.5, 99.6, 96.6, 88.96, and 100% were restored with compost in
238 contaminated soil for alfalfa, ryegrass, maize, white clover, and wheat. White clover and alfalfa
239 (Fabaceae family) showed some variation in germination, while ryegrass (Poaceae family)
240 followed a different trajectory. Poaceae family plants, wheat, and maize are confirmed to offer the
241 same germination pattern in other conditions of soil amendments.

242 Fresh and dried biomass of wheat and ryegrass was affected more when cultivated in contaminated
243 soil. Reduction in fresh biomass was 78.9 and 77.7%, while in dried biomass was 81.4 and 80.9%
244 for wheat and ryegrass. Significantly less reduction was noted for white clover in both fresh and
245 dried biomass, 62.4 and 57.9%, respectively. Biochar amendment in alfalfa and white clover, while
246 compost in ryegrass and maize, improved plants' fresh and dried biomass (Table 2). No difference
247 in wheat was observed with the additions of any of these amendments. Further, no significant
248 improvement in the fresh (1.74 ± 0.05 g in biochar amended and 1.91 ± 0.40 g in compost
249 amended) and dried biomass (1.26 ± 0.04 with biochar and 1.25 ± 0.58 with compost) was
250 observed for wheat, cultivated in contaminated soil (Table 2).

251 The negative impact of TPHs contamination on the roots of the selected plant was observed. The
252 maximum percentage decrease between plants cultivated on contaminated (3.66 cm) versus grown
253 on non-contaminated soil (13.57 cm) (73.0%) was observed in white clover. Wheat, ryegrass, and
254 alfalfa roots were 66.5, 56.2, and 50.4% abridged, respectively, compared with root lengths of
255 plants cultivated on clean soil (Table 2). A minor decrease was noted for maize, 35.33 cm in clean
256 soil and 20.55 cm in contaminated soil. Addition of biochar with alfalfa and white clover while
257 compost with ryegrass, maize, and wheat, in contaminated soil, significantly improved root
258 lengths. The root length recoveries (%) for compost were 98.2 in ryegrass, 86.2 in maize and 46.8
259 in wheat, and for biochar was 99.8 for alfalfa, and 61.1 for white clover (Table 2). The highest
260 negative effect of TPHs contamination on the plant's shoot length (59.57%) was found for wheat
261 (Table 2). Wheat shoot length reduced to 13.07 ± 1.07 cm when cultivated on TPHs contaminated
262 soil, from 32.33 ± 2.52 cm when in clean soil. Biochar addition significantly facilitated alfalfa,
263 white clover, and wheat to recover shoot size up to 71.7, 68.8, and 50.41%, respectively. On the
264 other hand, compost enhanced shoot lengths recovery significantly for ryegrass and maize, 95.9
265 and 70.1%, respectively (Table 2).
266 Reduction in chlorophyll content (mg g^{-1}) was observed in all plants when cultivated in
267 contaminated soil (Table 2). Statistically, a significant reduction was noted in white clover, 80.9%,
268 while the lowest reduction was observed in ryegrass (38.0%). The addition of biochar and compost
269 improved chlorophyll content. No significant difference was observed between soil amendments
270 for maize and wheat. In all plants, the addition of biochar was found to show higher chlorophyll
271 contents, compared to the addition of compost, except for ryegrass (3.47 ± 0.25), in which compost
272 improved chlorophyll content up to 94.3%. Biochar-mediated recovery ranged from 90.4 to 35.9%,
273 with the highest ryegrass (3.33 ± 0.03) and lowest 1.88 ± 0.02, for maize (Table 2).

274

275 **3.2.2. Endophytic and rhizospheric CFUs and residual TPHs in soil**

276 Endophytic CFUs of plants cultivated on contaminated soil (CS), CS + Biochar (B), and CS +
277 Compost (C), were noted. Endophytic CFUs (ECFUs) of plants cultivated on clean soils were not
278 presented, as colonizing ECFUs were not TPHs tolerant. TPHs contamination significantly
279 reduced endophytic, as well as rhizospheric CFUs in all plants (Table 3). The endophytic root
280 CFUs of all plants, except for alfalfa, were higher in treatment with compost. For white clover, the
281 impact of biochar and compost showed no significant variation. Contaminated soil amended with
282 biochar showed improved root CFUs for alfalfa ($1.8\ E^7 \pm 3.6\ E^5$), While, in white clover, both
283 biochar and compost application to contaminated soil resulted in significantly higher root
284 endophytic CFUs ($2.0\ E^7 \pm 3.2\ E^5$ and $2.1\ E^7 \pm 3.7\ E^5$, in respective treatment). A similar trend
285 was observed in shoot endophytic CFUs. All plants showed significantly higher shoot endophytic
286 CFUs in compost, except for alfalfa and white clover. No statistically significant variance in white
287 clover between biochar and compost was noted. However, both treatments improved endophytic
288 counts. The alfalfa plant produced statistically higher shoot endophytic CFUs when amended with
289 biochar (Table 3). For rhizospheric CFUs, statistically higher CFUs were noted for contaminated
290 soil amended with compost in all plants except alfalfa and white clover. The alfalfa showed a
291 statistically higher rhizospheric count in biochar amended contaminated soil, and white clover
292 having no significant variation (Table 3).

293 Total petroleum hydrocarbons (TPHs) degradations were improved with the application of
294 amendments (Figure 2). The addition of biochar into contaminated soil without any vegetation
295 resulted in 27% higher TPH removal than negative control's (NC) CS treatment. The addition of
296 compost also lowered the load of TPHs by 6% compared to CS treatment. The highest TPHs

297 degradation was noted among different plants for white clover and alfalfa, 27.25 ± 0.25, and 26.21
298 ± 0.21 g TPHs kg^{-1} of soil amended with biochar. Similarly, with ryegrass, soil amended with
299 compost resulted in higher TPHs degradation, 27.37 ± 0.87 g TPHs kg^{-1} soil. Compared to CS, the
300 status of TPHs degradation in maize and wheat with the amendment was statistically higher, but
301 no significant variation between amended treatments was noted. Plants of ryegrass and alfalfa
302 performed well in contaminated soil compared with the other plants and NC (Figure 2).

303 **3.3. Statistical analysis, the model of factorial experiment, and PCA for collected data**

304 In this analysis, the main focus was to test our state hypotheses using the stated model. Analysis
305 of the model output indicated that soil amendments individually have statistically significant
306 effects on all studied parameters, with a p-value of 0.00 in all cases. Similarly, the selection of
307 plants was also found significantly important (with a very low p-value of 0.00) on all studied
308 parameters except for the root and shoot endophytic CFUs (Table 4). Hence our null hypothesis is
309 rejected that the applied factors have no independent effect, and indicating the significant impact
310 on plant growth, individually. The interaction of soil amendments and plant types was also
311 statistically significant for the most studied parameters by analyzing the model output. It rejected
312 the null hypothesis that applied factors have no interactive effect on most of the studied parameters
313 in this study, except in the case root and shoot endophytic CFUs (p-value = 0.60 and 0.66),
314 respectively (Table 4).

315 Further, in general, a positive correlation (R= 0.62, p= 0.00, n=54) between rhizospheric CFUs
316 and TPHs removal was found in this study. For the third hypothesis, $SS_{contrast}$ is 1000, F-ratio is
317 39.52, and the p-value is less than 0.0001. The hypothesis of no difference between the Poaceae
318 family and the Fabaceae family is rejected (Table 4). We conclude that these two families have a

319 significant difference in mean from the data compared to general. Hence it can be inferred that the
320 type of family can play a substantial role in TPHs degradation.
321 The application of biochar and compost on all plants showed variable interactions, which can be
322 visualized by seeing the variability of data collected for each plant using PCA. The PCA
323 application yielded two dimensions for all the plants. The details for the principal component
324 extraction based on the Eigenvalues are provided in Supplementary Table 1, the PC loading values
325 were provided in Supplementary Table 2. In contrast, the scattered plots were provided in
326 Figure3.1 and 3.2. For Alfalfa (Figure 3.1.a and b, for biochar and compost, respectively), root
327 CFUs, DW, and GP showed the highest data variability (66.03%) with biochar. In contrast, with
328 compost, the SL, GP, FW, RL, and rhizospheric CFUs were found to have higher data variability
329 (70.89%). Rhizospheric CFUs, FW, RL, and RL in biochar, and Rhizospheric CFUs, FW, RL,
330 TChl, DW, and RL with compost, were the parameters that resulted in the highest variation, 78.38
331 and 70.68%, respectively, with maize (Figure 3.1.c and d). With ryegrass (Figure 3.1.e and f, for
332 biochar and compost, respectively), the rhizospheric, root, and shoot CFUs and RL have higher
333 variance in the data (56.93%) when cultivated with biochar. In contrast, compost TChl, DW, and
334 RL constituted higher variation (71.53%). Application of biochar with white clover resulted in
335 significantly higher variation (66.79%) with the shoot and root CFUs, TChl, DW, RL, and SL. In
336 contrast, when grown with compost, SL, RL, FW, DW, and root CFUs showed higher variably
337 (64.20%). With wheat, upon cultivation with biochar, higher variability (64.20%) was noted for
338 SL, RL, FW, DW, and root CFUs. The biochar application with wheat resulted in higher variability
339 (55.39%) with rhizospheric, shoot and root CFUs, SL, and RL, while compost application resulted
340 in higher variability with TChl, DW, and RL. The response of soil amendments (biochar and
341 compost) application is variable concerning the difference studied plants.

342

343 ## 4. Discussion

344 The current study reports the efficacy of organic amendments and the use of phytoremediation to
345 treat TPHs. Plants' physiological parameters, microbial analysis, and residual TPHs were noted as
346 indicators for the benefit of a conclusive strategy for TPHs removal. It is well documented that
347 TPHs contamination in soil affects the physicochemical properties of soil (Han et al., 2016). Soil
348 contaminated with petroleum hydrocarbon is characterized by a high C/N ratio and limited
349 amounts of nitrates and phosphates compared to uncontaminated soil (Alavi et al., 2016). Plant
350 growth and germination are affected by the limited availability of N and P. Seed germination
351 depends on many factors such as soil type, plant type, and hydrocarbon contamination (Shahsavari
352 et al., 2013). This study revealed that percentage germination of ryegrass, maize, and wheat was
353 not affected in contaminated soil, but a statistically significant reduction was observed for alfalfa
354 and white clover (Figure 1). A possible explanation for this trend is that in the early germination
355 period, the seed itself contains sufficient nutrients required to break seed dormancy and have
356 tolerance capacity (Júnior et al., 2016). Large-size seeds show more percentage germination than
357 small seeds because large-size seeds can capture more food and nutrients (Lloret et al., 1999).
358 Ryegrass seed germination was reported to stay unaffected at 5% TPHs soil contamination (Issoufi
359 et al., 2006). Soil amendments, plant type individually, and their interaction are the essential
360 determinants for the TPHs removal, plant physiological parameter, and plant microbial interactions
361 and growth (Table 2, 3, and 4, and Figure 2).

362 Even though plants can perform well with the germination rate, it does not guarantee the fate of
363 holistic phytoremediation. While some plants have intrinsic capacity to tolerate contamination
364 loads of organic and inorganic contamination at early seed germination stages, the contamination

365 can induce damage to plant seedlings at the early developmental stages (Júnior et al., 2016). A
366 similar finding was reported by Shahsavari et al. (2013) that 1% of crude oil contamination
367 significantly affected the growth of wheatgrass, but it did not affect seed germination.
368 Hydrocarbon contamination alters the physicochemical properties of soil (Hussain et al., 2018;
369 Khan et al., 2019a). TPHs have the characteristic of hydrophobicity and alter the soil and water
370 interactions; thereby, affecting the transfer of gases, uptake of water, and available nutrients (Khan
371 et al., 2019b).
372 Physiological parameters studied for all plants were considerably reduced compared to clean soil
373 control (Table 2). Plants' physiological responses are not generalized, and each plant type performs
374 differently in retort to TPHs contaminated soil and amendments provided (Hussain et al., 2018;
375 Khan et al., 2019c). Similar findings were noted in the present experiment, with all plants being
376 affected with 4% TPH contamination. A trend was observed in plant physiological parameters.
377 Biochar improved the growth of plants belonging to Fabaceae (white clover and alfalfa), while
378 Poaceae plants (wheat, maize, and ryegrass) were found performing better in compost amendments
379 (Table 2). Rondon et al. (2007) proposed that biochar improves the growth of legumes through
380 biological nitrogen fixation, enhancing the plant-microbe interactions. It contributed to the better
381 performance of Fabaceae plants when contaminated soil was amended with biochar. Chen et al.
382 (1996) suggested that compost improves the growth of ryegrass (belonging to the Poaceae family),
383 as it contains high N, P, K, and other major and minor nutrients. It can be inferred that the
384 physiological parameters of wheat, maize, and ryegrass (Poaceae family) are improved with
385 compost amendment (Table 2). Further, soil amendment and plant type interaction were found
386 significant (Table 4), suggesting that specific soil amendments are meritorious for definite plant
387 types. Care must be taken when selecting amendments for facilitating phytoremediation.

388 Rhizospheric and endophytic plant-associated bacteria are essential for the remediation of polluted
389 soil (Mushtaq et al., 2020). Improvement in plant growth also enhances microbial abundance
390 (McLeod et al., 2016). Compost and biochar improve the microbial count as biochar decreases
391 leaching of nutrients, more water availability, enhances aeration of the soil, changes in soil pH,
392 and offers pores for sorption of toxic contaminants and refuge from bacterial grazers (Iqbal et al.,
393 2019). Compost acts as a nutrient and soil ameliorant by changing moisture content, pH, and soil
394 structure, therefore, improves the soil environment for indigenous hydrocarbon-degrading
395 microorganisms (Mushtaq et al., 2020; Khan et al., 2017). Microbes do not utilize biochar directly,
396 but the increase in number is due to improved physicochemical characteristics of soil (Hussain et
397 al., 2018). However, compost is an available source of nutrients to microbes, besides increasing
398 soil physicochemical characteristics. In the present study, the condition in which plant growth was
399 improved, also found to attain higher endophytic and rhizospheric counts (Table 3). The individual
400 effect of soil amendments was statistically significant for the endophytic and rhizospheric count.
401 At the same time, interaction is only substantial for rhizospheric microbial abundance (Table 4).
402 Therefore, it can be inferred that with specific soil amendments and appropriate plants,
403 improvement in the rhizospheric CFUs occurred.

404 In the present study, unplanted contaminated soil, deprived of any amendments, showed 0.18%
405 hydrocarbon from the initial concentration. Barrutia et al. (2011) also reported that the TPHs status
406 remains constant in such treatments. Biochar removed 27.24% of TPHs from the aged-
407 contaminated soil compared to contaminated soil with no amendment (Figure 2). In this study,
408 enhanced removal of TPHs was significantly affected by soil amendment individually. It can be
409 explained as in biochar amended soil strong sorption of TPHs facilitated remediation. In compost-
410 containing treatments, increased degradation was due to microbes providing nutrients, enhanced

oxygen, and improved soil texture. The results are also well supported by the finding of Hussain et al., (2018). Another explanation for removing TPHs from the soil is the contribution of light n-alkanes volatilization due to rhizodegradation, as Gallego et al., (2007) proposed.

Similarly, planted treatments significantly improved TPHs degradation as compared to unplanted control. Plant type was found to affect the degradation process individually (Table 4). Observations of *Lolium multiflorum* (ryegrass) and *Medicago sativa* (alfalfa) further concerted the findings, which considerably reduced the concentration of TPHs from contaminated soil, 59.55 35.21%, respectively. Additionally, the interactive effect of soil amendment and plant type was also found significant (Figure 3.1 and 3.2, Table 4), suggesting that the use of appropriate plants along with proper soil amendment can exacerbate the TPHs removal in soil.

Appropriate plants and amendments are pivotal for the conclusive application of phytoremediation for any contaminate (Khan et al. 2020). The present study explored the use of grass plants (belonging to the Fabaceae and Poaceae family) and soil amendments (including biochar and compost) in TPHs contaminated soil. A three-step characterization is required for the definitive biological treatment of contaminated sites (Azubuike et al., 2016). The first and foremost importance is to study the treatability of the contaminants through different biological methods. Subsequently, if needed, environmental conditions like soil modification, microbial amendments, vegetative cover, and contaminant dilution need to be modified to optimize the removal/degradation of contaminants. Finally, the site must be characterized as well because these attributes influence the control and operation of engineering processes manipulating microbial activities in the subsurface. Therefore, it is essential to study the long-term exposure to confirm the distribution/translocation/fate of contaminants, plants' response to contaminant stress, and the

433 possibility of maneuvering the environmental matrices in favor of contaminants, notably TPHs
434 phytoremediation.

435 **5. Conclusion**

436 The potential of each type of grass is different, and not all types of grasses are suitable to obtain
437 prolific results of phytoremediation. It is, therefore, vital to identify the approaches with which
438 optimal benefits can be attained. We conclude that wheat, maize, and ryegrass (belonging to the
439 Poaceae family) perform better in phytoremediation of TPHs when contaminated soil is amended
440 with compost. At the same time, biochar was found more suitable for white clover and alfalfa.
441 Additionally, ryegrass and alfalfa are better than other studied plants for treating TPHs as they
442 performed statistically higher removal. As biochar and compost can facilitate the biodegradation
443 process of TPHs, individually and interactively, with the appositionally selected plant,
444 consideration for their use must be made when designing holistic TPHs phytoremediation strategy.
445

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450

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455

456 ## 8. Authors Contributions

457 Conceptualization- Yousaf U, Khan AHA, and Yousaf S.

458 Methodology- Yousaf U, Khan AHA, and Farooqi A.

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463 Supervision- Yousaf S.

464 Project administration- Yousaf U, and Yousaf S.

465

466 ## 9. References

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159 After plant harvesting, TPHs were determined using the method defined by Hussain et al. (2018).
160 The quantification of TPH (C10-C40) was performed using the Gas Chromatograph model
161 Hewlett Packard 5890 (series II) coupled with a flame ionization detector (FID). The whole unit
162 of GC-FID and was also equipped with HP automatic liquid injector 7673. The instrument operated
163 in SIM mode, particularly as ion monitoring mode. An already baked column (30 m×0.25 mm)
164 glazed with 0.25 μm 5% phenyl and 95% methyl polysiloxane as the stationary phase for
165 hydrocarbons analysis. The temperature for both detector and injector was set at 300 °C and 280
166 °C, respectively. Helium as carrier gas throughout this procedure was used, and the quantification
167 of TPH via MS ChemStation was performed. The GC oven was programmed according to the
168 following program, 60 °C for 60 seconds, 20 °C min-1 up to the level of 340 °C, and lastly kept at
169 340 °C for 10 min. The results of TPHs concentrations (75 days) were presented in percentages
170 (%). All the calculated hydrocarbons concentrations were expressed on a dry matter basis. The
171 microbial abundance of crude oil tolerating rhizospheric bacteria was estimated (2016a). Briefly,
172 serial dilutions (100 μl of 10^{-1} to 10^{-5} dilutions) of separated soil samples were made and
173 subsequently spread on nutrient agar media having 2% crude oil. Then, plates were incubated at
174 30 °C for 24 hrs, and colonies were counted.

175

176 **2.4. Statistical analysis and model of a factorial experiment**

177 Statistical analysis was performed on the R software environment for statistical computing. In the
178 current investigation, the treatment combinations were the varying levels of investigating factors.
179 We are using two factors;
180 (i) Factor one is the soil amendment type.
181 (ii) Factor two is a plant type.

182 The complete set of treatments is applied at random to the experimental unit in 'r' replicates. The

183 collection of remedies to investigate are used in experimental units. Let us consider the model;

184 $$f(x_1, x_2) = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2$$

185 Where the treatment factors display a factorial structure. For a simple factorial structure, we

186 consider two factors, x_1 (soil amendment type) and x_2 (plant type) say, with 'a' and 'b' levels each,

187 respectively. There are "a×b" treatments. As discussed above, the two factors were soil amendment

188 type and plant type run in "r" replicates. We consider this structure if 'Y_{ijk}' is the observation at i^{th}

189 level of factor soil amendment type to j^{th} level of factor plant type in the kth replicate. The model

190 for data can be expressed as;

191 $$Y_{ijk} = \mu + \eta_i + \gamma_j + (\eta\gamma)_{ij} + \varepsilon_{ijk}$$

192 Where μ represents the overall mean effect, η_i is the effect of ith level of soil amendment type 'i'

193 and γ_j effect of j^{h} level of plant type 'j'. $(\eta\gamma)_{ij}$ are interaction effects among plant type 'i' and soil

194 amendment type 'j'. The random error is ε_{ijk} represents experimental error. By using this model,

195 we are testing the hypothesis;

196 i) Whether the factors, soil amendment type, and plant type significantly affect their varying

197 levels?

198 ii) What are the significant interactions of applied factors against studied parameters?

199 iii) Comparisons among families of plants.

200 For the third hypothesis, i.e., comparisons among families of plants, we test the following contrast

201 in plant parameters ANOVA (hypothesis at α= 1% level of significance is rejected). In the

202 remaining two experiments, this test is meaningless as factors are not independent. This contrast

203 can be tested. Similarly, if factors would have been independent. For the test, our null hypothesis

204 is;

Authors Contributions

Conceptualization- Yousaf U, Khan AHA and Yousaf S

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Project administration- Yousaf U, and Yousaf S

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Ethical Approval, Consent to Participate, and Consent to Publish

Not applicable

Graphical Abstract

Poaceae family

Fabaceae family

Triticum aestivum (Wheat)

Zea mays (Maize)

Loilum multiflorm (Ryegrass)

Medicago sativa (Alfalfa)

Trifolium repens (White clover)

Compost

Biochar

Compost with Poaceae plants, while biochar with Fabaceae plants resulted in improved plant growth parameters and TPHs phytoremediation.

Compost and biochar alone removed 6.01% and 27.24% TPHs, respectively.

The interactive effect of soil amendments and plant types was also noted for studied parameters and TPHs degradation.

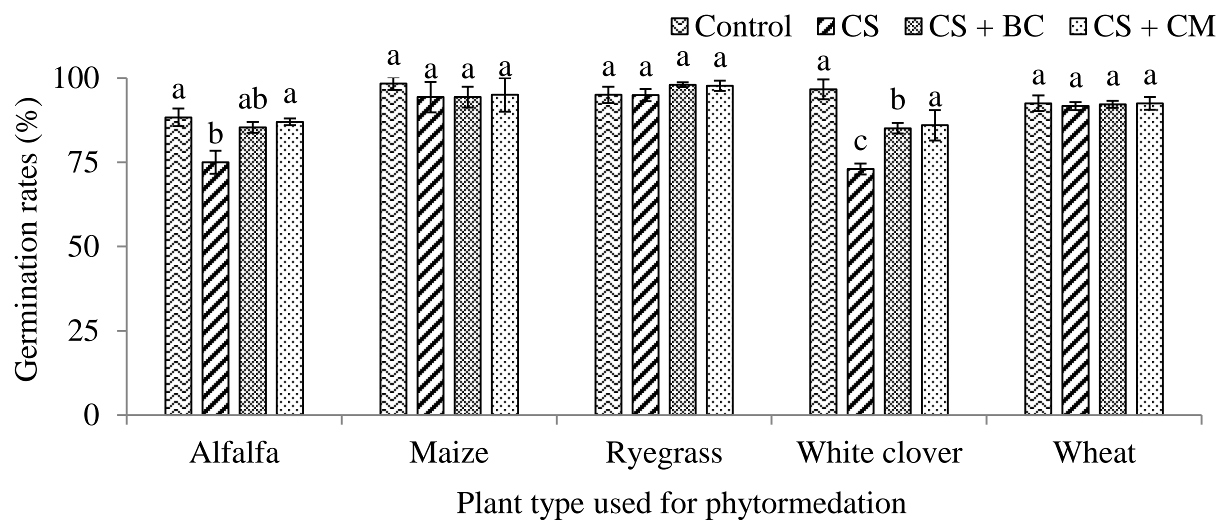

Figure 1. Germination rates of cultivated plants used for TPHs phytoremediation with different treatments. CS; Contaminated soil, CS+BC; Contaminated soil + Biochar, and CS+CM Contaminated soil + Compost. Data presented for each case in all parameters are in means, with n = 3 ± SD

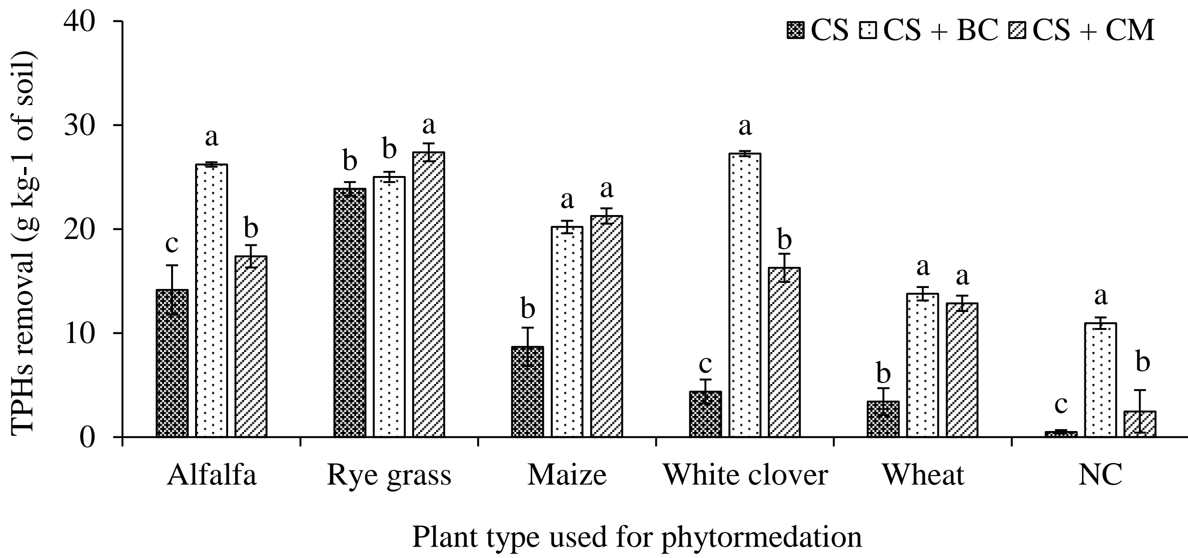

Figure 2. TPHs phytoremediation by different plants with reference to different treatments. CS; Contaminated soil, CS+BC; Contaminated soil + Biochar, and CS+CM Contaminated soil + Compost. NC meant negative control. Data presented for each case in all parameters are in means, with n = 3 ± SD

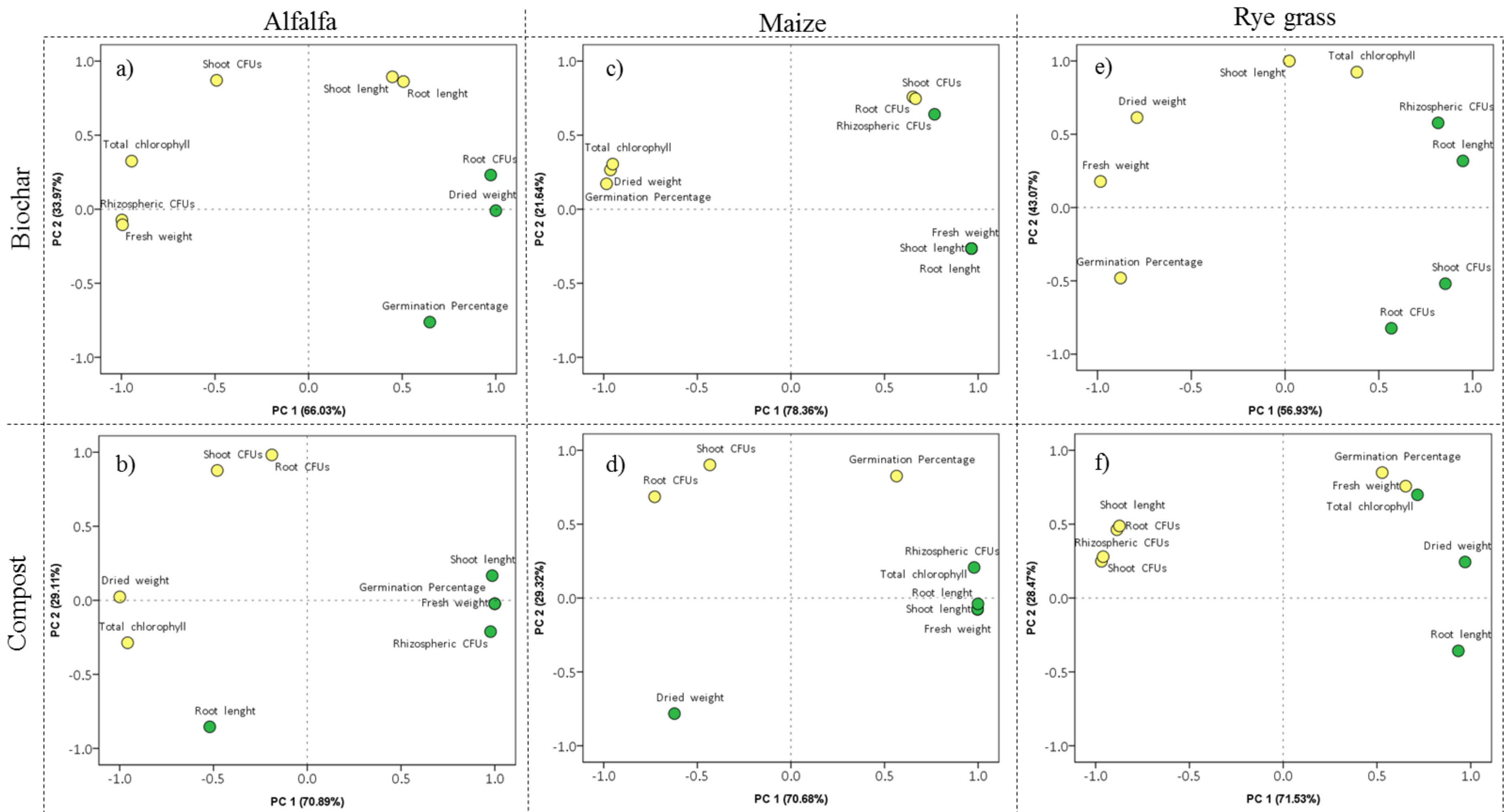

Figure 3.1. Principle component analysis (PC) plots for the studied parameters of plants, including Alfalfa (a, and b), Maize (c, and d), and Ryegrass (e, and f) for treatments with biochar and compost, respectively. In PC plots, the green dot has a higher loading value in PC 1, while yellow has a higher loading value in PC 2.

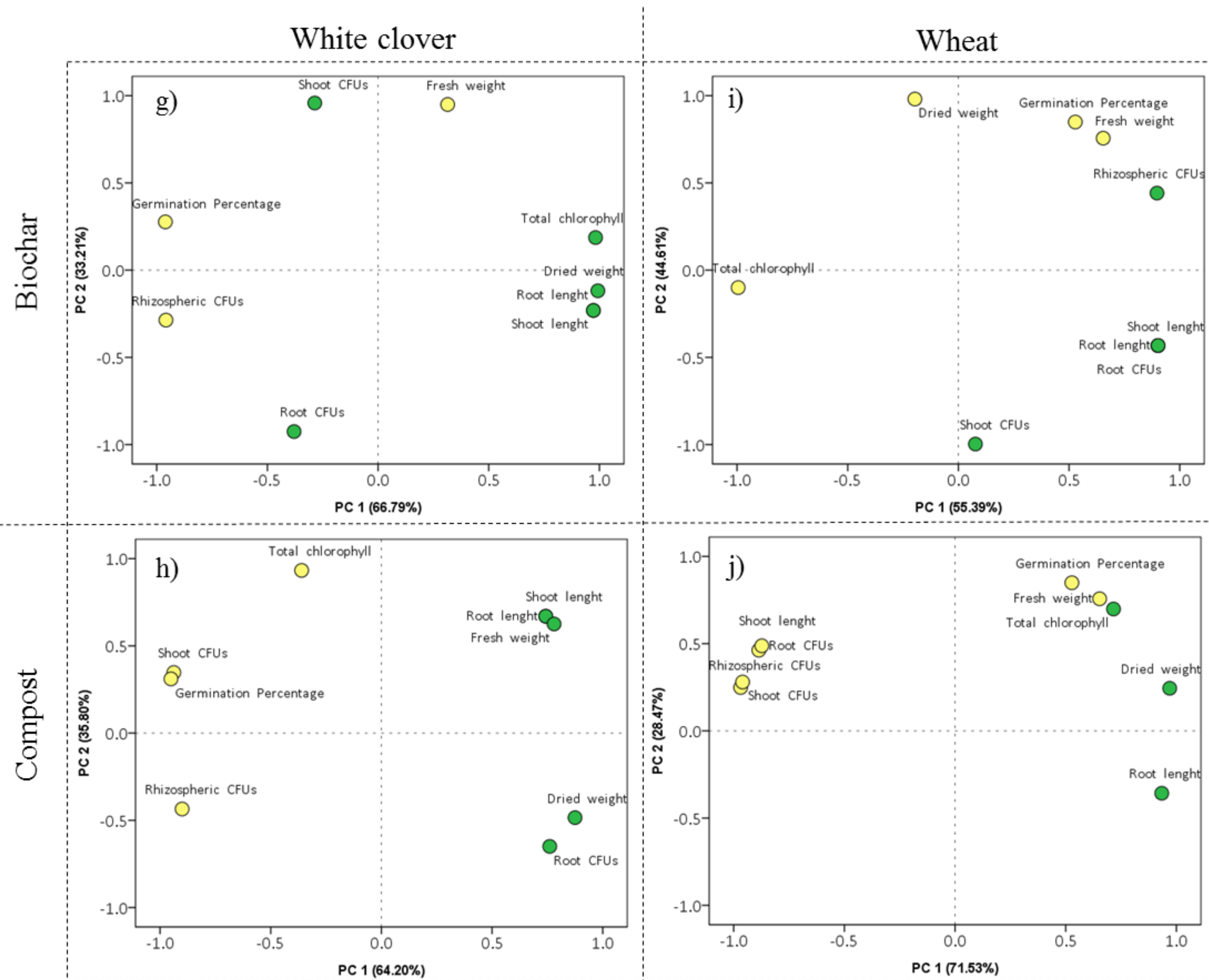

Figure 3.2. Principle component analysis (PC) plots for the studied parameters of plants, including wheat (f, and g), and White clover (i, and j) for treatments with biochar and compost, respectively. In PC plots, the green dot has a higher loading value in PC 1, while yellow has a higher loading value in PC 2.

Table

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Table 1. Details of plants' root characters

Botanical name	**Common name**	**Family**	**Seed roots**	**Postembryonic roots**	**Shoot roots**
Triticum aestivum	Wheat	Poaceae	Seminal roots	Nodal (crown or adventitious) roots	Not present
Zea mays	Maize	Poaceae	Embryonic primary and seminal roots	Crown roots	Brace roots
Trifolium repens	White clover	Fabaceae	Seminal roots	Nodal (crown or adventitious) roots	Not present
Medicago sativa	Alfalfa	Fabaceae	Seminal roots	Nodal (crown or adventitious) roots	Not present
Lolium multiflorum	Ryegrass	Poaceae	Seminal roots	Nodal (crown or adventitious) roots	Not present

Table 2. Physiological parameters of plants cultivated in different soil conditions

Parameter	Soil type	Alfalfa	Ryegrass	Maize	White clover	Wheat
Plants' fresh weight (g plant^{-1})	Clean soil	2.03 ± 0.46[a]	7.23 ± 0.38[a]	36.17 ± 1.04[a]	6.71 ± 0.17[a]	7.27 ± 0.21[a]
	CS	1.14 ± 0.16[c]	1.61 ± 0.27[c]	13.48 ± 1.21[b]	2.52 ± 0.14[c]	1.53 ± 0.09[b]
	CS + BC	2.02 ± 0.25[a]	2.63 ± 0.32[b]	14.24 ± 1.01[b]	6.62 ± 0.28[a]	1.74 ± 0.05[b]
	CS + CM	1.70 ±0.20[b]	7.21 ± 0.59[a]	34.07 ± 0.54[a]	4.34 ± 0.19[b]	1.91 ± 0.40[b]
Plants' dried weight (g plant^{-1})	Clean soil	0.91 ± 0.13[b]	4.73 ± 0.49[a]	15.50 ± 2.00[a]	4.40 ± 0.36[a]	5.70 ± 0.10[a]
	CS	0.74 ± 0.05[b]	0.90 ± 0.04[c]	5.53 ± 0.13[b]	1.85 ± 0.05[c]	1.06 ± 0.03[b]
	CS + BC	1.70 ± 0.20[a]	1.65 ± 0.31[b]	6.41 ± 0.13[b]	4.24 ± 0.15[a]	1.26 ± 0.04[b]
	CS + CM	0.90 ± 0.20[b]	5.30 ± 0.41[a]	14.52 ± 0.98[a]	3.53 ± 0.15[b]	1.25 ± 0.58[b]
Root length (cm)	Clean soil	15.86 ± 2.24[a]	26.15 ± 0.35[a]	35.33 ± 1.53[a]	13.57 ± 1.93[a]	42.67 ± 3.06[a]
	CS	6.94 ± 2.55[c]	12.95 ± 1.15[c]	20.55 ± 0.15[c]	3.66 ± 0.09[c]	14.27 ± 4.87[b]
	CS + BC	15.83 ± 3.33[a]	22.20 ± 1.47[b]	23.36 ± 4.09[c]	8.30 ± 0.50[b]	18.34 ± 2.03[b]
	CS + CM	10.29 ± 0.54[b]	25.70 ± 2.49[b]	30.47 ± 2.63[b]	5.46 ± 1.04[c]	20.00 ± 2.00[b]
Shoot length (cm)	Clean soil	3.97 ± 0.25[a]	12.30 ± 0.30[a]	34.33 ± 2.52[a]	6.00 ± 1.02[a]	32.33 ± 2.52[a]
	CS	2.00 ± 0.20[c]	8.47 ± 0.45[b]	16.73 ± 1.25[d]	2.82 ± 0.68[c]	13.07 ± 1.07[c]
	CS + BC	2.85 ± 0.63[b]	7.30 ± 1.10[b]	19.85 ± 3.45[c]	4.13 ± 0.70[b]	16.30 ± 2.50[b]
	CS + CM	2.47 ± 0.15[c]	11.80 ± 1.70[a]	24.09 ± 1.19a[b]	3.29 ± 0.11[c]	13.67 ± 2.52[c]
Chlorophyll content (mg g^{-1})	Clean soil	7.66 ± 0.20[a]	3.68 ± 0.12[a]	4.71 ± 0.19[a]	4.84 ± 0.14[a]	5.62 ± 0.61[a]
	CS	4.58 ± 0.03[d]	2.28 ± 0.03[c]	1.36 ± 0.02[c]	0.92 ± 0.13[d]	1.53 ± 0.25[c]
	CS + BC	6.81 ± 0.22[b]	3.33 ± 0.03[b]	1.88 ± 0.02[b]	3.33 ± 0.16[b]	2.02 ± 0.02[b]
	CS + CM	5.29 ± 0.27[c]	3.47±0.25[ab]	2.06 ± 0.02[b]	1.77 ± 0.15[c]	2.77 ± 0.23[b]

[1]Contaimnated soil, [2]Contaminted soil + Biochar, and [3]Contaminted soil + Compost

Similar small letters in the same column (for each plant) within the same studied parameter of the experiment are statistically insignificant

Significantly highest mean is represented as "a" column-wise followed by later alphabets for lower means

Data presented for each case in all parameters are in means, with n = 3 ± SD, post hoc test applied Duncan MR Test, p = 0.05

*a non-vegetative control with contaminated soil only

Table 3. TPHs tolerant bacterial CFUs counts of each treatment upon TPHs exposure cultivated in different conditions

Parameter	Soil type	Alfalfa	Ryegrass	Maize	White clover	Wheat	NC*
Root endophytic CFUs[+] (CFUs g^{-1})	CS[1]	$8.6\ E^{6} \pm 4.0\ E^{4\ c}$	$8.9\ E^{6} \pm 2.6\ E^{4\ c}$	$8.4\ E^{6} \pm 3.0\ E^{5\ c}$	$8.1\ E^{6} \pm 3.5\ E^{4\ c}$	$7.5\ E^{6} \pm 4.0\ E^{4\ c}$	
	CS + BC[2]	$1.8\ E^{7} \pm 3.6\ E^{5\ a}$	$1.7\ E^{7} \pm 4.9\ E^{5\ b}$	$1.5\ E^{7} \pm 3.5\ E^{5\ b}$	$2.0\ E^{7} \pm 3.2\ E^{5\ a}$	$9.1\ E^{6} \pm 5.0\ E^{4\ b}$	
	CS + CM[3]	$1.7\ E^{7} \pm 4.7\ E^{5\ b}$	$3.4\ E^{7} \pm 1.6\ E^{6\ a}$	$3.0\ E^{7} \pm 3.2\ E^{5\ a}$	$2.1\ E^{7} \pm 3.7\ E^{5\ a}$	$1.6\ E^{7} \pm 4.0\ E^{5\ a}$	
Shoot endophytic CFUs[+] (CFUs g^{-1})	CS	$6.3\ E^{4} \pm 2.1\ E^{3\ c}$	$6.8\ E^{4} \pm 2.0\ E^{3\ c}$	$5.9\ E^{4} \pm 2.5\ E^{3\ c}$	$5.5\ E^{4} \pm 5.0\ E^{3\ b}$	$4.7\ E^{4} \pm 2.5\ E^{3\ c}$	
	CS + BC	$1.9\ E^{5} \pm 2.0\ E^{3\ a}$	$1.8\ E5 \pm 2.0\ E^{3\ b}$	$1.6\ E^{5} \pm 4.5\ E^{3\ b}$	$2.1\ E^{5} \pm 9.6\ E^{3\ a}$	$7.2\ E^{4} \pm 2.5\ E^{3\ b}$	
	CS + CM	$1.8\ E^{5} \pm 4.0\ E^{3\ b}$	$3.8\ E^{5} \pm 1.0\ E^{4\ a}$	$3.1\ E^{5} \pm 1.0\ E^{4\ a}$	$2.1\ E^{5} \pm 7.6\ E^{3\ a}$	$1.7\ E^{5} \pm 3.5\ E^{3\ a}$	
Rhizospheric CFUs[+] (CFUs g^{-1})	CS	$9.3\ E^{7} \pm 1.5\ E^{6\ c}$	$9.5\ E^{7} \pm 1.5\ E^{6\ c}$	$9.2\ E^{7} \pm 1.5\ E^{6\ c}$	$9.0\ E^{7} \pm 1.0\ E^{6\ b}$	$8.1\ E^{7} \pm 6.3\ E^{6\ c}$	$3.7\ E^{4} \pm 2.5\ E^{3\ c}$
	CS + BC	$1.4\ E^{8} \pm 3.5\ E^{6\ a}$	$1.3\ E^{8} \pm 2.5\ E^{6\ b}$	$1.1\ E^{8} \pm 3.5\ E^{6\ b}$	$1.6\ E^{8} \pm 5.7\ E^{6\ a}$	$9.6\ E^{7} \pm 4.0\ E^{6\ b}$	$7.1\ E4 \pm 3.1\ E^{3\ b}$
	CS + CM	$1.3\ E^{8} \pm 1.5\ E^{6\ b}$	$2.7\ E^{8} \pm 1.8\ E^{7\ a}$	$2.0\ E^{8} \pm 4.2\ E^{7\ a}$	$1.5\ E^{8} \pm 2.1\ E^{7\ a}$	$1.2\ E^{8} \pm 2.5\ E^{6\ a}$	$7.9\ E^{5} \pm 5.5\ E^{4\ a}$

[1]Contaimnated soil, [2]Contaminted soil + Biochar, and [3]Contaminted soil + Compost

[+]Root and shoot endophytic CFUs are in gm of fresh plant material, while rhizospheric CFUs are gm of 2.5 mm sieved soil

Similar small letters in the same column (for each plant) within the same studied parameter of the experiment are statistically insignificant

Significantly highest mean is represented as ”a” column-wise followed by later alphabets for lower means

Data presented for each case in all parameters are in means, with n = 3 ± SD, post hoc test applied Duncan MR Test, p = 0.05

*NC = Negative control (a non-vegetative control with contaminated soil only)

Table 4. Statistical model's two-way ANOVA summary comparison of individual and interactions in the studied parameter of plants

Source		Sum of Squares*	df[a]	Mean Square	F value	Significance
Soil amendments (SA)	Germination Rates	555.51	3	185.17	7.32	0.00
	Shoot length	717.22	3	239.07	79.84	0.00
	Root length	1682.29	3	560.77	106.95	0.00
	Plant fresh weight	308.29	3	102.95	399.314	0.00
	Plant dried weight	281.48	3	93.82	314.31	0.00
	Total chlorophyll	79.52	3	26.51	626.78	0.00
	Soil residual TPHs	1211.53	2	605.76	127.33	0.00
	Rhizospheric CFUs	4.83 E^{16}	2	2.41 E^{16}	75.44	0.00
	Root ECFUs[b]	1.85 E^{15}	2	9.27 E^{14}	35.11	0.00
	Shoot ECFUs	2.82 E^{11}	2	1.41 E^{11}	38.60	0.00
Plant	Germination Rates	1610.88	4	402.72	15.92	0.00
	Shoot length	4121.51	4	1030.37	344.09	0.00
	Root length	3496.05	4	874.01	166.69	0.00
	Plant fresh weight	3201.93	4	800.48	3112.29	0.00
	Plant dried weight	938.31	4	234.57	785.825	0.00
	Total chlorophyll	103.76	4	25.94	613.35	0.00
	Soil residual TPHs	2211.11	4	552.78	116.19	0.00
	Rhizospheric CFUs	1.54 E^{17}	4	3.84 E^{16}	119.95	0.00
	Root ECFUs	5.71 E^{13}	4	1.43 E^{13}	0.54	0.71
	Shoot ECFUs	7.13 E^{9}	4	1.78 E^{9}	0.48	0.74
SA * Plant	Germination Rates	677.92	12	56.49	2.23	0.03
	Shoot length	573.99	12	47.83	15.97	0.00
	Root length	1045.63	12	87.14	16.62	0.00
	Plant fresh weight	681.43	12	51.53	200.37	0.00
	Plant dried weight	709.05	12	59.08	197.94	0.00
	Total chlorophyll	19.06	12	1.58	37.56	0.00
	Soil residual TPHs	547.28	8	68.41	14.38	0.00
	Rhizospheric CFUs	3.23 E^{16}	8	4.04 E^{15}	12.63	0.00
	Root ECFUs	1.69 E^{14}	8	2.11 E^{13}	0.80	0.60
	Shoot ECFUs	2.14 E^{10}	8	2.67 E^{9}	0.73	0.66
Residuals	Germination Rates	1011.81	40	25.29		
	Shoot length	119.78	40	2.99		
	Root length	209.73	40	5.24		
	Plant fresh weight	10.28	40	0.25		
	Plant dried weight	11.94	40	0.29		
	Total chlorophyll	1.69	40	0.04		
	Soil residual TPHs	185.542	39	4.76		
	Rhizospheric CFUs	1.25 E^{16}	39	3.20 E^{14}		
	Root ECFUs	7.92 E^{14}	30	2.64 E^{13}		
	Shoot ECFUs	1.09 E^{11}	30	3.65 E^{9}		

A p-value lower than 0.05 in sig. the column is considered statistically significant

*Type I sum of square

[a]df =Degree of freedom

[b]ECFUs= Endophytic CFUs