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Biochar Effect on the Benzo[a]pyrene Degradation Rate in the Cu Co-Contaminated Haplic Chernozem under Model Vegetation Experiment Conditions

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Abstract: The research of the fundamentals of the behavior of behavior in the soil–plant system during their co-contamination is of high interest because of the absence of technologies for the creation of effective, environmentally friendly and cost-effective remediation methods, as well as integrated systems for predicting the quality of soils co-contaminated with HMs and PAHs. The unique model vegetation experiment was studied with Haplic Chernozem contaminated by one of the priority organic toxicants, benzo[a]pyrene (BaP), applied alone and co-contaminated with Cu with the subsequent vegetation of tomato (*Solanum lycopersicum*) and spring barley plants (*Hordeum sativum* Distichum). Biochar obtained from sunflower husks was used as a sorbent for the remediation of the contaminated soil. It was established that by increasing the BaP amount applied to the soil, the rate of BaP degradation improved. The effect was enhanced in the presence of biochar and decreased in the case of joint co-contamination with Cu, which is especially expressed for the soil of tomato plants. The half-degradation time of the BaP molecule varied from 8 up to 0.2 years for tomatoes and barley.

Keywords: PAHs; benzo[a]pyrene; soil; Haplic Chernozem; model experiment; barley; tomato; combined soil pollution; copper oxide (II)

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1. Introduction

As a result of urbanization and industrialization ongoing processes, up to 10 km^2 of soil fertility on the European continent is declining daily [1]. Significant damage to the soils of agricultural areas is caused by their contamination with toxic and carcinogenic substances, inorganic pollutants, such as heavy metals (HMs), and organic ones, such as polycyclic aromatic hydrocarbons (PAHs) [1,2]. As a result of fertile soil pollution, there is a danger of the migration of ecotoxicants into agricultural products, food, and animal tissues, including that of humans. It also reduces the economic value of soils, limiting the potential for their use, which ultimately jeopardizes the food security of the entire European continent. The creation of environmentally friendly and cost-effective approaches of remediation is very relevant, considering the scale of territories subject to technogenic impact, as well as integrated systems for predicting the soil quality contaminated with HMs and PAHs.

Due to its carcinogenic activity, benzo[a]pyrene (BaP) is considered as the most dangerous representative of PAHs [3]. In the territory of the Russian Federation, governmental

standards have been established that regulate its content in all natural environments. The maximum permissible concentration (MPC) of BaP in soil is 20 μg kg^{-1} [4], which does not exceed the background content of pollutants in Russia [5,6]. In soils concentrated near industrial enterprises, the concentration of this pollutant can reach more than 1000 μg kg^{-1} [7]. BaP refers to persistent environmental pollutant. However, some groups of microorganisms are able to metabolize this toxicant into simple compounds [8,9].

The bioavailability and rate of pollutant degradation in soil is operated by a variety of biotic and abiotic factors. The presence of other ecotoxicants in the soil can act as a factor limiting or facilitating BaP transformation [10–14]. Often, a high content of PAHs, including BaP, is accompanied by elevated concentrations of HMs in soils compared to clarke. One of the most toxic and widespread heavy metals is copper. For agricultural land in southern Russia located near industrial areas, the Cu content exceeds the clarke of soils [15,16]. However, there is no consensus on the effect of HMs on the behavior of PAHs in soil, and there are practically no comprehensive studies on soils contaminated with the most dangerous representatives of PAHs and HMs, such as BaP and Cu.

Soil pollution with only HMs or PAHs promotes the inhibition of growth and active accumulation of pollutants in the plant [17,18]. These effects are associated with the uptake of hazardous chemical pollutants by plants and are often enhanced by combined soil contamination with HMs and PAHs [17,19]. However, Zhang et al. noted the limited penetration of PAHs into corn during soil contamination with Pb [20].

The presence of HMs in the soil can reduce the rate of PAH degradation. The higher the concentration of the Mn, Cr, Co, Ni, Cu, Zn, Pb mixture, the lower the PAH biodegradation rate [11,14]. A decrease in the rate of soil self-purification from fluoranthene and phenanthrene up to 54% was observed in the presence of Cu [10,13]. In the example of aqueous solutions contaminated with Cu, Zn, Fe, and Al, the degradation rates of naphthalene, phenanthrene, anthracene, and fluoranthene were inhibited [21]. In this case, an important aspect is the HM concentration in soils. Thus, soil cultivation with 10 mg L^{-1}, Cd, and Cu significantly reduced the potential for the self-purification of soils from fluorene, while Zn and Pb had a relatively weak effect at a concentration of 100 mg L^{-1} [12]. In general, there is a trend towards a decrease in the biodegradation of PAHs under HM co-contamination. However, Henry showed that the presence of HMs in the soil does not significantly affect BaP degradation, while Wang et al. showed an increase in pyrene biodegradation in the soil in the presence of Cd [22,23].

A decrease in the pollutant content in the soil can be activated by plants. First, plants are capable of accumulating pollutants. In this regard, the decrease in the pollutant amount in the soil is controlled by plant species tolerance to HMs and PAHs [24]. Secondly, plant root secretion promotes the active growth of PAH-degrading microorganisms [25,26].

The most common concept for the purification of soils contaminated with Cu and BaP is sorption remediation. Often, this method involves the application of carbon sorbents into the soil based on agricultural waste, which significantly reduces the cost of the sorbent manufacturing process and contributes to the development of waste-free production. The biochar's application helps to reduce the concentration of PAHs in the soil by firmly binding the pollutant to the surface of the sorbent [27,28], as well as stimulating the growth of the number of PAH-degrading microorganisms [8,9,29–31]. The biochar application into soil contaminated with PAHs reduces the concentration of pollutants by 40–80% compared to soil without the sorbent for up to 1–2 years [6,7,32]. The use of biochar in soils contaminated with Cu leads to a decrease in metal mobility [33]. The sorbent effect significantly depends on the dose of its application and the level of soil contamination. Often, such studies are devoted to only one type of pollutant and do not reflect their mutual influence on each other, especially in the conditions of growth of different crops.

Thus, in order to develop effective approaches to the remediation and systems for predicting the ecological state, one should take into account the quantitative and qualitative composition of pollutants, as well as the physiological characteristics of plants growing in the contaminated soil. This is especially important for soils with a high agro-industrial

potential, such as Haplic Chernozem. The purpose of this study is to study the effect of Cu, biochar, and various cultivated plants on the rate of BaP degradation in soil.

The purpose of the research is the determination of the biochar effect on the benzo[a]pyrene degradation rate in the Cu co-contaminated Haplic Chernozem under model vegetation experiment conditions.

2. Materials and Methods

2.1. The Object of the Study

The object of the study was the top layer 0–20 cm of Haplic Chernozem, sampled from the specially protected natural area "Persianovsky protected steppe". The soil cover of the protected area was a fallow area with Haplic Chernozem. The soil properties are shown in Table 1. The soil used was characterized by a high neutral pH, enriched in C_{org}, exchangeable Ca^{2+} and Mg^{2+}, and was a heavy loam.

Table 1. Physical and chemical properties of Haplic Chernozem.

Physical Clay	Silt	C_{org}	Ca^{2+}	Mg^{2+}	pH	CuO
%			cM(+)/kg^{-1}			mg kg^{-1}
48.3 ± 2.1	29.8 ± 1.8	3.8 ± 0.1	30.0 ± 0.7	4.1 ± 0.2	7.36 ± 0.05	48

All reagents used for the model experiment were purchased from Sigma-Aldrich, Burlington, MA, United States. Solvents and reagents were HPLC grade and included ethanol (96%, analytical), n-hexane (99%, analytical), potassium hydrate (98%, analytical), acetonitrile (99.9%, analytical), NaOH (97%, analytical grade), and anhydrous Na_2SO_4, BaP standard in acetonitrile (99%, analytical grade).

Biochar was obtained from sunflower husks using proprietary technology with a final pyrolysis temperature of 500 °C. The method of biochar production is explained in the study by Minkina et al. [34].

2.2. Experiment Design

Haplic Chernozem was cleaned of plant residues and sifted through a sieve with a 3 mm diameter hole for the experiment set up. To each 2 L pot volume, 2 kg of soil with a closed drainage system was added. The experiment was conducted using contaminated soil and all subsequent years' plants were grown in the contaminated first-year soil. To ensure a uniform distribution of pollutants, the soil was placed into the vessels in layers of 400 g each, after which a suspension of CuO and an aqueous solution of BaP in acetonitrile were added. According to the scheme of the experiment, the concentrations of pollutants applied into the soil were 400, 800, and 1200 μg kg^{-1} and 300, 2000, and 10,000 mg kg^{-1} for BaP and CuO, respectively. (Table 2). The doses of pollutants introduced into the Haplic Chernozem corresponded to the levels observed in the soil of the chernozem zone [7,15,32]. Soil incubation with pollutants was 3 months. Subsequently, according to the experimental scheme, biochar was added at a dose of 1% for the remediation of soil contaminated with 400 μg kg^{-1} of BaP separately and together with 300 mg kg^{-1} CuO and 5% for the remediation of soil contaminated with 800–1200 μg kg^{-1} BaP and 2000–10,000 mg kg^{-1} CuO.

The choice of different doses for the remediation was based on the results of the studies by Sushkova et al., Kołtowski and Oleszczuk, and Wu et al. [6,35,36]. The use of a sorbent at a dose less than 2% by weight of the soil for soils contaminated with individual compounds of the PAH group in amounts up to 400 μg kg^{-1} was recommended. Accordingly, for soil contaminated with BaP more than 400 μg kg^{-1}, the recommended dose of biochar application was 5% of soil dry weight. Incubation with sorbents was 3 months. Upon completion of the soil incubation with pollutants and the sorbent, two rows of seeds of the spring barley (*Hordeum Sativum* Distichum) variety, "Warrior", and tomato (*Solanum Lycopersicum*) early ripe variety, "White filling" 241, were sown. The number of spring

barley plants in 1 vessel was 20 pcs, and tomato—3 pcs. The experiment was conducted in 4-fold replication. The cultivation of the test crops was conducted until the spring barley and tomatoes had fully ripened. Subsequently, the soil was selected for analysis. Cultures were grown twice with a difference between crops of 1 year. In the soil of the model experiment, the moisture content was maintained at the level of 60% of the total field capacity during the entire study period [37].

Table 2. Scheme of a model experiment with barley and tomatoes.

Sample Name	Concentration		
	BaP	CuO	Biochar
	μg kg^{-1}	mg kg^{-1}	%
The control	0	0	0
Control + biochar 1%	0	0	1
Control + biochar 5%	0	0	5
BaP400	400	0	0
BaP400 + Cu300	400	300	0
BaP400 + biochar 1%	400	0	1
BaP400 + Cu300 + biochar 1%	400	300	1
BaP800	800	0	0
BaP800 + Cu2000	800	2000	0
BaP800 + biochar 5%	800	0	5
BaP800 + Cu2000 + biochar 5%	800	2000	5
BaP1200	1200	0	0
BaP1200 + Cu10,000	1200	10,000	0
BaP1200 + biochar 5%	1200	0	5
BaP1200 + Cu10,000 + biochar 5%	1200	10,000	5

2.3. BaP Extraction from Soil

BaP was extracted from soils using subcritical water [38]. A sample of 1 g of soil was placed in an extraction cartridge, and 8 mL of bi-distilled water was added and hermetically screwed on both sides. A manometer with a built-in emergency pressure-relief valve was connected to the cartridge so that the pressure inside the cartridge did not exceed 100 atm. The cartridge was placed in a thermostat and heated to 250 °C for 30 min. After cooling the system, the cartridge was unscrewed, the contents were filtered 3 times through a paper filter with a blue ribbon into a glass conical flask to a clear solution, and, each time, the filter was washed with 2 mL of distilled water. BaP was re-extracted three times from the obtained aqueous extract with n-hexane (analytical grade). To perform this, 5 mL was poured into the flask, closed with a glass stopper, and shaken on a shaker for 15 min. The layers were separated on a separating funnel with a volume of 50 mL sequentially in three stages with the next portion of hexane.

The combined hexane extract was passed through a funnel with calcined anhydrous sodium sulfate, after which the extract was evaporated in a pear-shaped flask on a rotary evaporator at a water bath temperature of 40 °C to obtain a dry residue. The resulting residue was dissolved in 1 mL of acetonitrile with stirring for 30 min, and the BaP concentration in the extract was determined by high-performance liquid chromatography (HPLC). The completeness of the BaP extraction was determined by the matrix spike method, for which a 1 g sample of soil was placed in the flask of a rotary evaporator and a certain amount of a standard solution of BaP in acetonitrile was added to create BaP concentrations in the sample of 10, 20, 40, 80, 160, and 320 μg kg^{-1}. Following the evaporation of the solvent for 30 min under room temperature conditions, the analyte was kept at 7 °C for a day, and then the sample was analyzed by HPLC according to a certified method [39] using a 1260 Infinity Agilent fluorometric detection system (USA). All the research results were performed in 3-fold analytical repetition.

To determine the degradation rate of BaP in the soil of the model experiment, the degradation constant (Kc, year^{-1}) (1) and half-degradation time (T_{50}, years) (2) were calculated:

$$K_c = -\ln(C_t/C_i)/t \quad (1)$$

where C_i—initial BaP concentration in the soil (μg kg^{-1}), C_t—BaP content in soil over time (μg kg^{-1}), and t—time (years).

$$T_{50} = 0.693/K_c \quad (2)$$

The statistical processing of the obtained results included Student's *t*-test, and regression analysis was performed using the SigmaPlot 12.5 program. The results are considered significant at *p*-level < 0.05. For the regression analysis, we used the linear regression Equation (3) and Equation Power (4):

$$y = y_0 + ax \quad (3)$$

$$y = ax^b \quad (4)$$

3. Results

It was established that, in the first year of the study in the control variant of Haplic Chernozem, the content of BaP did not exceed the MPC and amounted to 18 μg kg^{-1}. The sorbents' application contributed to a decrease in the pollutant concentration, which is especially pronounced in the soil of barley plants (differences are significant at $p < 0.05$). Under the same conditions for the pollutants and biochar application, the BaP content in the soil of tomato plants was significantly lower than that of barley plants (Figure 1). The effect intensified over the time, and by the second year the content of BaP decreased up to 57% from its initial concentration in the soil of the experimental variant with tomato plants.

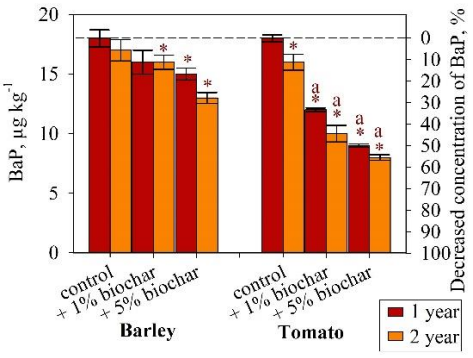

Figure 1. The BaP content in the soil of the control variants of the model experiment with and without the introduction of biochar. Note: * A statistically significant difference in the BaP content in Haplic Chernozem with and without biochar application (control) is noted. The letter "a" shows statistically significant differences between the content of BaP in the soil of pots with barley plants and with tomato plants under the same doses of pollutants and sorbent application conditions. The significance of the differences between the means was obtained by calculating the Student's *t*-test at *p*-level < 0.05.

After the first year of the study, it was shown that the pollutants' application increased the BaP content in Haplic Chernozem. This is typical for the soil in which both the barley and tomatoes were grown. With an increase in the applied BaP amount, its content in the soil increased. However, after the first growing season, the results obtained are 5–10%, 15–23%, and 20–30% lower than the initially applied 400 μg kg^{-1}, 800 μg kg^{-1}, and 1200 μg kg^{-1} and correspond to 349–384 μg kg^{-1}, 626–701 μg kg^{-1}, and 804–902 μg kg^{-1}, respectively. In the polluted soil with the tomato plants, the pollutant content was noticeably lower compared to the soil with barley plants. The differences increased with an increase in the initial concentration of the applied pollutants and the use of biochar, as evidenced by the results of the Student's criterion calculation (Figure 2). The presence of Cu

in the soil had the opposite effect. It was shown that, in the soil of the experimental variants with the combined application of BaP and CuO, the content of BaP was significantly higher than in the soil that was contaminated with only BaP (Figure 2).

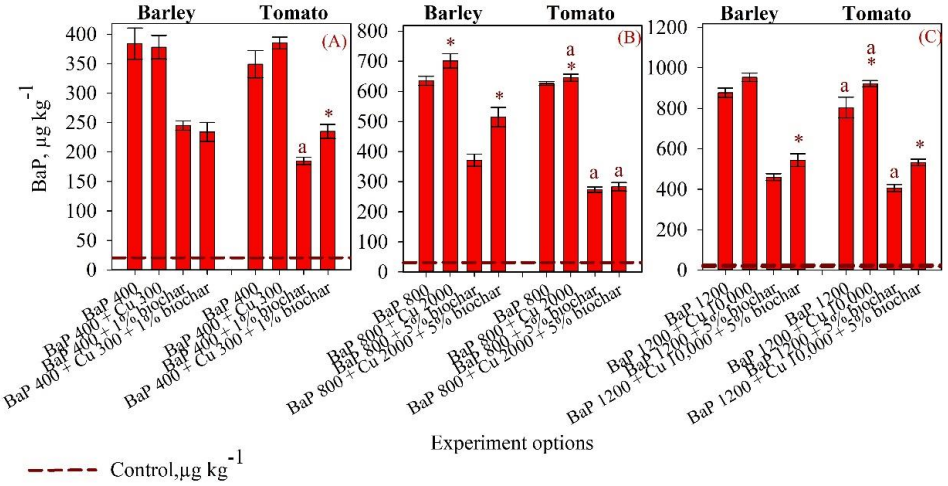

Figure 2. BaP content in Haplic Chernozem contaminated with 400 μg kg^{-1} of BaP alone and co-contaminated with 300 mg g^{-1} of CuO (**A**), 800 μg kg^{-1} of BaP alone and together with 2000 mg g^{-1} CuO (**B**), and 1200 μg kg^{-1} of BaP alone and together with 10,000 mg g^{-1} of CuO (**C**) after the first plant growing season. Note: * A statistically significant difference in the BaP content in Haplic Chernozem contaminated with BaP and co-contaminated with BaP and CuO was noted. The letter "a" shows statistically significant differences between the content of BaP in the soil of pots with barley plants and with tomato plants under the same doses of pollutants and sorbent application conditions. The significance of the differences between the means was obtained by calculating the Student's *t*-test at *p*-level < 0.05.

In the second year of the experiment, the decrease in the BaP content continued for the soils for all experiment variants. The regularities of the decrease in the BaP content of the soil were similar to the first year, but more expressed. Thus, the largest decrease, by 69%, compared to the first year, was observed in the variant with the a 1200 μg kg^{-1} BaP dose application into the soil of the experiment conducted with tomato plants. The minimum decrease, by 8%, was recorded for soil initially contaminated with 400 μg kg^{-1} of BaP and 300 mg kg^{-1} of CuO for tomato plants (Figure 3).

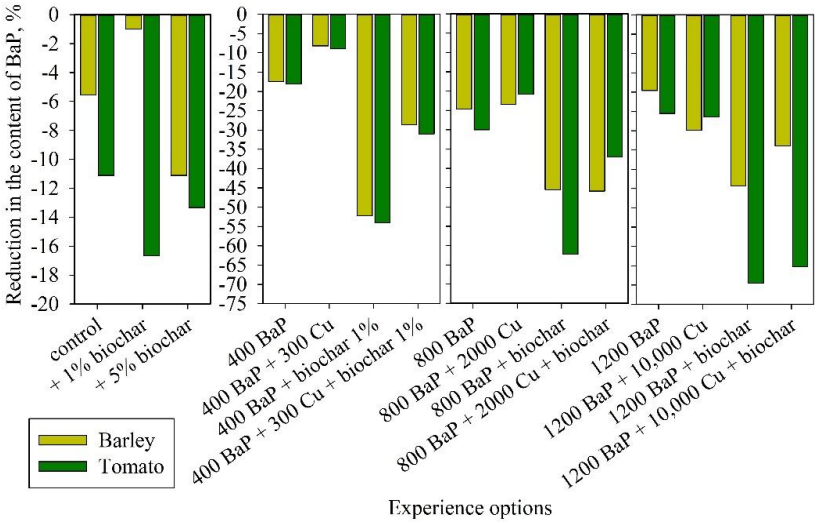

Figure 3. BaP content decreasing in the soil of different experiment variants after the second season of plant vegetation.

The calculation of the BaP degradation constant showed that the rate of pollutant degradation in the soil increased with an increase in its initial concentration in Haplic Chernozem. It was noted that the Kc of BaP for the soil with tomato plants was higher than in the soil with barley plants. In this case, the rate of pollutant degradation decreased in the presence of Cu, as well as over time (Figure 4).

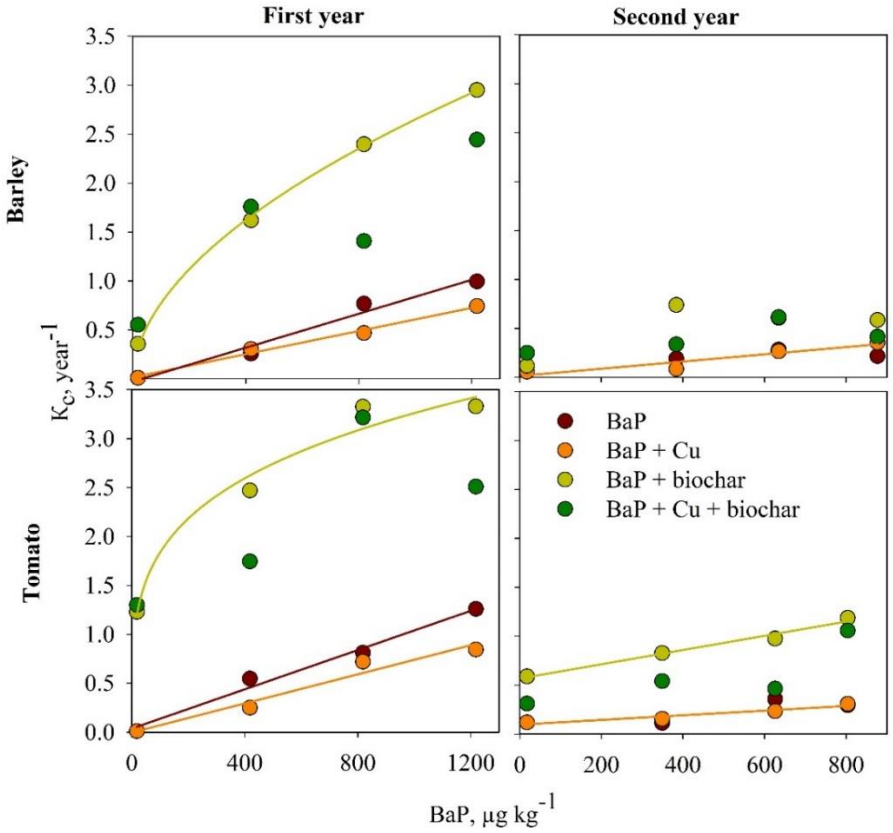

Figure 4. BaP degradation constant (Kc) in the Haplic Chernozem of model vegetation experiment. Note: the line of approximation on the figure indicates the presence of a relationship between the initial BaP concentration and the BaP degradation constant in Haplic Chernozem.

In accordance with the results of the Kc calculation, the half-degradation time of BaP increased with a decrease in the pollutant concentration in the soil over time, and the CuO application dose. The predicted half-degradation time of the pollutant was higher for the soil in with barley plants compared to the one with tomato plants. The difference in T50 results between the soil for barley and tomatoes reached 3.5–3.8 times in the first vegetation season and 2.3–2.5 times in the second vegetation season (Figure 5). In the second year of the experiment, the maximum half-degradation time of BaP in the soil of the barley plants corresponded to more than 8 years, and for tomato less than 3 years, under the condition of initial soil contamination of 400 μg kg^{-1} of BaP together with 300 mg kg^{-1} of Cu. The minimum value was established for soil initially contaminated with 1200 μg kg^{-1} of BaP with biochar used at a dose of 5%. Here, the half-degradation time of the BaP molecule was 1.2 years for barley and 0.2 years for tomato (Figure 5).

Experiment options	Barley		Tomato	
	Year of experiment			
	1	2	1	2
Control	-	12.12	-	5.88
Control+biochar 1%	1.94	5.88	0.56	1.18
Control+biochar 5%	1.25	2.13	0.33	0.85
BaP400	2.70	3.61	1.27	6.18
BaP400+Cu300	2.27	8.10	2.78	4.50
BaP400+biochar 1%	0.43	0.94	0.28	0.84
BaP400+Cu300+biochar 1%	0.39	2.05	0.40	1.28
BaP800	0.90	2.44	0.85	1.94
BaP800+Cu2000	1.48	2.60	0.96	2.98
BaP800+biochar 5%	0.29	1.14	0.21	0.71
BaP800+Cu2000+biochar 5%	0.49	1.13	0.22	1.49
БаП1200	0.70	3.17	0.55	2.35
BaP1200+Cu10,000	0.93	1.95	0.82	2.25
BaP1200+biochar 5%	0.23	1.18	0.21	0.58
BaP1200+Cu10,000+biochar 5%	0.28	1.67	0.28	0.66

Figure 5. BaP half-degradation time (T50) in the Haplic Chernozem of model vegetation experiment; years.

4. Discussion

BaP is a persistent environmental pollutant. Its destruction in Haplic Chernozem is complicated due to the high sorption capacity of this type of soil. Nevertheless, in the course of a complex model vegetation experiment, the following factors contributing to a decrease in BaP content were identified: (1) the level of soil contamination with BaP, (2) the presence of biochar in the soil, (3) the type of crop growing on the soil, (4) the presence of Cu in the soil, and (5) time.

Under the various concentrations of the effect of hydrocarbons in the soil, changes in microbial communities and their diversity were observed [40]. Moreover, these changes occurred in the first days of soil contamination and were accompanied by an increase in the number of PAH-degrading microorganisms in the soil, which affected the rate of BaP degradation [41–43]. The idea is based on the inhibition of the natural microbiome and filling the vacated ecological niche with PAH-degrading microorganisms, for which PAHs, including BaP, can act as the only source of carbon, such as Gram-negative bacteria [44]. Accordingly, than higher the concentration of the introduced pollutant, the more expressed the redistribution of the microbiological communities' composition in the soil and the stronger the rate of BaP degradation. Furthermore, in the process of BaP degradation and/or the process of the pollutant "aging" in the soil, the amount of the substrate available to PAH-degraders decreased, which lead to a gradual decrease in their amount in the soil, and, accordingly, to the decrease in the BaP degradation rate.

Reducing the PAH content, including BaP, in the soil due to the biochar application by various origins has been shown in many studies [8,9,27–31]. The modern understanding of the carbonaceous sorbent's role in the detoxification of polluted soils is based on several direct and indirect mechanisms of interaction between PAHs and biochar. The direct mechanisms include the sorption of pollutants by the developed surface of the biochar. At the same time, the processes of the strong binding of PAHs traditionally consist of van der Waals forces, π–π bonds, and hydrogen–π and cation–π bonds [45]. As a rule, only one

type of connection prevails out of those that have been listed. The mediated interaction occurs through the colonization of biochar pores by microorganisms [9].

Plant-root exudates enhance PAH degradation by increasing the amount of PAH degraders and hydrocarbon-degrading genes [26]. The differences in the BaP content in the soil of the model experiment under the influence of different crops are most likely caused by the physiological features of the chemical composition of the plant-root exudates by different species. Therefore, Guo et al. showed that the effect of corn-root secretion on the reduction in PAH concentration in the soil was higher than that of soybeans [24]. In turn, Davin et al. demonstrated that exudates of different plant species are released at different rates, which can also affect the rate of BaP degradation [25].

The influence of all the above-mentioned effects and mechanisms contributing to the BaP degradation in the soil decreases in the presence of HMs. Gram-negative bacteria attract metal cations, as a result of which the adsorption of PAHs by microorganisms decreases [44]. This may be due to the fact that the electrostatic attraction between the surface of microbes and heavy metal ions is much stronger than the van der Waals interaction between the bacterial shell and PAHs [46]. Thus, the presence of Cd and Hg inhibits the abundance of PAH-degrading microorganisms [13,47], while Cu, Zn, Fe, and Al inhibit the abundance of metal-resistant PAH-degrading microorganisms [21]. In the presence of Cu in the soil, the incomplete degradation of phenanthrene can occur with the possible formation of its toxic metabolites [10]. The biosorption of heavy metals by bacteria negatively affects the growth of its biomass, biodegradation of PAHs, and the total accumulation of lipids; the effect of HM decreases in the following: Cd > Ni > Pb > Cu > Zn > Fe [48].

The decrease in the biochar effect on the decrease in the BaP content in the soil is due to a change in the charge of the sorbent surface due to complexation with Cu^{2+} of hydration shells, which directly compete with organic pollutants for the area of the biochar adsorption surface [49,50].

The ability of various plants to absorb PAHs varies depending on the species [51]. This may indirectly indicate a greater tolerance of tomatoes towards BaP compared to barley in the model experiment. The results of studying the effect of HMs on the PAH uptake by plants are not unambiguous. The impact of Cr, Cu, and Pb reduced the uptake of PAHs by cruciferous plants, which may be due to a commutative inhibitory effect on root adsorption or the cation–π interaction [52]. In another study, the presence of Cu in the soil stimulated the accumulation of organic pollutants in spinach through the formation of PAH–Cu^{2+} complexes, which can penetrate into defective roots through the apoplastic pathway [53].

5. Conclusions

Thus, in the course of a complex model vegetation experiment with Haplic Chernozem artificially contaminated with BaP, Cu, and applied biochar in different doses, the main regularities of the decrease in the content of a dangerous carcinogen of the PAH group, BaP, were shown. It was found that, with an increase in the amount of pollutant introduced into the soil, the rate of BaP degradation increased. The effect was enhanced in the presence of biochar and decreased in the case of joint co-contamination with Cu, which was especially expressed for the soil of tomato plants. Over time, these processes weakened. The half-degradation time of the BaP molecule varied from 8 up to 0.2 years for tomato and barley plants.

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