The Effect of Kerosene Pollution on the Cellulolytic Activity of Albic Retisols and Arenosols (Aridic): A Laboratory Experiment

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Abstract—The results of a laboratory experiment modeling the effect of kerosene contamination on the cellulolytic activity of microbiocenosis of the Albic Retisol (Kaluga oblast, Russia) and Arenosol (Kyzylorda oblast, Republic of Kazakhstan) humus horizons are described. Cellulolytic activity is assessed according to the rate of weight loss in linen cloth fragments during the incubation for 0–3, 3–7, and 7–13 months. The intensity of cellulolytic activity in the unpolluted Albic Retisol is higher as compared with the Arenosol, which is determined by low acidity and an elevated content of organic matter and nutrients. The soil pollution with kerosene to 10 g/kg causes a reversible change in cellulolytic activity of both Albic Retisol and Arenosol (Aridic). A high load of kerosene (≥25 g/kg) inhibits cellulolytic activity in both soils over 13 months of observation.

Keywords: soil pollution, easily hydrolyzed organic matter, biochemical oxidation

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INTRODUCTION

A sustainable functioning of terrestrial ecosystems is to a considerable degree determined by microbiological processes in soil. The input of pollutants frequently suppresses soil biological activity and, as a consequence, causes either degradation or death of the entire biogeocenosis. One of the processes that determine the intensity of biological cycle of matter in an ecosystem is the transformation of organic residues, which considerably depends on the activity of cellulolytic organisms.

Cellulolytic activity (CA) in soil is frequently used as an indicator for the biochemical oxidation of organic substances [4, 11, 12, 30]. This characteristic is widely used for an integral assessment of soil ecological functions and the intensity of microbiological processes [7, 9]. Several test objects, such as filter paper [6, 43, 53, 54], cotton cloth [8, 49], or linen [35, 36], are used for estimating CA. The intensity of cellulose decomposition is determined according to the change in the density of test object [42, 51], area of the remaining material [8], and its weight not consumed by microorganisms over a certain incubation period [35, 36].

The CA value is determined by a set of natural ecological and anthropogenic factors. Many researchers report a considerable effect of hydrothermal conditions and properties of pollutant on the biological activity of soils. The CA intensity increases with approaching the optimal environmental conditions [1, 8, 15, 26, 35, 39, 51]. In the (semi)arid areas with Kastanozems and Calci sols, the season of biological activity is rather short because of moisture deficiency, which leads to a decrease in CA intensity. Analysis of the data on the composition and abundance of the cellulolytic microorganisms demonstrates that they are mainly represented by fungi in soils of cold humid landscapes versus more southern latitudes, where the share of actinomycetes is higher [28].

The CA of anthropogenically disturbed soils depends on their natural (background) level of biological activity [17, 18, 24]. The soil pollution by heavy metals or benzo(a)pyrene decreases the biological activity [21, 30, 40], whereas application of certain doses of mineral fertilizers, brown coal, and hydrocarbons, on the contrary, frequently increases its [2].

Several studies focused on the influence of fertilizers, methods for farmland tillage, and pasture degradation on CA [3, 5, 32]. An increase in the nitrogen content in soil results in an increase of CA [31].

In soils of urban landscapes, CA decreases when the annual leaf litter is taken off and the content of pollutants increases [17, 18, 24].

The effect of hydrocarbon pollution on CA has been most frequently studied in the soils contaminated with oil, mazut, gasoline, and motor oils [21, 23, 24] and less frequently, with kerosene [10]. A high migration ability of kerosene and its toxicity for biota determined the need to assess the allowable load of hydrocarbons of this type on the components of ecosystems,
especially, the areas adjacent to airfields and spaceports [19, 25, 27, 37, 46, 47].

The goal of this work was to assess the CA dynamics in the kerosene-polluted soils of forest and desert landscapes under controlled hydrothermal conditions.

OBJECTS AND METHODS

The laboratory experiment was performed with the samples of humus horizon of Albic Retisol (Loamic) and Protic Arenosol (Arlicid) (Table S1). The soils were sampled in the zones of humid landscapes of broad-leaved–coniferous forests (Borovsk raion, Kaluga oblast, Russia) near the Ermolino airport and the arid desert landscapes (Kyzylorda oblast, Kazakhstan) in the territory of Baikonur spaceport.

Albic Retisol is more favorable for soil microbiontopses as compared with Arenosol. In particular, the ammonium content in the former is 10.6-fold higher and the phosphorus content, 3.9-fold higher. A high content (almost 10-fold higher) of organic carbon in the humus horizon of Albic Retisol and weakly acid pH are favorable factors for the development of microorganisms. The humus horizon of Arenosol has a highly alkaline pH, which determines a decrease in the biomass and diversity of soil microorganisms.

The samples of humus horizon with a weight of approximately 20 kg were taken at a natural moisture, sieved (mesh, 3 mm), freed from roots and other inclusions, and air-dried. Before the laboratory experiment, the samples were moistened to 60% of the maximum field capacity using a sprayer. Distilled water was supplied in small portions and soil was thoroughly mixed after adding each water portion to provide a uniform imbibition. The amount of supplied water was controlled by weighing. Soil moisture was measured before adding kerosene by gravimetry with drying samples at 105°C and amounted to 22.71 ± 0.53% for Albic Retisol and 5.21 ± 0.25% for Arenosol. After the required moisture content was reached, the samples in polyethylene bags were left for 3 days at a temperature of 18–22°C to allow the moisture to evenly spread over the sample volume and microbiogenesis to become activated [16]. Mixing was performed gently to preserve the soil structure. Each sample was divided into six parts to use one as a control, while the remaining five samples were treated with different doses (1, 5, 10, 25, and 100 g/kg soil) of kerosene (TS-1 according to the state standard GOST 10227-86). Hydrocarbons were added uniformly over the sample volume using a sprayer to maintain homogeneization. The amount of applied kerosene was controlled by weighing. The kerosene loads were selected according to the data on its effects on vegetation [47] and microbiogenesis [13]. The polluted and control soil samples were placed into 500-cm³ glass containers with leak-proof iron lids. Each kerosene dose was added to one container. The bulk density of Albic Retisol and Arenosol samples was 0.92 ± 0.09 and 1.47 ± 0.04 kg/dm³, respectively.

Test objects (fragments of linen cloth with a size of 6 × 4 cm previously air-dried and weighed using an analytic balance to the fourth decimal place; average weight, 0.7 ± 0.1 g) were placed into the soil samples in glass containers in triplicate (Fig. S1). The experiment was conducted for 13 months at a temperature of 18–22°C. Temperature was controlled on a daily basis with a mercury thermometer. The containers were opened once in 5 days for ventilation and control of moisture by gravimetry. If the weight decreased, it was restored by adding distilled water with a sprayer (Table S2).

CA was monitored over the entire experiment at the intervals 0–3, 3–7, and 7–13 months.1 At the end of each interval, test objects were withdrawn and replaced with a new batch. The withdrawn linen cloth fragments were thoroughly washed from solid phase, air-dried, and weighed. CA was calculated as the mean rate of the loss in weight of test object relative to its initial weight over the selected observation interval (0–3, 3–7, and 7–13 months) in mg/(g day). The data on the destruction of linen cloth were additionally compared to the ranges of the scale of cellulose decomposition intensity, proposed by Zvyagintsev [16], expressed as percentage recalculated for 3 months.

A Statistica software package was used for data processing and a nonparametric Mann–Whitney U-test for independent variables, for assessing the significance of differences.

RESULTS AND DISCUSSION

Analysis and statistical processing of the data have shown that the CA of individual pollution variants weakly varies in both soils: the variation coefficient CV = 8–18% at all observation points. The maximum values, CV = 51%, are recorded in the most polluted variants of Arenosol (Arlicid) for the observation interval of 7–13 months.

CA of unpolluted soils. The intensity of cellulose decomposition in unpolluted Albic Retisol humus horizons over the experiment correspond to medium, high, and very high level according to Zvyagintsev’s scale. The CA in Albic Retisol decreased 1.7-fold by the end of experiment from 9.2 to 5.3 mg/(g day) (p-value, 0.08). These values are close to the in situ data on the Haplic Chernozems of the Central Russian Upland [36] and Crimean Haplic Calcisols [8] (Tables 1 and 2).

As for Arenosol, the intensity of cellulose decomposition was maximal at the beginning of experiment and further remained within the medium range. At the

1 These intervals were determined by the limitations in the access to laboratory because of the COVID-19 quarantine, which prevented a regular (each 3 months) control.
next observation intervals (3–7 and 7–13 months), CA dropped to a very low level. These values for Albic Retisol are significantly smaller ($p$-value, 0.08; $n = 3$). The most pronounced differences were observed for the intervals of 0–3 and 7–13 months: CA decreased from 3.87 to 0.98 mg/(g day) ($p$-value, 0.08). The differences between the two first intervals are insignificant ($p$-value, 0.66) (Tables S3 and S4).

CA decrease by the end of the experiment in both soils is most likely associated with the transition of microbiocenosis into homeostasis under laboratory conditions as well as possible decrease in the content of available nutrients necessary for cellulolytic organisms. The last assumption is confirmed by the fact that the CA in Albic Retisol, richer in nutrients, decreased 1.7-fold versus a 3.9-fold decrease in poorer Arenosol. Lower CA values in Arenosol as compared with Albic Retisol—2.4-fold for 0–3 months and 5.4-fold for 7–13 months—are explainable with the specific features of the corresponding microbial communities. A small input of plant residues in Arenosol and a high pH value have an adverse effect on the development of cellulolytic microorganisms. Several researchers believe that soil micromycetes, better adapted to Albic Retisol, are the leading participants in cellulose decomposition [29, 37].

**Table 1.** CA in soils at different kerosene loads, mg/(g day)

| Duration, months | CA in soil, mg/(g day) at a kerosene load, g/kg |
|------------------|-----------------------------------------------|
|                  | 0 (control) | 1 | 5 | 10 | 25 | 100 |
| Albic Retisol    |             |   |   |    |    |     |
| 0–3              | 9.24 ± 0.27 | 6.50 ± 1.07 | 4.65 ± 0.57 | 2.36 ± 0.41 | 2.45 ± 0.08 | 2.86 ± 0.32 |
| 3–7              | 6.18 ± 0.67 | 7.30 ± 0.20 | 5.50 ± 0.89 | 2.66 ± 0.42 | 2.92 ± 0.24 | 0.40 ± 0.14 |
| 7–13             | 5.30 ± 0.06 | 5.59 ± 0.52 | 5.04 ± 0.50 | 5.50 ± 0.09 | 4.15 ± 0.73 | 3.22 ± 0.15 |
| Arenosol (Aridic) |             |   |   |    |    |     |
| 0–3              | 3.87 ± 0.29 | 2.06 ± 0.33 | 1.24 ± 0.19 | 1.05 ± 0.09 | 0.86 ± 0.02 | 0.78 ± 0.36 |
| 3–7              | 2.15 ± 0.14 | 1.89 ± 0.04 | 0.83 ± 0.04 | 1.15 ± 0.02 | 0.72 ± 0.09 | 1.29 ± 0.02 |
| 7–13             | 0.98 ± 0.15 | 1.40 ± 0.18 | 1.96 ± 0.32 | 0.76 ± 0.04 | 0.21 ± 0.11 | 0.76 ± 0.08 |

See Table S3 for significance of differences.

**Table 2.** Intensity of cellulose decomposition in soils at different kerosene loads, percentage of the initial weight of test objects calculated per 3 months of incubation

| Duration, months | Kerosene load, g/kg |
|------------------|---------------------|
|                  | 0 (control) | 1 | 5 | 10 | 25 | 100 |
| Albic Retisol    |             |   |   |    |    |     |
| 0–3              | 83 ± 2.5    | 58 ± 9.6 | 42 ± 5.2 | 21 ± 3.7 | 22 ± 0.7 | 26 ± 2.9 |
| 3–7              | 56 ± 6.0    | 66 ± 1.8 | 49 ± 8.0 | 24 ± 3.7 | 26 ± 2.2 | 4 ± 1.3  |
| 7–13             | 48 ± 0.6    | 50 ± 0.5 | 45 ± 4.5 | 50 ± 0.8 | 37 ± 6.5 | 29 ± 1.4 |
| Arenosol (Aridic) |             |   |   |    |    |     |
| 0–3              | 35 ± 2.6    | 19 ± 2.9 | 11 ± 1.7 | 9 ± 0.8  | 8 ± 0.1  | 6 ± 0.7  |
| 3–7              | 19 ± 1.3    | 17 ± 0.3 | 7 ± 0.4  | 10 ± 2.0 | 6 ± 0.8  | 12 ± 0.2 |
| 7–13             | 9 ± 1.4     | 13 ± 1.7 | 18 ± 2.8 | 7 ± 0.4  | 2 ± 1.0  | 7 ± 0.5  |

Schedule of cellulose decomposition intensity: <10, very weak; 10–30, weak; 30–50, medium; 50–80, strong; and >80, very strong [16].

CA decrease by the end of the experiment in both soils is most likely associated with the transition of microbiocenosis into homeostasis under laboratory conditions as well as possible decrease in the content of available nutrients necessary for cellulolytic organisms. The last assumption is confirmed by the fact that the CA in Albic Retisol, richer in nutrients, decreased 1.7-fold versus a 3.9-fold decrease in poorer Arenosol. Lower CA values in Arenosol as compared with Albic Retisol—2.4-fold for 0–3 months and 5.4-fold for 7–13 months—are explainable with the specific features of the corresponding microbial communities. A small input of plant residues in Arenosol and a high pH value have an adverse effect on the development of cellulolytic microorganisms. Several researchers believe that soil micromycetes, better adapted to Albic Retisol, are the leading participants in cellulose decomposition [29, 37].

**CA of the Albic Retisol polluted with kerosene.** Addition of kerosene to Albic Retisol inhibited CA during the first 3 months of incubation. Under a low (1–5 g/kg) load, the intensity of cellulose decomposition decreased as compared with the control from a very high to high level and CA decreased from 9.2 to 5.6 mg/(g day) ($p$-value, 0.03). Addition of kerosene at a dose of 10–100 g/kg decreased the intensity of cellulose decomposition to a weak level and CA dropped to 2.7 mg/(g day) ($p$-value, 0.02). In the next intervals (3–7 and 7–13 months), the CA in the variants with a low load was comparable to the control values ($p$-value, 0.70–0.37). The intensity of cellulose decomposition remained at a high level. In the cases of medium and high kerosene loads, CA was lower than the background values ($p$-value, 0.02–0.05) and decreased to weak and very weak levels.

By the end of the experiment (7–13 months), the intensity of cellulose decomposition in the control and all variants of pollution corresponded to a medium level (29–50%). In the case of low kerosene pollution,
CA was comparable to the control variant versus a high pollution (>25 g/kg), when the biological activity remained 1.3–1.7-fold lower as compared with the control. Thus, the CA in Albic Retisol restores under the kerosene load of up to 10 g/kg during 1 year. Analysis of the residual amounts of kerosene demonstrated its absence (<100 mg/kg) after 7 months in the variants with 1 and 5 g/kg. After 13 months, kerosene was detectable only in the variants with 25 g/kg (270 ± 47 mg/kg) and 100 g/kg (1057 ± 314 mg/kg). Thus, the CA restoration is determined by a decrease in the kerosene content during the experiment, which can be associated with both its partial evaporation during the ventilation of containers and biological transformation of technogenic hydrocarbons.

These results agree with the data of a long-term field experiment on simulation of the effect of kerosene on CA in Albic Retisol: CA decreased during the first year followed by its intensification and restoration to background levels after 4 years [10]. A comparable inhibition of the microorganisms decomposing cellulose was observed in the natural oil-polluted and artificial soils [20, 33]. A relatively weak response to the kerosene pollution at a load of 1–5 g/kg and a strong response to a load of 25–500 g/kg were also observed for the mixed forest soils in the Russian Far East [47].

CA of the Arenosol (Aridic) polluted with kerosene. The kerosene pollution of Arenosol decreased CA with an increase of the load during the first 3 months of the experiment. According to Zyaginetsv’s schedule [16], the intensity of cellulose degradation in all variants of the load was weak and very weak. At a low kerosene load, CA amounted to 1.65 mg/(g day); at a medium load, to 1.05 mg/(g day), and at a high, to 0.83 mg/(g day), which differs significantly from the unpolluted variant (p-value, <0.03).

These data agree well with the CA observed in the soils of Kazakhstan subboreal deserts under an integrated technogenic impact (mechanical turbation, pollution with nitrogen-containing propellants, and combustion of vegetation): the CA there decreased twofold and more as compared with the background [46]. A decrease in the CA under inconsiderable (1 g/kg) hydrocarbon pollution has been reported for Chernozem, Arenosol, and Cambisol [20].

During the interval of 3–7 months, the CA in the soils polluted with 1 to 10 g/kg of kerosene became comparable to the unpolluted soil (p-value, 0.08). Large doses of hydrocarbons inhibited CA (p-value, 0.03). In the third interval (7–13 months), CA stimulation was observed (p-value of 0.03) at loads of 1 and 5 g/kg. The CA in these variants exceeded the levels of the control 1.4- and 2.0-fold, respectively. Addition of kerosene to the soils poor in organic matter stimulates the microbial oxidation of hydrocarbons. This part of microbiocenosis, which also contains a considerable amount of micromycetes, transforms technogenic hydrocarbons to low molecular weight organic compounds [37]. Presumably, an increase in the share of micromycetes and the enrichment of soil with readily hydrolyzable organic substances increase CA.

At high kerosene loads (≥25 g/kg), CA was inhibited and very weak (p-value, 0.03).

The CA in the Albic Retisol polluted with kerosene was higher as compared with Arenosol (p < 0.02, n = 18) for all doses and observation intervals, which is explainable with the initially higher soil biological activity in the soils with higher nutrient content [8, 38].

**Dynamics of the kerosene content in Albic Retisol and Arenosol during the experiment.** The rate of cellulose decomposition in soil is mainly determined by micromycetes [29, 41, 44, 45]. The growth of a part (peripheral zone) of the mycelium underlies this process rather than the whole mycelium, i.e., the growth of the hyphae that directly contact the cellulose fibrils [29, 48, 50]. The saturation of linen cloth with kerosene interferes with this process. Analysis of the residual kerosene content in soil clearly demonstrates the correlation between CA restoration after the decrease in this kerosene content. The content of kerosene in soil over the observation period decreases in both soils (Table S5). Kerosene content was undetectable 3 months after the incubation in both soils at the minimum contamination level (1 g/kg). At a load of 5 g/kg, 4 and 6% of the initial kerosene amount remained in Albic Retisol and Arenosol, respectively. In the variants with a medium load (10 g/kg), 25% of the initial kerosene mass was recorded in Albic Retisol and slightly more than 28%, in Arenosol. The level of residual pollution at the maximum load (25 and 100 g/kg) varied from 61 to 79% of the initial hydrocarbon contamination. The absence of kerosene was observed in both soils after 7 months at the loads of 1 and 5 g/kg. And its content did not exceed 3% of the initial amount at 10 g/kg. By the end of the experiment, kerosene remained (1–2% of the initial amount) only in the variants with high concentrations. A decrease of kerosene contamination in the course of experiment is most likely associated with both its partial evaporation during the ventilation of containers and the biodegradation (transformation) of easily hydrolyzable organic substances.

The presence of hydrocarbons also decreases the availability of mobile nitrogen, phosphorus, and potassium compounds for microbiocenosis, which is confirmed by a decrease in the correlation coefficients between the content of these elements and CA [14]. Soil organic matter content and appropriate pH also have a positive effect on CA. In particular, soils form the following sequence according to the CA resistance to mazut pollution: Haplic Chernozems > Cambisol > Arenosol [20].

**CONCLUSIONS**

The laboratory experiment has shown a low variation of CA and its high information value for all vari-

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EURASIAN SOIL SCIENCE Vol. 55 No. 2 2022
nants of kerosene loads, suggesting that this characteristic can be used as an indicator in the assessment of biological activity of soils polluted with hydrocarbons. The CA intensity in unpolluted Albic Retisol is higher as compared with Arenosol due to weakly acidic environments and increased nutrient and organic matter content. Estimation of CA dynamics demonstrates that an increase in the level of hydrocarbon contamination to 10 g/kg causes a reversible CA inhibition in both Albic Retisol and Arenosol. High kerosene loads (≥25 g/kg) inhibit CA in both soils over 13 months of observation. It is necessary to take into account the period of biological activity of soil microbiocenosis in the corresponding landscape when extrapolating the results of this experiment to natural and anthropogenically transformed soils to estimate the rate of their self-restoration.

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**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**SUPPLEMENTARY INFORMATION**

The online version contains supplementary material available at https://doi.org/10.1134/S1064229322020119.

**Fig. S1.** Cellulolytic activity of soils in a laboratory experiment: (a) initial state of linen tissue (test object) and (b) linen tissue after three months of incubation.

**Table S1.** Properties of soil humus horizons.

**Table S2.** Initial water content in the studied soil samples with due account for the added kerosene.

**Table S3.** Cellulolytic activity in soils grouped according to kerosene load (rate of mass loss, mg/g soil per day).

**Table S4.** Significance of differences between cellulolytic activities of contaminated soil samples relative control samples according to the Mann-Whitney U-test.

**Table S5.** Kerosene content in the studied soil samples at the end of the experiment, % of the initial content.

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