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Influence of the biochar on petroleum hydrocarbon degradation intensity and ecological condition of Haplic Chernozem

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Abstract

It was evaluated the impact of biochar on the petroleum hydrocarbons degradation intensity and the ecological condition of Haplic Chernozem. The study was conducted in the model experiment conditions with Haplic Chernozem contaminated with 5% of petroleum hydrocarbons with application of 10 and 20% biochar. The number of soil bacteria, the activity of catalase and dehydrogenases, germination ability and radish root and shoot length, soil respiration considered to evaluate biological activity. It has been established that 10% biochar application led to intensification of petroleum hydrocarbons degradation up to 17% compare to contaminated soil. Upon adding 10% biochar the CO_2 emission increased up to 70-85% on the 18-19th days, then reduced by the 28-30th days till soil emission with the application of biochar in the amount of 20% from soil mass. Activity of dehydrogenases of Haplic Chernozem were stimulated after application of 10% biochar up to 49% compared to control. Biochar application in the doses of 10 and 20% increased the number of soil bacteria up to 209 and 203%, respectively. Application of 20% biochar intensified the germination and early growth of radish seeds. The germination ability, length of radish shoots and roots increased to 44, 66 and 44%, respectively, compare to control. The biochar application in petroleum contaminated soil increased the activity of catalase, dehydrogenases and the number of soil bacteria. Biochar using in doses 10% and 20% contributes to acceleration of petroleum hydrocarbons degradation and improvement of the soil ecological condition.

Keywords: Biochar, petroleum hydrocarbons contamination, soil, bioremediation, residual content of petroleum hydrocarbons

Introduction

Soil and agricultural land contamination with petroleum hydrocarbons constantly increases at the growth of petroleum hydrocarbons consumption. According to the data of the Ministry of Energy of Russia, the production of petroleum hydrocarbons is annually over 550·109 kg (ME, 2021). At the present time, sales and petroleum hydrocarbons export into the commonwealth of independent states (CIS) and non-CIS countries have increased by more than 240·106 ton every year (Neftegaz, 2021). In the case of petroleum hydrocarbons ingress into the soil, all physical, biological, and ecological functions of this soil are violated. According to the concept "Strategic Directions for Stable Social and Economic Development of Agroindustrial Complex of Russia" presented in the Presidium of the Russian Academy of Sciences in 2017, the fundamental change is required in land relation system and production ecological and adaptation to climate changes (Ushachev, 2017). The climate change due to greenhouse gas emission officially executed thanks to concept of "4 ppm", carbon retention and its sequestering for the soils of Russia and the world as a whole are required. At anthropogenic load, for example, at spillages of petroleum hydrocarbons, the introduced carbon

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disturbs the balance in the natural ecological system. The up-to-date methods of petroleum hydrocarbons contaminated soil detoxification consist of acceleration in petroleum hydrocarbons degradation in the soil with soil fertility restoration and quality (Volchko et al., 2013; Zhang et al., 2016; Al-mansoory et al., 2017).

Applying organic fertilizers changes the texture, structure, size distribution of pores and density, gas exchange, water-retaining capacity, plant growth, and soil structure (Downie et al., 2009; Lu et al., 2010; Popova et al., 2019; Abbaspour et al., 2020). Carbon sequestering and contaminant immobilization using the organic absorbing material (activated carbon, biochar and carbon nanotubes) is restored of the contaminated soils (Wang et al., 2018). Compared with other carbon-bearing materials, biochar as a sorbent has shown the best results to provide the possibility of its use to restore the contaminated sediments of soil. For example, the cost of activated carbon is about 10 USD·m^{-2}, while the cost of biochar is about 2 USD·m^{-2}. Biochar as a main restorative agent is widely used for removal and decontamination of organic compounds, heavy metals and inorganic contaminants (NH_4, PO_4 and NO_3) in wastewaters, as well as a soil additive for immobilization or isolation of organic contaminants, heavy metals (Ye et al., 2017; Yin et al., 2017; Wang, Wang, 2019). Biochar is obtained by biomass pyrolysis (agricultural wastes, wood wastes and wastewater mud) under anaerobic condition. The physical and chemical properties of biochar significantly influence its capability to immobilize contaminants from sediments and soils (Suliman et al., 2016). In biochar, the functional groups are represented by carboxyl (COOH), hydroxyl groups (OH), amine groups ($-NH^{4+}$) and aromatic compounds (–C=C–), which determine interaction mechanisms between biochar and contaminants in bottom sittings (Hung et al., 2020). Pyrolysis temperature and raw biochar biomass structure are the main factors associated with the functional groups (Leng and Huang, 2018). Availability of such groups allows ensuring higher immobilization capacity of biochar in the bottom sittings (Suliman et al., 2016). Biochar's influence on the ecological soil condition at different types of contamination has been studied insufficiently (García-Delgado et al., 2015; Malyan et al., 2019; Soudejani et al., 2019; Abbaspour et al., 2020).

The work objective is an evaluation of the influence of biochar on petroleum hydrocarbon degradation intensity and the ecological condition of Haplic Chernozem Calcic.

Material and Methods

Soil samples

Haplic Chernozem Calcic was selected as the subject of the research. The sampling place is arable soil (top layer- 0-10 cm) of the Botanical Garden of the Southern Federal University, Rostov on Done (sampling point: 47°14'17.54″N 39°38'33.22 ″E). Haplic Chernozem Calcic in the south of Russia is the most fertile soil in Russia. Restoration and retention of soils after anthropogenic load are especially relevant. For the restoration of petroleum hydrocarbons contaminated Haplic Chernozem Calcic functions by petroleum hydrocarbon degradation, the organic carbon-bearing fertilizer - biochar - was used.

Biochar preparation

Biochar is pure charcoal produced during pyrolysis of birch wood (A grade), with carbon content of at least 85% (GOST 7657-84, 1984). The product is made by birchwood (*Betula alba*) pyrolysis at temperature of 360-380°C without access of oxygen by retort units. Biochar was applied based on 10 and 20% to the mass of the soil.

Modelling of experiment

After sampling, the prepared common Haplic Chernozem Calcic was dried, sorted out for plant root removal and screened through a metal sieve with a diameter of a mesh of 2 mm. Soil samples was placed in vegetative pots (mass of soil 200 g) in triplicate. Petroleum hydrocarbon with a concentration of 5% of the soil mass (w/w) was applied to moistened soil (up to 60% from soil mass, (w/w)). Soil moisture content was kept within the whole period of incubation. The experiment scheme is given in Figure 1.

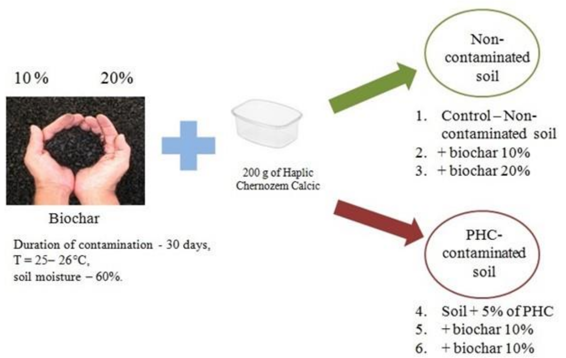

Figure 1. Scheme of the experiment for the introduction of biochar concentration of 10 and 20% into non-contaminated and into the petroleum carbohydrates-contaminated Haplic Chernozem Calcic
Note: PHC, petroleum hydrocarbon

The petroleum hydrocarbon was kindly provided by Novoshakhtinsk's Refinery (Novoshakhtinsk, Rostov region) and used for contamination modelling. This petroleum hydrocarbon was characterized as light petroleum hydrocarbon (density of 0.818 g/m^3). Other properties were summarized as follows: the sulfur mass fraction of 0.43%, mechanical impurities mass fraction of 0.0028%, water mass fraction of 0.03%, at chloride salt concentration of 40.1 mg/dm^3.

Indicators of ecological condition

Upon completion of petroleum hydrocarbon contaminated soil and bioremediation agent exposition residual content of petroleum hydrocarbon and the number of biological indices were determined characterizing the ecological condition of the soil after bioremediation: activity of dehydrogenases and catalase, phytotoxic indices, soil respiration (CO2 emission), the number of soil bacteria (Kazeev et al., 2016).

The residual petroleum hydrocarbon content was determined by the infrared spectrometry method using carbon tetrachloride as an extracting agent (PND F 16.1: 2.2.22-98, 1998).

For evaluation of the ecological condition, the soil phytotoxicity (germination ability, length of roots and spouts), carbon dioxide emission, the activity of soil enzymes (catalase and dehydrogenases) were determined. The condition of soil without soil, but with the application of similar amounts of bioremediation agents to the petroleum hydrocarbon contaminated Haplic Chernozem Calcic was evaluated to determine the toxic level of the proper bioremediation agents.

Soil phytotoxicity was assessed using standard procedures to evaluate the plant shoot and root lengths. The phytotesting of contaminated Haplic Chernozem Calcic was performed using the garden radish (*Raphanus sativus* var. radicula Pers) cultivar "Rubin".

When increasing the exposition term due to petroleum hydrocarbon degradation CO_2 content increases in soil air. When adding bioremediation agents, the degradation rate increases. The intensity of soil degradation was evaluated by means of gas analyzer TESTO-535 with error of ±50 ppm. CO_2 emission in mg/kg for soil was recalculated by Equation (Kadulin et al., 2017):

$$C = \frac{Dc}{dt} \times \frac{V}{S} \times 1000 \times 60 \quad (1)$$

where Dc - carbon dioxide emission, ppm
dt - time period, within which the measurement was performeds
V - chamber volume, m^3
S - chamber cross-section area, m^2

The activity of enzymes of oxidoreductase class (catalase, dehydrogenases) was studied by per A.Sh. Galstyan, F.Kh. Khaziyev (Kazeev et al., 2016). The enzyme activity was studied as per the recommendations of Galstyan (1978) at natural pH of the soil.

The catalase activity (H_2O_2: H_2O_2 – oxidoreductase, EC 1.11. 1.6) was determined by the gasometric method proposed by Galstyan (1978) considers the quantity of decomposed peroxide during reaction with soil by volume of extracted oxygen displacing water from the burette. The enzyme activity was expressed in ml O_2 g^{-1} min^{-1}.

The activity of dehydrogenases (substrate: NAD(P) – oxidoreductase, EC 1.1.1) was determined as per Galstyan (1978) on the restoration of tetrazolium chloride salts in triphenylformazan. The enzyme activity was expressed in mg triphenylformazan 10 g^{-1} 24 h^{-1}.

The number of soil bacteria was determined by the luminescence microscopy method considering the number of soil bacteria after staining with acridine orange (Kazeev et al., 2016). The results were expressed in 10^9 bacteria in 1 g^{-1} soil (Equation 2).

$$M = \frac{4 \times A \times H \times 10^{10}}{p} \quad (2)$$

where M - number of cells per 1 g of soil
A - the average number of cells within one field of vision
H - dilution index
P - the area of the field of vision, μm^2

Statistical analyses

Statistical processing of the data obtained was carried out using the software package of STATISTICA 12.0. Statistics (mean values, dispersion) were determined, and the reliability of different samples was established by using dispersion analysis (Student t-test).

Results

The residual petroleum hydrocarbon content in haplic chernozem calcic after application of the biochar

The efficiency of bioremediation agent application and change in the Haplic Chernozem Calcic ecological condition was evaluated based on petroleum hydrocarbon content in the Haplic Chernozem Calcic remaining after incubation (Figure 2).

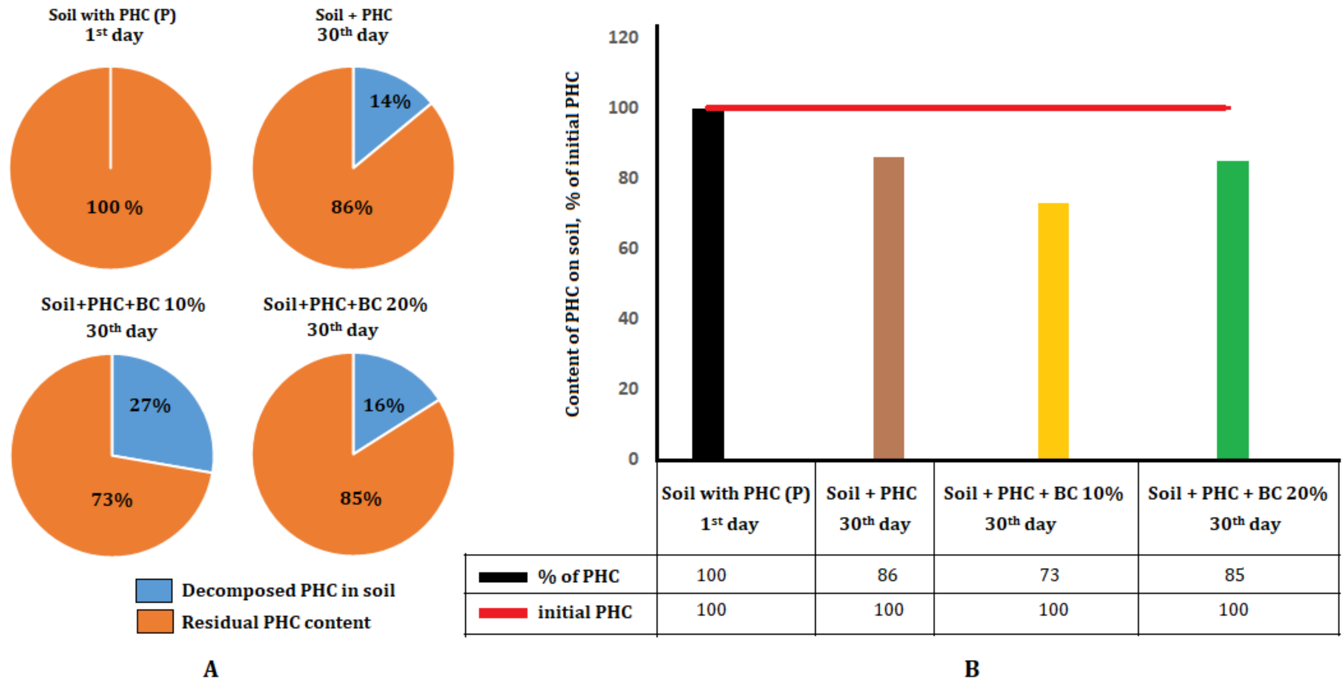

Figure 2. Change in the petroleum hydrocarbon content on the 30th day of the experiment after the introduction of biochar (10 and 20% of the soil mass (w/w)) into the petroleum carbohydrates-contaminated Haplic Chernozem Calcic, % of the PHC-contaminated soil: A) Proportion of decomposition of petroleum hydrocarbons after biochar application; B) Change in oil content, % of the original. Note: BC, biochar; PHC, petroleum hydrocarbon

Petroleum hydrocarbon content after incubation with bioremediation agents was changed to a variable degree. Maximum decrease of petroleum hydrocarbon content by 17% lower than in the petroleum hydrocarbon contaminated variant has been detected in the sample with biochar in the amount of 10% from soil mass. Such an effect is conditioned by the physical structure of the bioremediation agent. By composition, porosity and area of surface biochar is similar to the activated carbon, but it has a wider range of feedstock (Doumer et al., 2016). Within the last years biochar is actively used as an organic fertilizer during restoration of the soil fertility and agricultural functions. In the case of petroleum hydrocarbon and petroleum hydrocarbon product contamination, biochar application has an effect on microbiological activity stimulation. The preparation based on brown coal "Gumikom" is sufficiently efficient during bioremediation of petroleum hydrocarbon contaminated soils (Yagafarova et al., 2016).

Change in the number of soil bacteria of non-contaminated and petroleum hydrocarbon contaminated chernozem after application of the biochar

The number of soil bacteria in pure soil without petroleum hydrocarbon after biochar application in the amount of 20% from soil mass (w/w) has increased by 243% from the control accordingly (Figure 3). The final calculation of the number of soil bacteria was performed using Equation 2. When applying biochar in the amount of 10% from soil mass, the number has increased only by 15%.

Upon applying biochar in amount of 10 and 20% from soil mass (w/w), the number of soil bacteria was 209 and 203% (143 and 138% from petroleum hydrocarbon contamination) from the control.

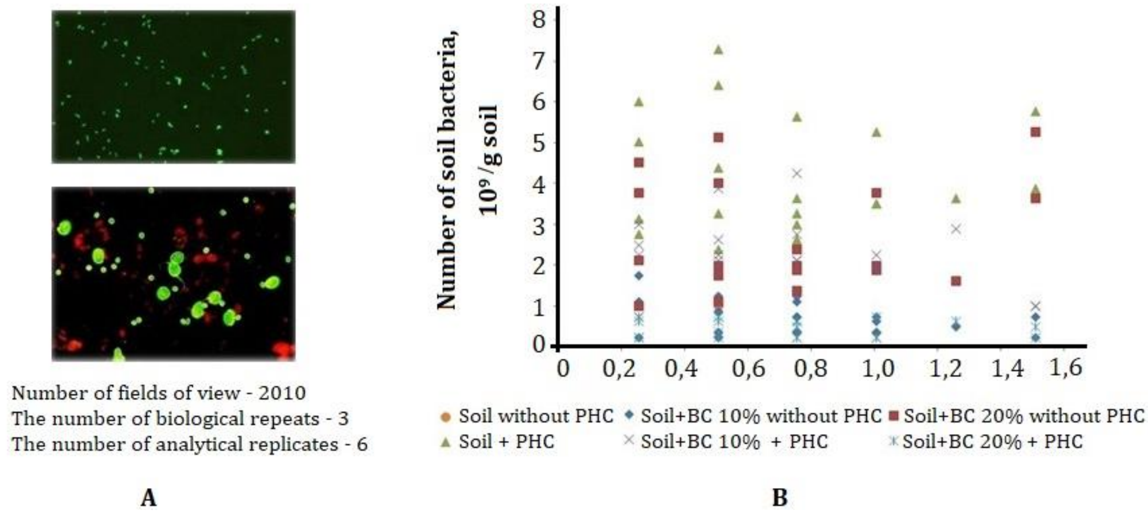

Figure 3. Changes in the number of soil bacteria after the introduction of the biochar (10 and 20% of the soil mass (w/w)) into petroleum hydrocarbon-contaminated Haplic Chernozem Calcic in comparison with clean soil, % of control: A) magnification of the view of bacteria under a 40X fluorescent microscope; B) the number of soil bacteria.
Note: BC, biochar; PHC, petroleum hydrocarbon

Change in carbon dioxide emission of uncontaminated and contaminated haplic chernozem calcic after application of the biochar

As a result of natural transformation and degradation process, petroleum hydrocarbon decomposes in the soil very slowly. When adding bioremediation agents, the rate of petroleum hydrocarbon degradation increases and causes the formation of carbon dioxide and water vapours. The biochemical condition of the soils is evaluated not only by the activity of soil enzymes and microbiological indices but also by the products characterizing petroleum hydrocarbon decomposition (carbon dioxide and water vapours). In soils, the carbon dioxide emission is an index, which allows evaluating its air conditions, as well as providing indirect presentation about its microbiological activity (Galstyan, 1961).

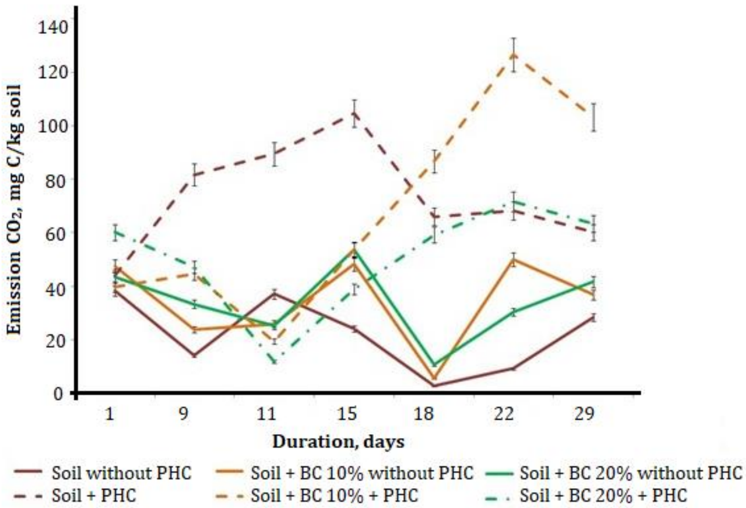

Figure 4. Change soil respiration (CO_2 emission) when adding biochar (10 and 20% of the soil mass (w/w)) to petroleum hydrocarbon-contaminated Haplic Chernozem Calcic in comparison with clean soil, % of control
Note: BC - biochar; PHC, petroleum hydrocarbon

For correct evaluation of petroleum hydrocarbon degradation to simple decomposition products (carbon dioxide and water vapors), uncontaminated and petroleum hydrocarbon contaminated soil samples were analyzed (Figure 4). The final calculation was performed by Equation 1. After the 1st day of the experiment, CO_2 emission in the petroleum hydrocarbon contaminated Haplic Chernozem Calcic and when adding biochar didn't significant. On the 18th day, the growth of CO_2 emissions was observed in the variants with biochar in amount of 10% from soil mass by 70-85% in relation to petroleum hydrocarbon contamination and then decreased to soil with biochar application in amount of 20% from soil mass – 222-362 ppm. In amount of 20% from soil mass, biochar didn't have a stimulating influence on biota and didn't cause inhibition of the biota activity compared with petroleum hydrocarbon contamination.

Change in the catalase activity of uncontaminated and contaminated haplic chernozem calcic after application of the biochar

The enzyme activity of soils was evaluated by the catalase and dehydrogenase activity (class oxidoreductases). Enzymes of class oxidoreductases shall be informatively used in soil contamination of different chemical substances in the south of Russia (Kolesnikov et al., 2011, 2012; Minnikova et al., 2019, 2020).

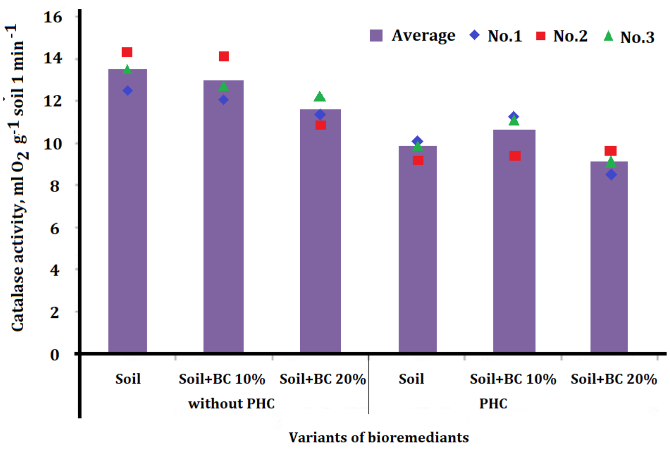

Figure 5. Changes in catalase activity upon introduction of the biochar (10 and 20% of the soil mass (w/w)) into petroleum hydrocarbon-contaminated Haplic Chernozem Calcic compared to pure soil, ml O_2 g $^{-1}$ soil 1 min^{-1}.
Note: BC, biochar; PHC, petroleum hydrocarbon

Change in the catalase activity in the pure soil without petroleum hydrocarbon contamination when applying a large amount of biochar (20% from soil mass (w/w)) was inhibited by 15% (Figure 5). With an increase in the concentration of biochar up to 20% from soil mass (w/w), the activity of catalase does not change in comparison with oil-contaminated soil. When petroleum hydrocarbon applying the catalase activity decreased by 27%. After the introduction of biochar in the amount of 10 and 20% of the soil mass (w/w), Haplic Chernozem Calcium contaminated with petroleum hydrocarbons, the activity of catalase decreased by 22 and 33% of control, respectively. After biochar was added 10% of the soil mass (w/w), an increase in activity by 10% was observed.

Change in the dehydrogenases activity of uncontaminated and contaminated haplic chernozem calcic after application of biochar

The dehydrogenases activity changed on different sides (Figure 6). In non-contaminated soil, at the amount of 20% from soil mass stimulation by 10% in relation to the control was observed. In the petroleum hydrocarbon contaminated Haplic Chernozem Calcic, the dehydrogenase activity was higher when applying biochar in the amount of 10 % from soil mass by 49% from the control (30% from petroleum hydrocarbon contamination). Petroleum hydrocarbon application to the soil increased the dehydrogenases activity conditioned oxidation-reduction processes in the contaminated soil using hydrogen and carbon ions (Lopes et al., 2021). Enzymes of the oxidation-reduction group are associated with carbon and nitrogen and faster response to the application of carbon-bearing compounds than enzymes involved in the sulphur and phosphorus cycle.

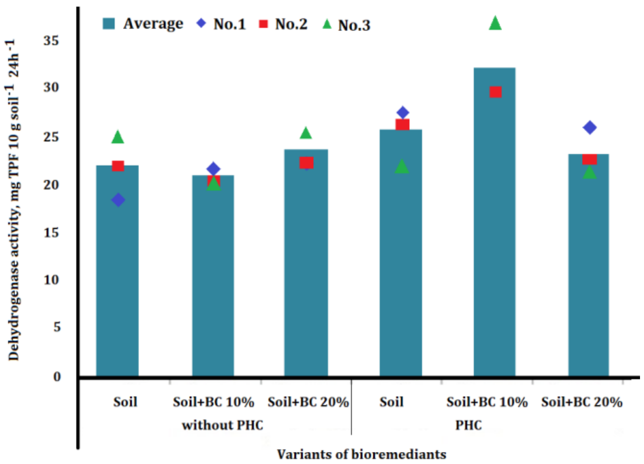

Figure 6. Changes in the activity of dehydrogenases upon the introduction of biochar (10 and 20% of the soil mass (w/w)) into petroleum hydrocarbon -contaminated Haplic Chernozem Calcic compared to control soil, mg TPF 10 g soil^{-1} 24 h^{-1}
Note: BC, biochar; PHC, petroleum hydrocarbon, TPF, triphenylformazan

Change in the ıntensity of early growth and development of radish seeds in uncontaminated and contaminated haplic chernozem calcic after application of the biochar

Taking into account the results of change in the enzyme activity and CO_2 emission the influence on the plants as sensitive test objects were considered. For evaluation of soil toxic level after bioremediation agent application the sensitive phytotest (radish seeds) was used. Radish use allows fast response to petroleum hydrocarbon contaminated Haplic Chernozem Calcic toxic level change within a short time, particularly, when using nonorganic bioremediation agents (Minnikova et al., 2019, 2020).

The toxic level was evaluated based on the germinating ability of radish seeds and its morphological indices: length of shoots and roots (Figure 7). In non-contaminated soil germinating ability of radish seeds didn't change after biochar adding. In the petroleum hydrocarbon contaminated soil germinating ability decreased by 45%. When adding biochar in 10 and 20% from soil mass, germinating ability increased by 17 and 44% from the control accordingly (by 32 and 65% in relation to the petroleum hydrocarbon contaminated soil).

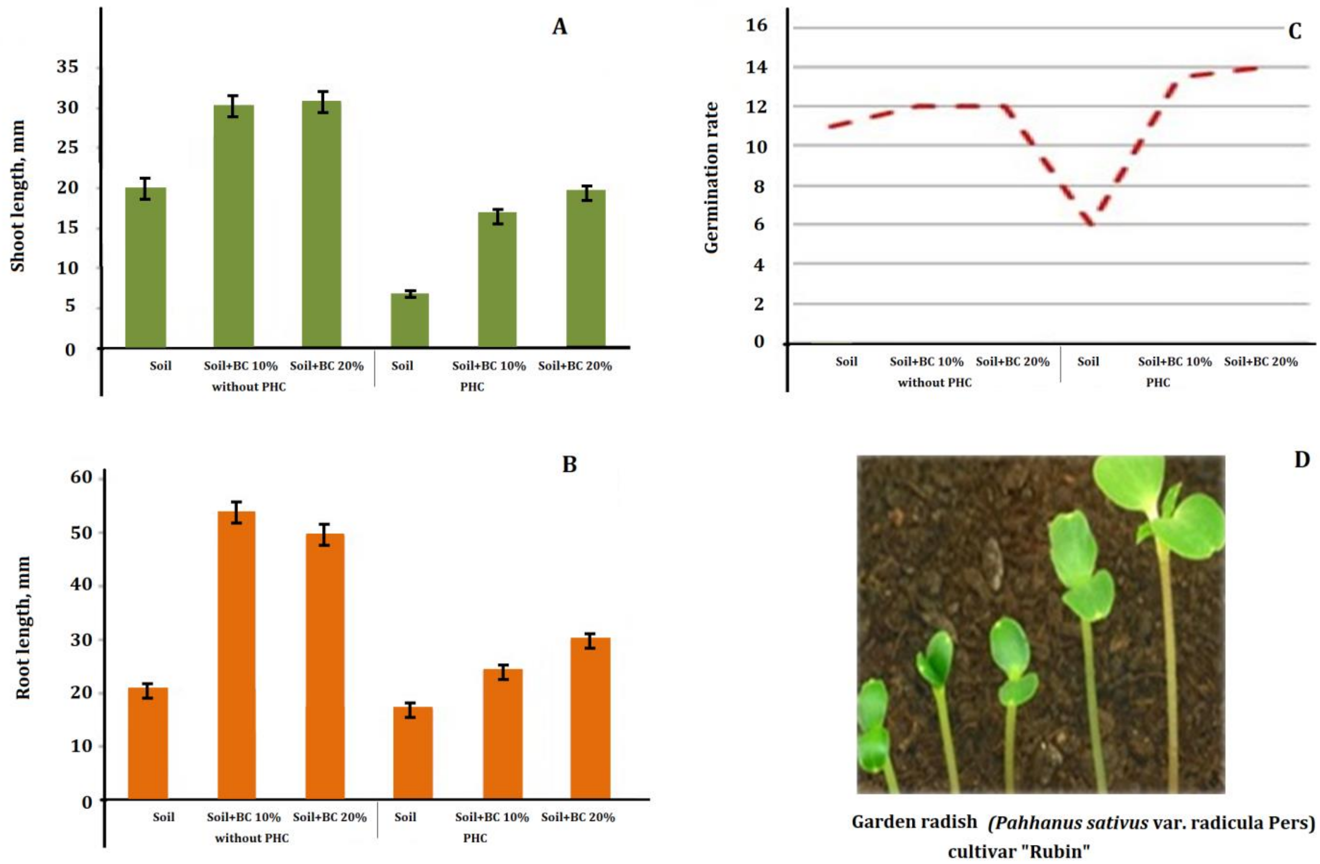

Figure 7. Changes in the intensity of initial growth (length of shoots (A) and roots (B)) and indicators of seed germination (germination (C), view garden radish Garden radish (Raphanus sativus var. radicula Pers) cultivar "Rubin" (D)) after addition of the biochar (10 and 20% of the soil mass (w/w)) to petroleum hydrocarbon-contaminated Haplic Chernozem Calcic in comparison with uncontaminated soil.
Note: BC, biochar; PHC, petroleum hydrocarbon

The indices of early radish growth intensity after cultivation in the uncontaminated and petroleum hydrocarbon contaminated soil are shown in Figure 6. In pure petroleum hydrocarbon uncontaminated soil, the length of sprouts with biochar in the amount of 10 and 20% from soil mass was higher than the control by 53 and 55%. When adding petroleum hydrocarbon in the soil, a decrease of growth sprout by 67% in relation to the control was observed. When adding biochar in petroleum hydrocarbon contaminated soil, growth in the length of sprouts by 51 and 66% was observed for biochar in amount of 10 and 20% from soil mass accordingly.

The length of radish roots has changed based on a similar scenario. In uncontaminated soil, stimulation of the root length is detected upon biochar application in amount of 10 and 20% from soil mass by 159 and 138% higher, than the control. In oil contaminated soil without biochar, the root length is lower by 18%. Applying biochar in amount of 10 and 20% from soil mass is higher by 17 and 44% from the control (by 35 and 63% from the petroleum hydrocarbon contaminated variant).

Biochar in the amount of 20% from soil mass to be by 24% higher than for biochar in amount of 10% from soil mass has a more favourable influence on the intensity of radish seed sprouting in case of petroleum hydrocarbon contamination. Stimulation of radish root and sprout growth in the soil with biochar is conditioned with a high concentration of organic matter and mineral elements.

Discussion

Restoration of the biological properties of the soil after petroleum hydrocarbon contamination is a long process (Figure 8). The restoration degree is evaluated based on stimulation of the soil biological indices: the intensity of early growth and development of radish seeds, carbon dioxide emission, number of soil bacteria (R=-0.61), change in activity of catalase (R=-0.79) and dehydrogenases (R=-0.92) versus to the control. The CO_2 emission is closely correlated with the catalase activity – R=0.75 and inverse correlation – with the length of radish sprouts and roots – R=-0.83 and 0.94. The immobilized bacterium on the biochar treatment was maintained at the highest level during the entire remediation compared with other ameliorants (Zhang et al., 2019). At 50 days, the FDA hydrolysis, dehydrogenases and polyphenoloxidases activity in the immobilized bacteria on biochar treatment (BIM) reached maximum values and were 22.71 mg g^{-1} $24h^{-1}$, 93.44 μg g^{-1} $24h^{-1}$ and 39.96 mg g^{-1} $24h^{-1}$, respectively, confirming that biochar not only can provide a favorable habitat but also sufficient nutrients for microbes to produce enzymes and biodegrade the contaminants. The enzymatic activities (as catalase) decreased the possible cause of such change may be due to the partial decomposition of biochar and accumulation of residues of refractory components inhibiting the activity of microorganisms, leading to microbial exfoliation and even death.

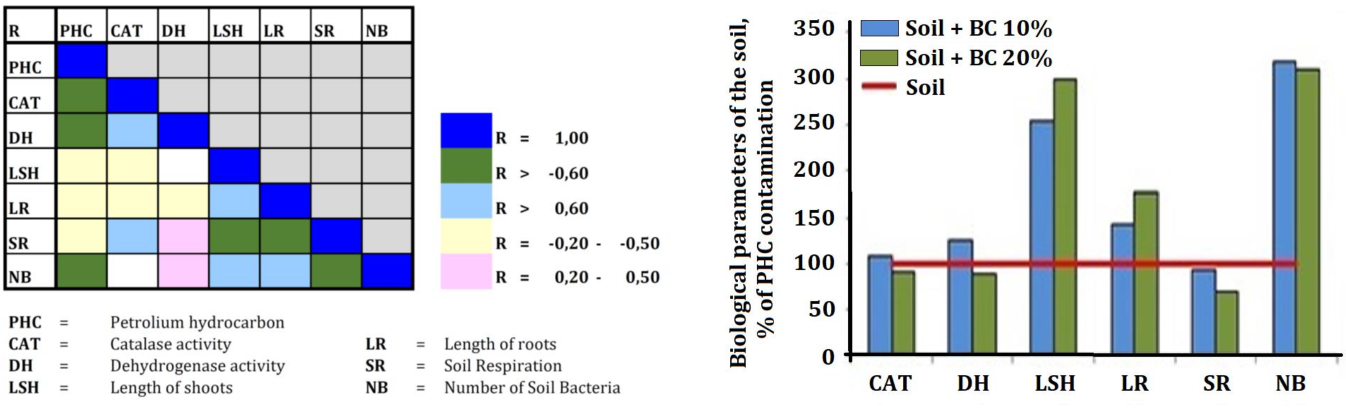

Figure 8. Correlation dependence between the petroleum hydrocarbon content and changes in the biological parameters of the Haplic Chernozem Calcic after the introduction of the biochar

The number of soil bacteria is associated with the length of shoots and roots by a direct relationship R = 0.97 and the length of roots R = 0.89. In order to develop a new method for remediation of petroleum hydrocarbon contaminated soils, salt tolerant *Corynebacterium* variable HRJ4 and biochar were used early (Zhang et al., 2016). Results indicate that the bacteria involving the biochar were very efficient in degradation of PAHs (PYR, NAP) mixture and n-alkanes (C_{16}, C_{18}, C_{19}, C_{26}, C_{28}).

Thus, in the case of Haplic Chernozem Calcic petroleum hydrocarbon contamination, the use of biochar in 10% is the most efficient, as it is environmentally reasonable. Higher concentration increases by germination ability and length of plant roots and sprouts and decrease of petroleum hydrocarbon content in the soil. Decomposition of low petroleum hydrocarbon concentration, 5% from soil mass, was evaluated in the research. These data are confirmed by Mukome work with coauthors when studying 0.25% petroleum hydrocarbon contamination with biochar application on the basis of pine saw dusts and straw (Mukome et al., 2020).

This research has demonstrated that biochar on the basis of pine saw dusts in the soil is efficient together with fertilizer even at low concentration of light crude petroleum hydrocarbon in the soil. However, when using biochar from wheat straw, the significant difference between the control soil with crude petroleum hydrocarbon and treated by biochar applying in amount of 1% from petroleum hydrocarbon amount in the soil (Han et al., 2016).

Biochar has displayed a good potential to remediation of petroleum hydrocarbons owed to its wide availability of the necessary feedstock, sustainable nature, high efficiency, large internal surface area and desirable physicochemical surface properties (Zahed et al., 2021). Using biochar can include carbon sequestration, improving soil fertility, reclamation, and processing of agricultural wasting.

Conclusion

Biochar use contributes to accelerating petroleum hydrocarbon degradation and improving Haplic Chernozem Calcic ecological condition after petroleum hydrocarbon contamination. Use of biochar in the amount of 10 % from Haplic Chernozem Calcic mass (w/w) is more reasonable than biochar amount of 20% mass (w/w). Petroleum hydrocarbon degradation intensity and stimulation of most of Haplic Chernozem Calcic biological activity indices were higher when using 10% from biochar concentration. A decrease in the concentration of petroleum hydrocarbons in the soil after the introduction of biochar increases the activity of catalase (R = -0.79), dehydrogenases (R = -0.92) and the number of soil bacteria (R = -0.61). The use of biochar accelerates the decomposition of petroleum hydrocarbons and improves the ecological state of the soil after contamination with petroleum hydrocarbons.

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