The influence of prehistory and chemical properties of soils on their allelotoxicity

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Abstract. The influence of prehistory and chemical properties of soils on their allelotoxicity was studied. 12 soil samples of East-European Plain, 6 seeds’ cultivars of spring wheat and seeds of barley, rye and triticale were used in the research. It was found that all studied cereals are inhibited by soil allelotoxins according to the revealed regularity. Soil samples from territories of agricultural use are characterized by a greater allelotoxicity compared with fallow and forest areas. Experimental data suggest that crop rotation applying not always able to reduce soil fatigue. For this reason, it is necessary to assess the real soil fatigue (soil allelotoxicity) when using the crop rotation.

1. Introduction
Soil fatigue is a well-known phenomenon in agriculture which leads to a soil fertility decrease. It is well observed under the monoculture cultivation. It is emphasized in A.M. Grodzinsky works that soil fatigue is based on allelotoxins accumulation in soils [1]. Sources of these compounds are plants and microorganisms excretions, as well as substances released during the decomposition of plant residues [2-6]. However, allelotoxins accumulation occurs not only in agricultural soils. The study of several thousands of soil samples that was carried out by N.A. Krasilnikov showed that almost all of them had a toxic influence on both higher plants and microorganisms.

Despite ubiquitous occurrence of allelopathic soil toxicosis the study of this phenomenon remains insufficient. The study of soil chemical composition does not allow to determine the existence of soil toxicosis and its value definitely [1, 7]. There is a number of reasons for this. Firstly, allelochemicals consist of various chemical groups. Based on chemical similarity the following 14 categories are classified: water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones; simple unsaturated lactones; long-chain fatty acids and polyacetylenes; benzoquinone, anthraquinone and complex quinones; simple phenols, benzoic acid and its derivatives; cinnamic acid and its derivatives; coumarin; flavonoids; tannins; terpenoids and steroids; amino acids and peptides; alkaloids and cyanohydrins; sulfide and glucosinolates; and purines and nucleosides [4, 6]. It should be mentioned that each category contains tens, hundreds or even thousands of compounds (phenolic compounds, for instance). Secondly, allelootoxins are a mixture of different substances in many cases. Single components' concentration of this mixture can be below the inhibition level while the total inhibition can be very strong [7]. Thirdly, some substances are not toxic themselves (sugars, for instance), but increase the impact of other toxins significantly [8]. Fourthly, toxins are fixed in the soils (primarily in the soil organic matter) by bonds with very different energy [1]. As a result, it is almost impossible to determine a part of the toxin molecules in the soil which will have a negative
impact on plants. For these reasons biotesting methods are basic for the soil toxicosis studying. In most cases, the study of soil extractions’ influence on seed development or the assessment of test-cultures set development in certain soils is carried out. This makes it possible to obtain information on soil toxicosis at a qualitative and semi-quantitative level. But even the use of such methods allowed to show that the data received in the early stages of seed germination have a high correlation with soils alleloxicity and fertility providing the yield of agricultural plants [9].

The goal of the work was to study the influence of prehistory and chemical properties of soils on their alleloxicity.

2. Objects and methods

The influence of soils on the change of seedling length in 7.5 g of seeds (~200 seeds) in the course of their germination in different soils was studied. The length of seedlings was determined using express method based on linear relationship between saturated volume of germinated seeds in water and the length of their seedlings [10]. Seeds germinated in soil or in sand were washed from substrate and placed by portions into 100–100 mL measuring cylinder with water placed on vibrating table, vibrated with frequency 50 Hz. After putting of every portion of germinated seeds into a cylinder, which formed cellular porous structure, small weight (8 g) in the form of rubber cork was placed atop for 15–20 s, and this resulted in compaction of the structure. After putting of all germinated seeds into a cylinder, small weight was placed atop, and additional compacting of the structure was made through tapping (30–40) of cylinder with seeds against a table. These procedures allowed formation of homogeneous structure, and lower boundary of weight allowed determining the saturated volume with accuracy up to 0.5 mL.

When carrying out experiments, 30 g of soil or sand were placed on the bottom of Petri dish 95 mm in diameter, then 7.5 g of seeds were placed in an even layer, and 30 g of soil or sand, respectively, were placed atop. Then water was added evenly from pipette on the dish. The experiment was carried out in sixfold repetition with subsequent statistical treatment of results. To minimize the error associated with the different quality of seeds 1000–1200 seeds were used in one experiment. As a result, the error of the experiment did not exceed 7% at a 95% confidence level.

Despite the existence of various phytotesting methods – the method of standard soil extracts, for instance – they do not allow to determine the precise value of soil alleloxicity since allelochemicals have different binding energy with soil adsorption complex and do not completely go into the water extracts [1]. Besides, some of soil bound allelochemicals enter into exchange reactions with substances that are produced by germinated seeds [1].

The influence of ratio “soil-seeds” on the seeds’ germination was also tested. The received data show that the use less than 40–g (20+20) of soil for the 7.5 g of seeds do not influence on the average alleloxicity value but the reproducibility of experiment results decreases significantly. 60 g of soil was chosen because of the experiments’ convenience.

To assess the soil alleloxicity it is necessary to choose «starting point» or in other words substrate without toxins. In our case, washed river sand was such substrate. Inhibition of seeds development in soils in comparison with sand can be expressed in % with a minus sign, i.e. the more inhibition the less its absolute value (table 1).

It should be noted that one of the main factors of seeds development rate is soil moisture. It is clear that seeds’ development is inhibited both by the deficit of water and the deficit of air. Thus, the comparison of substrates with different water-holding capacity can be correct in optimal water-air conditions for the development of seeds.

Alleloxicity determination was carried out for 12 substrates (table 2) and 6 spring wheat cultivars 2018 year harvest: «Liza», «Agata», «Lubava», «Zlata», «Rima», «Ester». Besides, the barley «Nur» cultivar, rye «Tatiana» cultivar and triticale «Nemchinovsky 56» cultivar were used in the work.

The seed surface sterilization was not carried out since experiments were aimed to be close as much as possible to natural conditions.
Soil samples both zonal row (Retisols, Luvisol, Chernozem, Kastanozem) and one type (Retisols) but with the different prehistory: soils under the crop rotation (№1-4) and fallow soils (№5, 6) were used in the study (table 1, table 2) [11]. The samples of Regosol under the monoculture (potatoes cultivated since 2015, №7) and Umbrisol under the forest (№8) were also used [11]. We supposed that a variety of objects allows us to understand better the reasons of soils allelotoxicity appearance. Soil samples №1-8 were taken from educational-experimental soil-ecological center of Moscow State University «Chashnikovo» so they represent a wide range of previous cultures’ influence on the seed germination and plant development of studied cereals. In addition, a number of soil samples of Russian plain was studied.

In the process of the research the following chemical characteristics were determined: pH (KCl), N<sub>total</sub>, P<sub>2</sub>O<sub>5</sub>available, K<sub>2</sub>Oavailable, C<sub>exchange</sub>, C<sub>total</sub>, S<sub>total</sub>. Measurement of pH and content in potassium (K<sub>2</sub>O<sub>exchange</sub>), phosphorus (P<sub>2</sub>O<sub>5</sub> available) and exchange calcium in soils was carried out by «CPC-3» photocolorimeter and a «FPM» flame photometer [12]. Available phosphorus and potassium were determined by the Kirsanov method [12]. The phosphorus determination N, S, C content in soils was determined on a CHNS analyzer Vario EL III, Elementar, Germany.

3. Results and discussion

Experimental data allows to make several conclusions. Firstly, different cultivars of spring wheat are inhibited by soils according to the revealed regularity (figure 1). Besides, it is worth mentioning that there is no chaotic reaction of cultivars on the allelotoxins complexes of various soils. There are almost no results when one cultivar is more resistant to allelotoxins of specific soil, and on other soil this cultivar is inhibited more strongly than others.

Table 1. The inhibition of spring wheat seeds, which is determined by the change in the total seedlings’ length of the seed array for 2 days on the soils in comparison with sand (%).

| № of the soils<sup>a</sup> | Prehistory           | The inhibition of spring wheat “Liza” cultivar | The inhibition of spring wheat “Agata” cultivar | The inhibition of spring wheat “Lubava” cultivar | The inhibition of spring wheat “Zlata” cultivar | The inhibition of spring wheat “Rima” cultivar | The inhibition of spring wheat “Ester” cultivar |
|---------------------------|----------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 1                         | Vetch-oat mixture    | –5 ± 5                                        | –24 ± 5                                       | –32 ± 5                                       | –34 ± 5                                       | –37 ± 5                                       | –56 ± 6                                       |
| 2                         | Mustard              | –16 ± 5                                      | –36 ± 6                                       | –48 ± 6                                       | –47 ± 6                                       | –60 ± 6                                       | –72 ± 7                                       |
| 3                         | Potatoes             | –20 ± 6                                      | –37 ± 5                                       | –57 ± 7                                       | –55 ± 7                                       | –61 ± 6                                       | –76 ± 7                                       |
| 4                         | Barley               | –21 ± 5                                      | –34 ± 5                                       | –53 ± 7                                       | –51 ± 6                                       | –61 ± 7                                       | –80 ± 6                                       |
| 5                         | Fallow               | 23 ± 6                                       | 16 ± 5                                        | 5 ± 5                                         | 5 ± 5                                         | 5 ± 5                                         | 5 ± 5                                         |
| 6                         | Fallow               | 0 ± 5                                        | –19 ± 5                                       | –1 ± 5                                        | –17 ± 5                                       | –5 ± 5                                        | –19 ± 5                                       |
| 7                         | Potatoes             | 0 ± 5                                        | –35 ± 6                                       | –29 ± 6                                       | –49 ± 6                                       | –49 ± 6                                       | –67 ± 6                                       |
| 8                         | Forest (litter)      | 0 ± 5                                        | –17 ± 6                                       | 2 ± 5                                         | –11 ± 5                                       | 9 ± 5                                         | 6 ± 5                                         |
| 9                         | Wheat                | –49 ± 7                                      | –52 ± 7                                       | –34 ± 6                                       | –42 ± 6                                       | –36 ± 5                                       | –65 ± 6                                       |
| 10                        | Potatoes             | –16 ± 6                                      | –34 ± 6                                       | –42 ± 6                                       | –41 ± 7                                       | –45 ± 6                                       | –65 ± 7                                       |
| 11                        | Wheat                | –42 ± 6                                      | –69 ± 6                                       | –76 ± 7                                       | –85 ± 7                                       | –86 ± 7                                       | –94 ± 7                                       |
| 12                        | Fallow               | –3 ± 5                                       | –17 ± 5                                       | –14 ± 5                                       | –10 ± 5                                       | –11 ± 5                                       | –13 ± 5                                       |

<sup>a</sup> Soil names according to WRB classification: 1-4 – Eutric Retisol (Aric, Loamic, Humic); 5,6 – Eutric Protostagnic Retisol (Aric, Loamic, Humic); 7 – Colluvic Aric Regosol (Dystric, Humic); 8 – Cambic Gleyic Umbrisol (Loamic, Humic); 9 – Eutric Glossic Retisol (Aric, Loamic, Humic); 10 – Vermic Calcic Chernozem (Loamic); 11 – Haplic Luvisol (Aric, Loamic, Raptic); 12 – Calcic Kastanozem (Anthic, Siltic)
The figure 1 shows that the order of cultivars’ inhibition by allelotoxins preserves approximately for each soil, although the ratio between the values of inhibition for different cultivars on soils can differ.

Based on revealed regularity the important fact should be noted. The cultivars that are more resistant to allelotoxins of one soil, will be more resistant to allelotoxins of other soils too. In this case, the choice of a sowing cultivar will be determined by the significance of its response differences to the allelotoxins complex of the studied soil. Thus, the data on the inhibition order of cultivars simplify greatly their selection for the sowing.

Table 2. Coordinates of sampling points.

| № of the soils\(^a\) | Coordinates       | № of the soils\(^a\) | Coordinates       |
|----------------------|-------------------|----------------------|-------------------|
| 1                    | Latitude: 56°2'1” N Longitude: 37°10’6” E | 7                    | Latitude: 56°2'27” N Longitude: 37°10’18” E |
| 2                    | Latitude: 56°2'1” N Longitude: 37°10’8” E | 8                    | Latitude: 56°2'33” N Longitude: 37°10’39” E |
| 3                    | Latitude: 56°1’59” N Longitude: 37°10’8” E | 9                    | Latitude: 56°22’26” N Longitude: 37°10’39” E |
| 4                    | Latitude: 56°1’60” N Longitude: 37°10’8” E | 10                   | Latitude: 53°29’16” N Longitude: 38°58’45” E |
| 5                    | Latitude: 56°2’22” N Longitude: 37°9’55” E | 11                   | Latitude: 53°96’12” N Longitude: 37°17’15” E |
| 6                    | Latitude: 56°2’22” N Longitude: 37°9’55” E | 12                   | Latitude: 49°6’37” N Longitude: 44°7’20” E |

\(^a\) The soil numbers correspond to the legend of table 1.

The experimental data for barley, rye, triticale and wheat on soils of zonal row (figure 2) show that inhibition order by allelotoxins which is observed for spring wheat corresponds for these cereals too.

Secondly, the results of experiments show that the lowest allelotoxicity value for the samples that were taken from the crop rotation fields is observed for the №1 soil sample where the predecessor was the vetch-oat mixture (table 1). However, according to crop rotation it is planned to sow wheat after potatoes on the №3 field, where the inhibition value is statistically higher for all 6 cultivars in comparison with samples of the №1 field. It allows to suggest that crop rotation applying without allelotoxicity control of a specific field can’t always give a positive result.

It attracts attention that soil allelotoxicity is more typical for agricultural lands regardless of the crops being grown (table 1). This regularity is common for all studied wheat cultivars (table 1, figure 1). The minimum values of allelotoxicity are characteristic for samples of fallow area (soil samples №5 and №12) as well as for litter under the forest (sample №8). The data of soil sample №5 show the lowest allelotoxicity values. They are statistically significant compared with samples №1-4, 9 for all studied cultivars. The most resistant to allelotoxins cultivar – Liza – is stimulated by the soil sample №5 on 23% whereas the least resistant cultivar – Ester – is inhibited by this sample on 7%. The cultivars and cultures order of resistance to allelotoxins is preserved approximately that is clearly seen from the figures 1 and 2.

The received data confirm the opinion that allelotoxicity phenomenon is not typical for the mature natural communities. The relationships between soil and plants in natural phytocenoses form during the evolution process. Therefore, they are well-balanced and relatively sustainable. The substance and energy that are produced in the photosynthesis redistributed and consumed sequentially by biogeocenosis components. Soil – is one of this system components. It is enriched by life and has
specific metabolism that is influenced by plants. During a unilateral impact on the soil, this metabolism is disturbed. Thus, disturbance of metabolism and energy in “soil-plant” system is a common reason of soil fatigue. Any agricultural activity leads to a shift in equilibrium in this system. The desire to obtain the highest possible yields causes a protective reaction of the ecosystem, which tries to return to the equilibrium state. Allelootoxins release is a mechanism that lowers the productivity of agrocenoses, preventing the losses of elements that are necessary for sustainable ecosystem development.

![Figure 1. Development of spring wheat seeds of various cultivars into soils compared with sand, determined by the increase in the total length of seedlings (%). The soil numbers correspond to the legend of table 1.](image)

Mechanism counteractions of ecosystems are based on the following phenomena. Firstly, monoculture cultivation leads to the changes in soil microorganisms’ composition with the increasing in phytopathogen share. Secondly, cultivation of almost any monoculture due to the high sowing density results in plant competition for resources by plants excretion of allelotoxins. This fact is confirmed in many studies on allelopathy [1, 9].

Besides, the test of various soil horizons allelotoxicity (table 1 samples № 5, 6) was carried out. The primary experimental results correspond to the known literature data about the increase in allelotoxicity with the deep [1, 9]. However, this issue requires further investigations.

In the process of studying the influence of soils chemical properties on allelotoxicity the following characteristics were determined: pH (KCl), N_total, P_2O_5 available, K_2O available, Ca_exchange, C_total, S_total (table 3). However, no correlation between inhibition and studied chemical properties could be found.
Figure 2. Development of various cultures’ seeds into soils compared with sand, determined by the increase in the total length of seedlings (%). The soil numbers correspond to the legend of table 1.

Table 3. Chemical properties of the studied soils.

| № of the soils<sup>a</sup> | Ca<sub>exch</sub>, mg/100g | pH (KCl) | N<sub>tot</sub>, % | P<sub>2</sub>O<sub>5</sub>available, mg/100g | K<sub>2</sub>O available, mg/100g | S<sub>tot</sub>, % | C<sub>tot</sub>, % |
|--------------------------|--------------------------|----------|-----------------|-------------------------------|------------------------------|----------------|--------------|
| 1                        | 208                      | 6,2      | 0,29            | 31,5                          | 36,95                        | 0,09           | 3,33         |
| 2                        | 216                      | 6,3      | 0,35            | 32,5                          | 25,9                         | 0,10           | 3,91         |
| 3                        | 167                      | 5,9      | 0,23            | 31,5                          | 29,8                         | 0,07           | 2,57         |
| 4                        | 117                      | 6,1      | 0,20            | 31                            | 22,05                        | 0,06           | 2,23         |
| 5                        | 83                       | 5,5      | 0,17            | 14,5                          | 6,48                         | 0,05           | 1,82         |
| 6                        | 67                       | 5,1      | 0,04            | 3                             | 3,25                         | 0,02           | 0,26         |
| 7                        | 67                       | 5,1      | 0,17            | 17,5                          | 7,15                         | 0,04           | 1,71         |
| 8                        | 117                      | 3,6      | 1,18            | 6                             | 11                           | 0,39           | 35,1         |
| 9                        | 133                      | 6,6      | 0,14            | 29                            | 19,45                        | 0,05           | 1,65         |
| 10                       | 316                      | 5,3      | 0,24            | -                             | -                            | 0,08           | 3,58         |
| 11                       | 100                      | 5        | 0,12            | 18,5                          | 15,55                        | 0,05           | 1,07         |
| 12                       | 150                      | 6,2      | 0,09            | -                             | -                            | 0,05           | 0,8          |

<sup>a</sup> The soil numbers correspond to the legend of table 1.

4. Conclusions
1. The study of various cultures and cultivars of cereals allowed to reveal the inhibition order of these crops by allelootoxins complexes. Despite the different ratio between the values of inhibition for different crops, their inhibition order on various soils preserves generally.
2. The soil samples from territories of agricultural use are characterized by a greater allelotoxicity compared with samples from fallow and forest areas.

3. The experimental data show that crop rotations are not always able to reduce soil fatigue. This makes it necessary to assess the real soil fatigue (soil allelotoxicity) under the crop rotation.

4. During the experiments, no correlation between inhibition and the studied chemical properties of soils was found.

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