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Comparison of the Impact of Drought on Soil with its Contamination with Cadmium and Reduced pH Level in Terms of Ecological, Toxicological and Microbiological Indicators

ANNA SERGEEVNA OLKOVA* AND EVGENIYA VLADIMIROVNA TOVSTIK[1]

Department of Ecology and Nature Management, Competence Center "Environmental Technologies and Systems", Vyatka State University, Russia, Kirov
**(e-mail: usr08617@vyatsu.ru; Mobile: +79531339321)*

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ABSTRACT

The aim of this work was to compare the contribution of heavy metal contamination (Cd, mobile form, 6.4±0.5 mg/kg), short-term drought (25 days) and pH decrease (4.8 pH_{KCl} units versus 6.6 pH_{KCl} units in control) to toxicological and microbial status of the experimental soil to conclude on the agricultural suitability on similar soils. Each functional load was modelled separately in a microfield experiment. It was shown that increased soil acidity led to maximum differences in toxicity indices from those of the control soil compared with the action of other unfavourable factors: 36.4% in the test for *Paramecium caudatum* and 56.8% in the test for *Escherichia coli* to control. The results of laboratory bioassays were consistent with the reaction of soil enzymes: the decrease in the urease activity in acidic soil reached 39.09%, the decrease in the catalase was 25.23% compared to the soil without load. At the same time, the community of soil microflora was subjected to restructuring under all types of load with the dominance of oligotrophic microflora, which was uncharacteristic for natural soil. Consequently, the degree of change in ecological, toxicological and microbiological characteristics of the soil decreased under the influence of factors in the following series: soil acidity > drought ≈ contamination.

Key words: Bioassay, soil enzymatic activity, ecological, trophic groups, microorganisms

INTRODUCTION

This ecological instability, including a combination of abnormal weather and climate situations and anthropogenic contamination, leads to the forced use of arable land in crop production, which has some deviations from the norm, for example, urban soils (Baghaie, 2023).

Drought, soil acidity and heavy metal (HM) contamination is among the most common problems in agriculture and urban greening. Drought is typical not only for tropical and sub-tropical climatic zones, but is also periodically recorded in countries with a temperate climate (Kim *et al.*, 2023). It causes changes in the soil environment, worsens many ecological and biological characteristics of the soil, reduces soil fertility, as well as plant productivity. Stress conditions caused by drought lead to a decrease in the number of microorganisms; provoke the transformation of the microbial complex of the soil (Bogati and Walczak, 2022).

Soil acidity is a significant abiotic factor that limits agricultural productivity and causes environmental problems worldwide (Gurmessa, 2021). One of the mechanisms of loss of productivity is the violation of the relationship "plant - soil microbiota". In acidic soils bacterial diversity can increase due to oligotrophs, while fungal diversity decreases (Tayyab *et al.*, 2021). Among HMs, cadmium (Cd) can be distinguished as the most common ecotoxicants that reduce soil quality and level its ecological functions. Its toxicity is higher than, for example, lead and copper. Cadmium in high doses reduces the enzymatic activity of the soil (Wang *et al.*, 2019), negatively affecting actinomycetes and micromycetes (Xiao *et al.*, 2020). However, low levels of stress caused by soil contamination with cadmium have been suggested as a plant growth promoter (Mengdi *et al.*, 2021).

Due to the lack of land resources, attitudes towards unfavourable soil factors are beginning to change. The first step towards

[1]Laboratory of Biotechnological Methods of Agricultural Plant Breeding, Federal Agricultural Research Center of the North-East named N.V. Rudnitsky, Russia, Kirov.

reassessing the importance of soils characterized by stress factors is the assessment of their fertility and the ecological status of the soil. The last set of parameters includes natural and anthropogenic factors of soil formation, the relationship of which is actively studied all over the world (Tepanosyan *et al*., 2018; Ye *et al*., 2019; Yang *et al*., 2020). This information is important both for the choice of soil reclamation technologies and for the selection of crops capable of growing under functional load conditions.

The combination of the analyzed scientific data and the existing problems in the field of land use, led to the next goal of our work to compare the contribution of soil contamination with HM (Cd), short-term drought and lowering of the pH level in the toxicological and microbial status of the soil according to the corresponding indicators to conclude on the agricultural suitability of similar soils.

MATERIALS AND METHODS

The experiment was carried out under conditions close to the field. Soddy-podzolic soil of medium loamy mechanical composition was laid in vegetation containers 1.5 × 1.3 × 0.3 m in size. The soil was sown with barley to create conditions for an agroecosystem with a monoculture. The experimental period was May-August 2021. During the experiment, in addition to natural precipitation, the plants were watered until the soil moisture content reached 50-65%. At the end of the experiment, the soil was freed from plants and sent for research.

The first experimental series simulated functioning of the "soil-plant" system with a toxic load in the form of uniform contamination of the soil with cadmium. Cadmium was introduced into the soil a month before sowing the seeds in the form of a solution of $Cd(CH_3COO)_2$. The content of mobile forms of cadmium in the soil was determined by the atomic absorption method, reaching 6.4±0.5 mg/kg by the time of seeding, which was 3.2 times higher than the approximately permissible concentration regulated for soils in Russia (SanPiN 1.23685-21, 2021).

The second experimental series was aimed at studying the consequences of a short-term drought. To do this, during the period of "exit to the tube - heading of barley", natural watering was stopped by isolating the container from above with a plastic film. The total drought time was 25 days.

The third series of experiments was carried out on the soil, close in its main characteristics to other options, but differed by a lower pH level of the water extract from the soil – 4.8 units, which was 1.7 pH units lower than the control. No pollutants were added to the soil. The soil without functional load served as control.

After the microfield experiment, soil bioassaying was carried out, the activity of its enzymes was assessed, and the number of microorganisms of various ecological and trophic groups was recorded.

Soil bioasaying was carried out by the eluate method. For this, aqueous soil extracts (1:4) were prepared and assessed by the chemotactic reaction of *Paramecium caudatum* and bioluminescence of *Escherichia coli* for 30 min.

The number of ecological and trophic groups of microorganisms in the soil was determined by standard methods by inoculation of appropriate dilutions of the soil suspension on nutrient media. Representatives of the zymogenic and oligotrophic ecological niches were taken into account in the soil. The zymogenic microflora included macrotrophic microorganisms capable of growing on rich nutrient media, and mesotrophs growing on simpler media, but containing the main biogenic elements. Oligotrophs were microorganisms that can grow on poor nutrient media.

The determination of the catalase activity was carried out by the gasometric method by the amount of molecular oxygen released when the soil contacted with hydrogen peroxide. The recording of indicators was done three times after 1, 3, 5 min, with further averaging of the data. The urease activity was determined by a spectrophotometric method based on measuring the amount of ammonia formed during the hydrolysis of urea by binding it into coloured complexes with Nessler's reagent.

Laboratory determination of indicators was carried out in triplicate. During statistical processing of the obtained data, the mean values (M) and standard deviation (δ) were determined. The significance of differences between the compared data series was assessed by Student's t-test at $P<0.05$. All values of the studied soil characteristics were presented relative to the values obtained for the control soil. This approach made it possible to compare different indicators with each other.

RESULTS AND DISCUSSION

The reactions of bacteria and ciliates to water extracts from their studied soils were opposite: the chemotaxis of *P. caudatum* was inhibited, while the bioluminescence of *E. coli* increased in the samples compared to the methodological control. This was probably due to the fact that the soil habitat was more characteristic of bacteria than protozoa.
The results obtained in relative values in percentage terms relative to the values for the control soil variant are presented in Fig. 1.

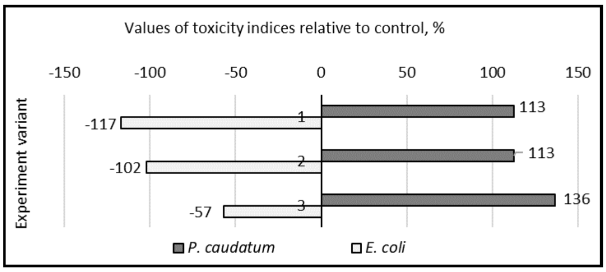

Fig. 1. Effect of cadmium contamination (1), drought (2) and acidic soil reaction (3) on soil toxicity according to *P. caudatum* and *E. coli* reactions.

When analyzing the results of the bioassay for P. *caudatum*, it turned out that the values of the toxicity indicator relative to the control were 12.73% in both variants (P<0.05). Soil samples which differed from the most favourable control variant by a lower pH level were the most toxic for ciliates among the three indices during short-term drought where toxic load was equal. The increase in the value of the toxicity index reached 36.4% to the control (P<0.05).
As in the case of protozoa, in the test on bacteria, the soil with an acidic reaction of the environment, in comparison with other types of functional load, most differed from the control variant. Stimulation of the test reaction in the nutrient soil extract, which is a characteristic of *E. coli*, decreased in this variant of the experiment by almost two times (56.8% of the control). For soil samples that experienced cadmium contamination and the effect of short-term drought, an increase in the bioluminescence of bacteria relative to the soil of the control variant was noted by 17% (P<0.05) and 2.2% (P>0.05), respectively.
It turned out that the responses of *P. caudatum* and *E. coli* correlated with each other with a high level of association (r=0.97). Therefore, using two laboratory express bioassays, it was shown that the acidic reaction of the soil environment had the greatest toxic effect, compared with cadmium and short-term drought.
The insignificant reaction of laboratory microorganisms to drought was quite explainable by the fact that these bioassays were developed primarily to indicate anthropogenic pollution, and the absence of acute toxicity of contaminated soil indicating tolerable stress levels on the applied concentration (6.4±0.5 mg/kg). It is known that almost every toxicant has a range of doses that do not cause damaging effects (Erofeeva, 2022). At the same time, the pH level of the environment quickly affects organisms through a change in the permeability of biological membranes, the impact on water-salt metabolism, and the transformation of the bioavailability of macro- and microelements (Animal Models in Yoxicology, 2016). This explains the most striking reaction of test organisms to increased soil acidity.
Such unexpected results required studying other soil properties associated with microorganisms in order to determine the level of formation of soil adaptation as a bioinert body to loads of various etiologies.
Fig. 2 shows the change in the activity of urease and catalase relative to the control depending on the studied factors.

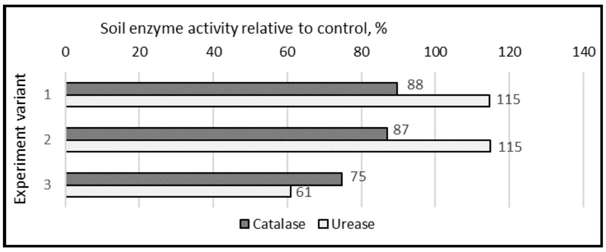

Fig. 2. Effect of cadmium contamination (1), drought (2) and acidic soil reaction (3) on the activity of soil enzymes.

The activity of catalase compared with urease was more stable under the influence of unfavourable factors. Inhibition of the catalase activity was observed in all variants, while the urease activity increased in response to two functional loads, and in one experimental variant it was below the control values.
The results of cadmium and drought were very

close to each other within assessing a single enzyme (P>0.05) when comparing indicators for a single enzyme). The catalase activity decreased relative to control by 10.28% in the soil contaminated with cadmium and by 13.09% in the soil subjected to a 25-day drought. The action of urease increased in comparison with the soil without load by 14.56 and 14.94% in the same series.

In the soil with high acidity, the reaction of enzymes turned out to be highest and it was in the zone of inhibition for both enzymes. At the same time, the decrease in the urease activity reached 39.09%, the decrease in catalase was 25.23% (P<0.05) for two enzymes, compared with the control.

Consequently, the factor of increased soil acidity had the maximum negative effect on the activity of soil enzymes compared to cadmium contamination at the level of 6.4±0.5 mg/kg of dry soil mass (mobile form) and medium-term drought (25 days). These data were consistent with the results of the microbiological tests given above, where also the toxicity indices were found to be highest when testing acidic soil.

The explanation for the significant effect of the acidic pH of the soil environment on enzymes can be seen in the mechanism of interaction between hydrogen ions and exoenzymes. Thus, it was reported that a slight change in pH prevented the ionization of functional groups of amino acids and the breaking of hydrogen bonds, as a result of which the folding of the polypeptide chain changed, which led to denaturation and the cessation of enzymatic activity (Msimbira and Smith, 2020). It is also known that various soil enzymes are sensitive to both natural factors of the soil environment (fluctuations in temperature, humidity, richness of macro- and microelements) and anthropogenic factors (chemical and physical pollution) (Zhu *et al.*, 2021; Erdel, 2022). A recent study showed that the activity of soil enzymes depended more on the average annual temperature than on the type of soil (Olkova and Tovstik, 2022). Thus, the data obtained in this work confirm that natural environmental factors, in particular soil acidity, have a stronger effect on soil enzymes than other stress factors, certainly outside the extreme values.

The number and ratio of ecological-trophic groups of microorganisms involved in the transformation of soil organic matter and ensuring the cycle of elements in ecosystems is an important parameter for assessing the state of the environment (Nikitin *et al.*, 2021). It turned out that soils experiencing different functional loads differed in these microbiological characteristics (Fig. 3a and b).

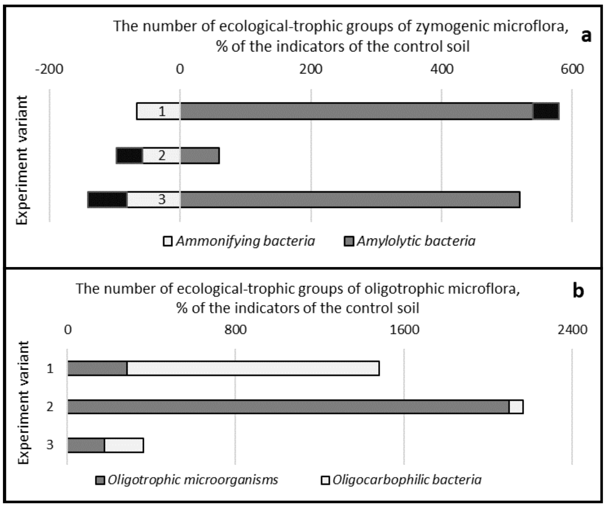

Fig. 3. Effect of cadmium contamination (1), drought (2) and acidic reaction of the soil environment (3) on the number of microorganisms in the zymogenic (a) and oligotrophic ecological niche (b).

The number of representatives of some groups of soil microorganisms belonging to the zymogenic microflora decreased as a result of the transferred load. So, the number of bacteria using organic forms of nitrogen (ammonifiers) decreased compared to the control in all experimental variants of the experiment (P<0.05). The suppression of the number of cellulolytics in the soil was noted under two types of load: as a result of drought by 40% (P<0.05) and with increased acidity by 60% (P<0.05). At the same time, the effect of cadmium on cellulolytics was stimulating: 40% higher than in the control soil (P<0.05). The number of amylolytic microorganisms that destroy starch and oligosaccharides was significantly higher in the soil without load: from 60 to 540% of the control at different functional loads (P<0.05). It is possible that the dominance of amylolytic microorganisms in comparison with other representatives of the zymogenic microflora is due to the fact that the oxidation of starch and oligosaccharides is a less energy-intensive process than the mineralization of cellulose and nitrogen-containing organic compounds.

The confirmation of this is seen in the significant development of oligotrophic microflora, the value of which, against the background of the revealed inhibition of amyllolytic and cellulolytic microorganisms, exceeds the control indicators by 22 times (the "drought" option, ($P<0.05$). Oligocarbophils received the maximum opportunity for development in the soil contaminated with cadmium. Their number in the soil under the influence of this load increased by 13 times compared to the control ($P<0.05$). The greatest number of soil oligotrophs was recorded under the influence of drought (mentioned above).

On the whole, the sensitivity of the ecological trophic groups of soil microorganisms to functional loads decreased in the following order: megatrophs > mesotrophs > oligotrophs. This causes a restructuring of the soil micro-biocenosis with the dominance of groups of organisms that are content with low-energy carbon sources. The response of the soil microbial community to soil acidification can be asynchronous, in particular, to soil acidification (Liu *et al.*, 2021).

The confirmation of these results can be found in the study (Dubrovskaya *et al.*, 2022), where low humidity did not limit the development of microorganisms. It was also shown that soil moisture had a positive effect on soil respiration, but it did not contribute to the daily fluctuations of the indicator, unlike temperature (Fekadu *et al.*, 2023). This suggests that microorganisms quickly respond to the temperature factor, and changes in response to humidity fluctuations occur gradually. Probably, hygroscopic moisture adsorbed on the surface of soil particles is enough for microorganisms to develop for quite a long time (Fomina and Skorochod, 2021).

In general, the results obtained give optimistic grounds to believe that many lands burdened with an unfavourable factor can correspond to quality lands in terms of their ecological, toxicological and microbiological indicators. Many brown fields of urban and industrial areas, arid agricultural regions, acidic soils of boreal zones can be included in economic activities and perform ecological functions, which are already practised (Phytoremediation, 2021).

CONCLUSION

The study showed that unfavourable factors such as contamination, drought and high acidity had a negative impact on the ecological and toxicological indicators of the soil. Soil toxicity for laboratory test organisms *P. caudatum* and *E. coli* increased in a series of applied functional loads: cadmium contamination (6.4 ± 0.5 mg/kg mobile form) = drought (25 days) < acidic soil reaction (4.8 pH units). Similar results were obtained in the study of the activity of soil enzymes. Catalase and urease had minimal activity in acidic soil, and the effects of cadmium and drought were similar. It follows from this that among the agricultural problems that adversely affect soil properties, acidity is the leading factor compared to heavy metal pollution and drought. Of course, this conclusion is valid outside the extreme values of the tested loads. So, in this work, the contamination was moderate (exceeding the permissible level by 3.2 times), The drought was short-term, the reaction of the soil environment was moderately acidic.

In part, the obtained results were also confirmed in the study of the number of individual representatives of ecological and trophic groups of soil microflora. Ammonifiers and cellulolytics reduced their number in the soil to the maximum as a result of drought and increased soil acidity. Other microorganisms, which can be attributed to mesotrophs and oligotrophs, on the contrary, received a numerical advantage in the microbial community. In general, the sensitivity of ecological-trophic groups of soil microorganisms to functional loads decreased in the following order: megatrophs > mesotrophs > oligotrophs. This caused a restructuring of the soil micro-biocenosis with the dominance of groups of organisms that were content with low-energy carbon sources.

Thus, it has been shown for the first time that the ecological, toxicological and microbiological characteristics of soils depend most of all on the optimal pH level of the soil environment and, to a lesser extent, on contamination and drought. This conclusion applies to the low degree of loads tested. It is with a low and moderate degree of manifestation of adverse factors that the question of the possibility of agricultural use of the soil is raised. Therefore, the results of our work can be useful when planning the reclamation of soils that are distinguished by some unfavourable factor for agriculture.

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