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Distribution of "Molnia" Pesticide Along Soil Profile and its Influence On Soil Organisms

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In this study, influence of "Molnia" insecticide (active agent lambda-cyhalothrin) widely used in Russia and many European countries was investigated. Using several microbial parameters (microbial biomass, basal respiration, urease and dehydrogenase activities) and *Avena sativa* root elongation inhibition, effects of migration of pesticide along soil profile in short time period (3 months) was estimated. It was shown, that microbial biomass is higher sensitive to pesticide presence than microbial basal respiration. Digging of the soil which lead to its aeration changed the reaction of microbes on pesticide presence, in both, increasing and decreasing sides. Urease activity was sensitive to "Molnia" pollution while cellulase activity did not change significantly after pollution. The most biological parameters analyzed decreased from the upper (0-20 cm) to the lower (40-60 cm) soil layers which was connected with organic carbon and oxygen content. *Avena sativa* roots were significantly inhibited (21%) in the middle layer of pesticide polluted digged-up soil whereas it was equal to the corresponding control value in the non digged-up polluted soil. This is due to higher migration ratio of the pollutant into the digged-up soil. In three years of experiment, the quality of polluted soil slightly restored, that can be explained by degradation of the pesticide.

Key words: Agricultural soil, Pesticide, soil restoration, Microbial parameters, Phytotoxicity, leakage of pollutants.

Pesticides are used in agriculture to protect yields from pest organisms of different systematic groups[1]. Despite on the fact that modern pesticides are more target-oriented and less persistent than ones used before, they still can be accumulated in soil and influence its fertility as well as its habitants[2]. Soil microorganisms are responsible for many soil processes, inclusive nutrient cycling, plant growth promotion and decomposition of contaminants, e.g. pesticides[3, 4]. From the other hand, do to their quick response, high sensitivity, ecological relevance and ability to present information that integrates many environmental factors, soil microbes can be used as indicators of sustainability of agricultural management ecosystems[5-9]. It was shown previously that pesticides affect microbial activity and diversity in soil. Thus, pesticide degrading populations grows, abundance of gram positive bacteria increases, while functional diversity of microbial community decreases under influence of pesticides. Soil microbial enzymes are reported to be sensitive to pesticide presence. Another soil parameter that sensitively reacts on pesticide implementation is phytotoxicity[10-14].

Many works were devoted to study of pesticide leaching process in soil. It was demonstrated that leaching behavior of pesticides depends on irrigation or rainfall amounts and their time distribution, chemical characteristics of pesticide and properties of soil[15-18]. According to

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Tiktak with co-authors (2012), leaching of pesticides can be linked with soil macropore system which, in its turn, is connected with organic matter and clay content[19].

The objective of this paper was to estimate the effects of pesticide "Molnia" (active substance – lambda-cyhalothrin) widely used in Russia against insects on soil. We've analyzed the leaching process of this pesticide in uncultivated and cultivated soils which differ in their porosity. Besides, we've estimated the influence of the pesticide leaked into depth, on soil microbial characteristics and fertility measured using phytotoxity. The experiment was conducted on the field experiment plot for 3 years. The chemical and biological properties of soil samples were analyzed separately in three soil layers: 0-20, 20-40 and 40-60 cm.

MATERIALS AND METHODS

In the field experiment, the haplic greyzem soil (with 60% loam, 36% silt and 4% clay, pH 6.5) was artificially contaminated by 20 g kg^{-1} of "Molnia" pesticide containing lambda-cyhalothrin in concentration 50 g l^{-1} (CAS name (*R*)-cyano(3-phenoxyphenyl)methyl (1*S*,3*S*)-rel-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate, CAS registration number 91465-08-6) This level of content of "Molnia" pesticide is reported to be common in the agricultural soils in Russia. The three experimental areas 5mX5m each were situated in the experimental plot of Kazan Federal University (Kazan, Russia). The first two areas were contaminated by pesticide in May 2010, and the other two control areas remained unpolluted. In August 2010 (after 3 months) we took the samples from the first polluted and first control area, and in August 2013 (after 36 months) we took the samples from the second polluted and second control area. To simulate the agricultural treatment of the soil, half of each area was digged-up immediately after beginning of the experiment. The samples were collected from five points in each area from three soil layers: 0-20 cm (upper layer), 20-40 cm (middle layer) and 40-60 cm (lower layer) to estimate the influence of oil migration along soil profile.

Before analysis of agrochemical parameters, soil was air-dried. Soil organic carbon was determined using wet oxidation according to ISO 14235:1998 [20].

Basal microbial respiration rates were determined according to[21], and microbial biomass (Cmic) - according to [22]. Cellulase activity was estimated by hydrolysis of carboxymethylcellulose according to the method described in[23] with modifications: soil (3 g) adjusted to 60% water holding capacity, 7.5 ml 1.15M phosphate buffer, 5 ml 1% carboxymethylcellulose and 0.5 ml toluene were incubated at 28°C for 24 h. The samples were filtered, 2 ml dinitrosalicylic acid reagent (10 g of 3,5-dinitrosalicylic acid, 16 g NaOH, 300 g K-Na-tartrate tetrahydrate in 1 l of distilled water) added to 4 ml filtrate, incubated at 95^0! for 10 min in a water bath, cooled and measured at 540 nm. Results were expressed as mg reducing sugars in 1 kg dry soil. Urease activity was estimated by hydrolysis of urea according to the method described in [12] with modifications: 4 ml phosphate buffer (pH 6,7) and 0.2 ml toluene were added to soil (2 g) adjusted to 60% water holding capacity, and after 5 min 4 ml 10% urea was added. The mixture was incubated at 37°C for 24 h. The samples were adjusted to 20 ml with 1M KCl, filtered. 1 ml filtrate was transferred to Falcon tube, adjusted to 50 ml with distilled water, than 2 ml Nessler's reagent was added, and extinction was measured at 400 nm. Results were expressed as mg ammonia perkg dry soil[24].

Phytotoxicity of soil samples was measured according to ISO 11269-1 (2012) using *Avena sativa* as a test-object. Tests were carried out in a chamber at 20± 2^0C under constant illumination (4000-7000 lx), with a 16:8 day-night light cycle. Assays were conducted in plastic pots containing 200 g of contaminated substrate moistened at 70% water-holding capacity. The moisture level was maintained constant by adding distilled water every day. Twenty seeds were placed intro the testing soil sample. After 10 days, the length of the plant roots was measured. The percentage of inhibition (I,%) for each of the dilutions was determined by comparing the average root length in each testing sample to the root length in the control.

We analyzed each sample a minimum of five times. For statistical analysis we used a randomized complete block design Analysis of Variance (ANOVA) with a = 0.05. Means were compared using a t-test at a = 0.05. Correlations

were analyzed using Product-Moment Correlation Coefficient in Origin 8.0. Cluster analysis of results obtained was carried out using R.Gui package.

RESULTS

The soil that was used in the experiment characterized by the average organic carbon content. In the upper soil layer (0-20 cm) it was estimated to be 4.2±0.6%, in the middle (20-40 cm) and lower (40-60 cm) layers it was equal to 2.1±0.4 and 0.4±0.1%, respectively. Pesticides are supposed to be active against pests during the vegetative season. The "Molnia" pesticide is designed to fight against insects; however, it can be potentially toxic to non-target organisms such as bacteria and plants[25]. That is why on the first stage of our work we checked the influence of the "Molnia" pesticide on soil microbiota (respiration activity, microbial biomass, enzyme activity) and soil phytotoxicity in short time period (3 months in summer). Besides, we estimated whether the pesticide affects in the upper soil layer only (where it is used) or there are negative changes in the deeper soil layers caused by pesticide leakage. The results of estimation of soil microbial biomass are presented on Fig. 1.

As shown on Fig. 1, microbial biomass decreased in control soil from the upper to the lower layer. This is typical for soils and was described by many authors previously[26, 27]. In the unpolluted soil that was digged-up, the level of microbial biomass is comparable with the control one, in the middle and lower layers the values exceed the control ones which is probably connected with better aeration. Microbial biomass in "Molnia" polluted samples is inhibited in 1.2-3.5 times in comparison to corresponding control values. The inhibition is less in the polluted digged-up samples. Probably, access of oxygen stimulates the indigenous microflora in these soil samples.

Further, we estimated the respiration activity in soil samples. As shown on Fig. 2, this parameter was slightly higher in non-polluted digged-up soil in comparison to control. The highest level of activity respiration (1.3 times higher than in corresponding control) was observed in polluted non cultivated soil.

Table 1. Enzyme activities of soil samples in 3 months after beginning of the experiment

Sample	Urease activity, % from C0-20	Cellulase activity, % from C0-20
C0-20	100,0±14,8	100±14
C20-40	65,1±9,7	118±17
C40-60	18±2	89±13
Cd0-20	108±16	77±11
Cd20-40	93±13	87±13
Cd40-60	20±3	51±7
P0-20	57±8	119±17
P20-40	47±7	58±8
P40-60	23±3	54±8
Pd0-20	56±8	88±13
Pd20-40	24±3	75±11
Pd40-60	14±2	91±13

Table 2. Changes of biological parameters of the soil samples during the experiment

soil sample	Microbial biomass, %		Respiration activity, %		Urease activity, %		Cellulase activity, %		Oat root length, %	
	3 months	3 years	3 months	3 years	3 months	3 years	3 months	3 years	3 months	3 years
Ñd0-20	97	105	119	107	108	101	77	107	103	98
Cd20-40	78	95	81	94	93	38	87	85	87	83
Cd40-60	74	41	30	33	20	25	51	69	69	67
P0-20	46	94	131	53	57	64	119	112	80	90
P20-40	24	36	80	33	47	48	58	96	84	86
P40-60	32	31	14	28	23	22	54	47	59	62
Pd0-20	86	64	77	21	56	67	88	109	81	95
Pd20-40	46	32	63	16	24	47	75	104	66	83
Pd40-60	25	34	44	22	14	25	91	75	66	62

This is probably connected with reaction of microorganisms on stress conditions. In polluted digged-up soil, in opposite, we observed the inhibition of the basal microbial respiration. This difference from control can also be explained by the negative influence of "Molnia" pesticide on soil microbial community. It has to be stressed, that negative impact of this insecticide was described previously. Thus, "Molnia" pesticide in concentration of 1.25 mg kg^{-1} soil caused 21% inhibition of soil microorganisms involved in N-cycle[25].

Since microbial enzyme activities are reported to be sensitive to pesticide pollution, we estimated the levels of cellulase (C-cycle) and urease activity (N-cycle) of soil samples. Results expressed in percentages from the level of corresponding enzyme activity in the upper layer of control soil are presented in Table 1.

As shown in Table 1, urease activity in unpolluted digged-up samples is slightly higher than in the control ones. This is probably connected with higher oxygen access in these samples. In the

Table 3. Scoping of the changes of biological parameters estimated in soil samples

Soil samples	Scopes based on changes within 3 years of experiment					Final scope for the sample
	Microbial biomass	Microbial respiration	Urease activity	Cellulase activity	Oat root length	
Cd0-20	-1	-1	0	1	0	0
Cd20-40	1	1	-1	0	0	
Cd40-60	-1	0	0	1	0	
Avarage for Cd	-1	0	-1	2	0	
P0-20	1	-1	0	0	0	1
P20-40	1	-1	0	1	0	
P40-60	-1	1	0	0	0	
Avarage for P	1	-1	0	1	0	
Pd0-20	-1	-1	1	1	1	1
Pd20-40	-1	-1	1	1	1	
Pd40-60	0	-1	1	-1	0	
Avarage for Pd	1	-1	0	1	0	

polluted samples, the level of urease activity was 43-45% lower than in corresponding controls. Aeration did not play important role for inhibition of urease activity in pesticide polluted samples. Cellulase activity differed significantly among replications. Generally, this parameter was more sensitive to digging than to pesticide presence.

Plants are one of the organisms habiting in soil and depending on its quality. Plants reflect the main agricultural and economical property of soil – its fertility[28-30]. Despite on the fact that pesticides are designed to protect plants from pests and to improve their growth and biomass uptake, these compounds still can have negative impacts on plants. In our previous studies we demonstrated, that root length is the sensitive biometric test function that can be used for phytotoxicity estimations. Therefore, on the next stage of our investigations we estimated the influence of "Molnia" pesticide on plant roots. Oat (*A. sativa*) was used as a test-object. The results are presented on Fig. 3.

As shown on the Fig. 3, length of the plants' roots in the upper layers of both non digged-up and digged-up pesticide polluted soils was inhibited in 19-20% in comparison to corresponding control. The length of the roots in the lower layers did not differ significantly from corresponding control, this is probably connected with the fact the pesticide did not leak so deep. It is interesting to compare the influence of the polluted soil samples of the middle layer on the roots of the test-plants. In non digged-up soil, the roots' length was equal to the control ones whereas in digged-up soil it was significantly inhibited (21%). This may be explained by the increasing of porosity in the digged-up soil and more easy migration of the pesticide along soil profile.

Pesticides are reported to be quickly biodegradable. However as reported by many authors, metabolites of some pesticides can be highly toxic for both target pests and non-target organisms[31-33]. Therefore, further we estimated the microbial parameters and phytotoxicity of the soil samples in three years after pesticide treatment and digging-up. In control soils we observed some changes in levels of the parameters investigated. The fluctuations of biological parameters are normal and described many times by other authors[11, 32, 34]. The levels of three parameters (microbial biomass, cellulase and urease activity) became less, they were estimated to be 186±25 and 101±14 mg g^{-1} 24

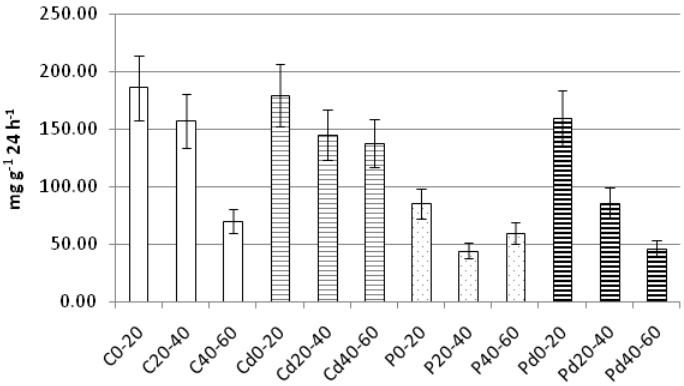

Fig. 1. Microbial biomass in soil samples in 3 months after experiment beginning

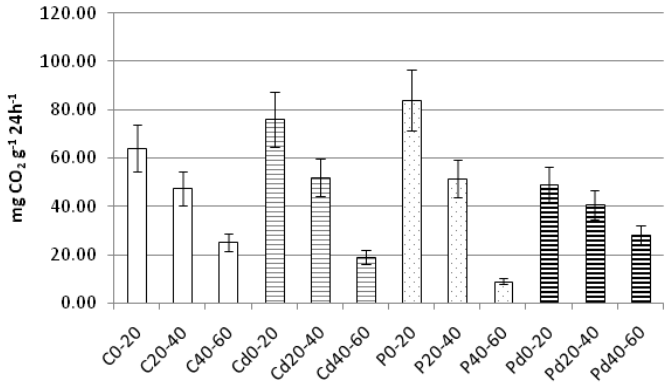

Fig. 2. Microbial basal respiration in soil samples in 3 months after experiment beginning

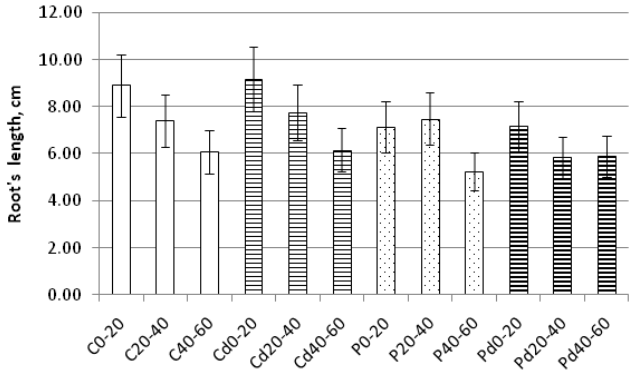

Fig. 3. Roots' length of oat plants grown in soils samples in 3 months after the beginning of the experiment

h^{-1}, 1239±185 and 1149±172 mg kg^{-1}, 234±35 and 159±23 mg NH_4 kg^{-1}, in 3 months and 3 years after the experiment beginning, respectively. The root length did not change significantly (8.91±1.25 and 8.99±1.34 cm) and microbial respiration increased (from 64±9 and 100±14 mg g^{-1} $24h^{-1}$) during the time of experiment. Further, we estimated the levels of the parameters mentioned above in the non-polluted digged-up, non digged-up polluted and digged-up polluted soil samples. To take into account the natural fluctuation of parameters, we expressed the values obtained in percentages from the C0-20 sample of the corresponding time of sampling. The results for the samples Cd, P and Pd are presented in Table 2.

To analyze the massive of the results obtained, we implemented the scoping. If the value of the parameter increased in 3 years more than 10%, the scope was equal to 1, if the parameters decreased more than 10%, the scope was equal to -1. If the parameter did not change significantly, the scope was equal to 0. The results of the scoping are presented in Table 3.

As demonstrated in Table 3, root length did not differ in all the treated samples, cellulase activity increased, and other parameters ranged differently during the experimental period. Generally, the quality of soil that was digged-up did not change in three years of experiment. The quality of pesticide polluted soil slightly restored which is probably connected with pesticide degradation.

CONCLUSION

In this study, the environmental hazard of widely used "Molnia" pesticide for non-target organisms (indigenous soil microflora and plants) was demonstrated. On the basis of analysis of microbial and plant parameters, it was shown that differences caused by the pesticide during 3 month are more significant in the upper soil layer in comparison to the middle and lower layers. This is probably because the pesticide mainly remains in the upper soil layer and insignificantly migrates along soil profile. Besides it was shown that digging-up of the soil promotes the leakage of the pesticide into deeper soil layers. In three years, biological parameters of the polluted soil improved, probably, due to degradation of the pesticide.

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