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Influence of pesticides on the biological activity of light chestnut soils in South-East Kazakhstan

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Abstract

The paper discusses the impact of pesticides on the biological activity of soils, as well as an environmental assessment of the state of light chestnut soils by the Kazakh Research Institute of Agriculture and Crop Production with the aim to establish diagnostic indicators that reduce biological activity. The study covers physical, chemical and biological properties of soils under crops of winter wheat in the light chestnut soil in the South-East of the Republic of Kazakhstan. The content of pesticides in soil samples was determined using the gas chromatography mass-spectrometric method. The paper shows results of the chromatographic analysis of soil samples regarding the content of pesticides. The study of changes of light chestnut soil biological activity was conducted to determine their relative resistance to pesticide contamination. Data obtained revealed the degree of light chestnut soil resistance to pesticide contamination. The study also identified species of soil invertebrates, as well as soil enzymes that should be used as bioindicators for the monitoring of the contamination with pesticides. Results obtained expand knowledge about changes in the biological activity of light chestnut soils due to pesticide contamination in the ecosystems of South-East Kazakhstan. In contrast to abundance indicators, the results suggest that the species composition of soil organisms can be used as a criterion for a qualitative assessment of the soil exposure to pesticides.

Key words: *biological activity, chromatographic analysis, ecology, light chestnut soil, pesticides, soil mesofauna*

INTRODUCTION

Contemporary agricultural production is impossible without the use of pesticides. While recognizing the unquestioned positive effect of their use in various spheres of human economic activity, data have been gradually collected to document their negative impact [CHAUVIN *et al.* 2015].

Soil pollution with pesticides has become a global phenomenon. Their seepage into the soil in large quantities primarily affects biological properties of soil: the number of animals living in soil decreases. Moreover, invertebrates die in the saprotrophic layer, a layer in which the consumption and decomposition of organic residues occurs. In contaminated soils, apart from microorganisms, the population of earthworms decreases. The latter are indicators of humus content and soil pH regulators. Calcium carbonate produced by earthworms in their glands neutralizes soil acids while passing through their digestive tract. Therefore, the coprolites secreted by them have a higher pH value than soil. Bacteria abundantly develop in coprolites, which are centers of specific associative microbial communities formation [ARORA, SAHNI 2016].

Regardless of the form and method of application, pesticides seep and accumulate in the soil and as such affect the soil biota. The need to study the interaction between pesticides and soil fauna is crucial to determine the role of soil organisms in creating soil fertility and soil detoxification from xenobiotics [MOREL *et al*. 2015]. Effects of long--term fungicide application on microbial properties in the tallgrass prairie soil show that Mycorrhizal fungi play an important role in altering the availability and flow of carbon in prairie soil and may influence the composition and abundance of soil biota groups. The process reduces the population of certain fungal feeders (*Tylenchidae*) by 13% and predatory nematodes (*Dorylamidae*) by 33%. Soil nematodes, earthworms and protozoa are affected by field application rates of the fungicide fenpropimorph and other herbicides [SMITH *et al*. 2000]. Tests of Bavistin and Dithane M-45 fungicides for the effect on soil mycoflora with special reference to mycorrhizal fungi of the sunflower crop showed that both fungicides had deleterious effect on the mycorrhizal spore number and percentage mycorrhizal root colonization [AGGARWAL *et al*. 2005]. The assessment of indicators showing earthworm sensitivity to pesticides by PELOSI *et al*. [2014] has shown that earthworms suffer from pesticides at all organisation levels. For example, pesticides disrupt enzymatic activity, increase individual mortality, decrease fecundity and growth, change individual behaviour, such as the feeding rate and decrease the overall community biomass and density. Insecticides and fungicides are the most toxic pesticides having impact on survival and reproduction for earthworms. Comparative acute toxicity of twenty-four insecticides to *Eiseniafetida* has been assessed by WANG *et al.* [2012]. It showed that neonicotinoids were super toxic to *E. fetida*, pyrethroids were very toxic and insect growth regulators (IGRs) were moderately toxic. However, antibiotics, carbamates and organophosphates induced variable toxicity responses in *E. fetida*. It was concluded that, irrespective of bioassay systems, earthworms were more susceptible to neonicotinoids than other modern synthetic insecticides. LC50 and LD50 studies revealed that earthworms are highly susceptible to insecticides causing immobility, rigidity and also showing a significant effect on biomass reduction, growth and reproduction by disrupting various physiological activities leading to the reduction of earthworm population and soil biodiversity. The effect of pesticides applied to soil has affected earthworm mortality, reproduction, metabolism and also enhanced the mechanism of bioamplification [MIGLANI, BISHT 2019].

Among other soil types, light chestnut soils have a relative degree of resistance to pollution in general. However, such soils have not always been able to withstand the impact of pollutants. Light chestnut soils play a significant role in agricultural production in South-East Kazakhstan as well as throughout the country. Therefore, a decrease in soil fertility because of contamination with chemicals is dangerous [ROMANOVA *et al*. 2019; TUKENOVA *et al*. 2020]. For most soil invertebrates, the resistance to increased pesticide content is quite large. Pesticides can change biological properties of soil, partially disrupt or lead to a complete loss of its fertility. In addition, pesticides also change more conservative features of ecosystem soils, such as humus state, structure, and pH. When soil is contaminated with pesticides, a decrease in the number of micro-mesofauna is observed. However, not all cases show a decrease in the number of soil invertebrates due to the death of sensitive invertebrates and the active development of resistant forms.

The impact of pesticides, as well as an environmental assessment of the state of soils in the biocenoses of the region's ecosystems, has been determined by establishing several diagnostic indicators that reflect biological activity. These indicators are widely used in the monitoring the ecosystem soil pollution with pesticides [CLUZEAU *et al*. 2012].

In contrast to the available works in the scientific literature, the novelty of our research is based on the study of the physicochemical and biological properties of light chestnut soils contaminated with pesticides in South-East Kazakhstan. In Kazakhstan, physicochemical and biological studies of soils are fragmented, while scientific knowledge of the management of modern soil-forming processes in traditional and agro-technical farming systems and soil fertility requires a systematic study of soil fauna in conjunction with physical-geographical, ecological and human factors.

As a result, the study has assessed the degree of sensitivity to various chemical elements and the impact of pesticides on the biological activity of soils.

MATERIALS AND METHODS

Field experiments were carried out at the LLP of the Kazakh Research Institute of Agriculture and Plant Growing, the Agriculture Department of Almalybak Settlement (chickpea and winter wheat crops) on rain fed lands.

Generally accepted testing methods were used to examine soil samples. Humidity was determined using the gravimetric method, total humus [SILFVERBERG 1957] and specific gravity by the pycnometric method, general porosity by the calculation method, and biological indicators, i.e. soil mesofauna, using the method of manual disassembly according to GILYAROV [1965].

Laboratory and analytical studies were carried out using methods generally accepted in biology and soil science. In addition, a number of modified copyright techniques were used. Following the recommendation of KHAZIEV [1990], the activity of soil enzymes was studied at the natural pH of the soil. With a 4-fold replication, the activity of inverters, urease and dehydrogenase were studied using the method of SHCHERBAKOV *et al.* [1993].

Quantitative determination of tebuconazole in samples was carried out by gas chromatography with mass spectrometric detection from liquid extracts in hexane. A gas chromatograph was used with a mass spectrometric detector 6890N/5973N (Agilent, USA), equipped with a Combi-Pal autosampler (CTC Analytics, Switzerland) with an automated injection of liquid samples. Chromatography was performed using a J&W DB-35ms capillary column (Agilent, United States) of 30 m in length, inner diameter of 0.25 mm, and a film thickness of 0.25 μm at a constant carrier gas velocity of 1.0 $cm^3 \cdot min^{-1}$. The volume of the sample introduced into the injector of the gas chromatograph was 3 mm^3. The sample was injected in the splitless mode at an injector temperature of 250°C. The program for heating the gas chromatograph column was as follows: initial temperature 40°C, heating to 220°C at 20°C·min^{-1} (shutter speed 3 min) and heating to 280°C at 10°C·min^{-1} (shutter speed 5 min). The total chromatographic time was 27 min. Temperatures of the quadrupole and the MSD ion source were 150°C and 230°C, respectively. The retention time of tebuconazole at given chromatographic parameters is 21.4 ± 0.2 min (Fig. 1). Agilent MSD ChemStation (version 1701EA) was used to control the instrument and process results. Data processing included determination of retention times and peak areas, as well as identification of peaks using their mass spectra. Mass spectra were identified by applying the Wiley 7th edition and NIST'02 libraries.

Mass spectrometric detection of tebuconazole was carried out in the monitoring mode of the selected m/z (SIM) ions – 250 and 125, and the ion registration time was 100 ms. The mass spectrum of tebuconazole is shown in Figure 2.

RESULTS AND DISCUSSION

The study found that when soils were contaminated by pesticide, in most cases the number of soil invertebrates in the studied light chestnut soils decreased. The most sensitive to soil pollution by tebuconazole were *Chrisomelidae*, *Elateridae*, *Chloropidae*, and *Pyraustidae*.

The morphological description of the section laid in the experimental plot is given in Table 1.

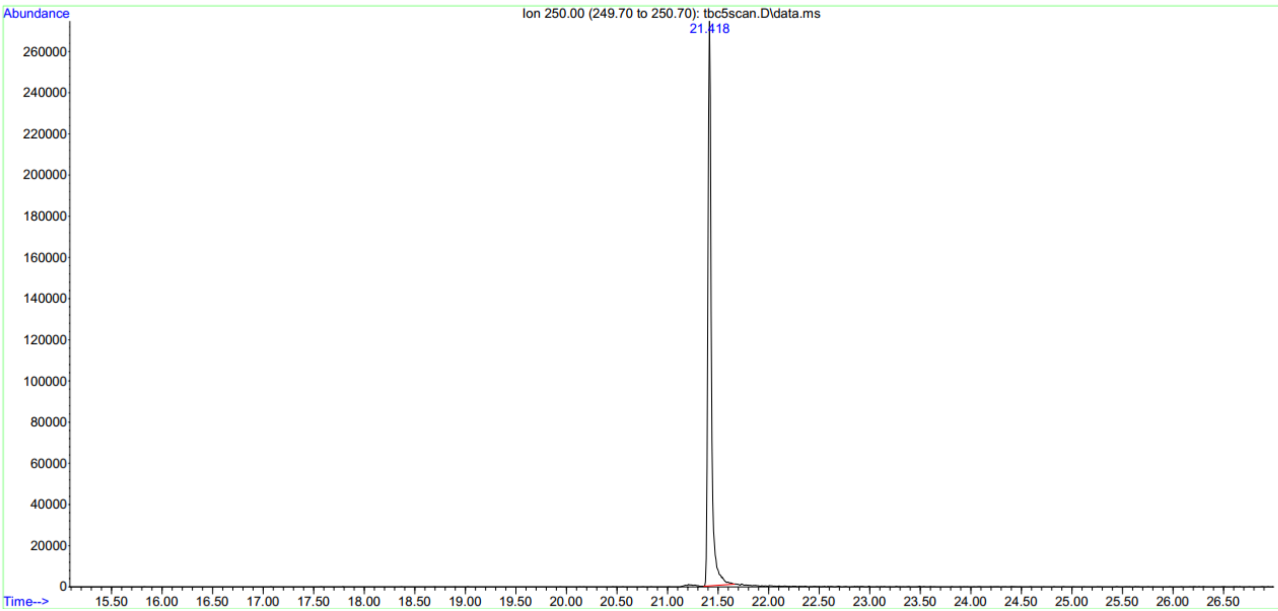

Fig. 1. Chromatogram of tebuconazole solution (C = 10 μg·cm^{-3}); source: own elaboration

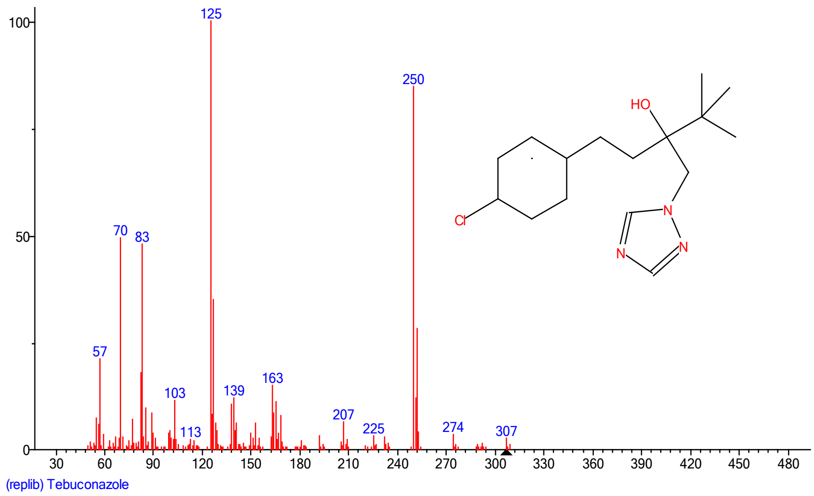

Fig. 2. Mass spectrum of tebuconazole; source: own elaboration using NIST'02 libraries

Table 1. Morphological description of the section in the experimental plot

Incision	Description
$A_{topsoil}$ $\frac{0-24}{24}$	brownish-grey, fragile or structureless, loose, medium-compacted, dryish, fine-crumbly, heavily rooted, heavy loamy, transition to the next horizon is clear in colour
A $\frac{24-32}{8}$	brownish-grey, moist, finely lumpy, denser than overlying, earthworm passages and their coprolites are found in abundance, porous, heavy loamy, transition to the next horizon is clear in colour, penetrated by roots
B $\frac{32-59}{27}$	greyish brown, moist, compacted, finely lumpy, porous, many moves of earthworms and their coprolites, as well as rodents, the moves of mice are filled with humus, cut by the roots of plants, heavy loamy, transition to the next horizon is gradual in colour
BC $\frac{59-103}{45}$	light grey, moist, wet below, porous, finely granular lumpy, coprolites of earthworms are rare, cut by the roots of plants, heavy loam, rare rust spots, with 103 cm ground water

Source: own elaboration.

The section was laid on the experimental fields of LLP Kazakh Research Institute of Agriculture and Crop Production, Department of Agriculture in the Almalybak Settlement.

It can be seen from the morphological description of the section that the profile of light chestnut soils is stretched, moisture increases with depth, rust spots are noted in lower horizons, the humus horizon is well structured, and the medium compacted.

According to the granulometric composition, the described soil belongs to heavy loamy, coarse dust. Particles larger than 3 mm are in most cases absent. The content of coarse particles is also insignificant. In the distribution of them along the profile, no definite pattern is observed. Coarse dust predominates. The distribution along the profile of the silt fraction indicates a noticeable predominance of fine particles in the middle and lower layers of the soil.

The heavy particle size distribution determines the unfavourable physical properties of the soil: stickiness in the wet state, compaction and hardening upon drying, which in turn leads to high resistance during plowing and to a clumpy surface of the field [MUSTAFAYEV 2020]. Light chestnut soil is characterized by moderate humus content.

The following regularity should be noted in the distribution of humus along the profile: its relatively high content in the upper horizon rises sharply, more than doubles, and then decreases upon transition to the next sub-arable horizon. A further decrease in the humus content occurs gradually, stretching to a considerable depth.

The content of gross nitrogen in the soil is low and amounts to 0.12%, which is why the ratio of humus carbon to total nitrogen is wide. In this case, it varies between 10 and 12, which is wider in comparison with zonal soils.

The gross content of phosphoric acid in the humus horizon does not go beyond 0.13–0.21%, which characterizes a low level of security.

The amount of CO_2 varies from 5.82 to 7.35%, with a minimum in the upper horizon and a maximum in the lower one. An increase in the percentage of CO_2 with depth occurs gradually, which is apparently associated with hydrogenic accumulation.

The results of the analysis of water extraction (Tab. 2) show that arable and subsurface horizons of the described soil are not saline, but at the same time there is a small but toxic amount of normal carbonates in lower horizons, which leads to weak salinization of the soil and an average degree of alkalinity of the soil solution. The dry residue in upper horizons does not exceed 0.166%. The maximum salt content is in the middle of the profile.

Table 2. Data of the water extract of the soil section of the light chestnut soil

Indicator	Value			
Depth (cm)	0–24	24–32	32–59	59–103
pH	7.8	7.9	8.1	8.3
Dry residue (%)	0.166	0.173	0.184	0.269
CO_3^{2-} – mEq – %	–	–	0.0038 0.125	0.0140 0.465
HCO_3^- – mEq – %	0.9788 0.0597	0.8361 0.0512	1.7740 0.1082	2.0330 0.1242
Cl^- – mEq – %	0.0790 0.0025	0.0674 0.0020	0.1174 0.0035	0.1493 0.0045
SO_4^{2-} – mEq – %	0.4201 0.0203	0.4880 0.0235	0.2920 0.0141	0.2910 0.0142

Source: own study.

This indicates the pulling up of water-soluble salts from deep horizons. The HCO_3^- ion prevails with a low content of sulphate and chlorine ions. It should be noted that normal carbonates can be found in the profile. Their amount along the profile is unevenly distributed. They are absent in the humus horizon (0–32 cm), their content in the parent rock is not high, and in the middle part of the profile it is within the toxicity range for plants (>0.001%).

The amount of sodium absorbed from bases in the humus horizon is less than 5% (Tab. 3). At the same time, it should be noted that with the increased content of magnesium in the profile and sodium in the lower part, the second half-meter layer allows us to classify these horizons as slightly saline.

Table 3. Composition and content of the absorbed base in light chestnut soil

Depth cm	The amount of absorbed bases (mEq per 100 g of soil)	Content of the amount (%)		
		Ca^{+2}	Mg^{+2}	Na^+
0–24	18.30	77.0	21.9	1.8
24–32	18.80	63.2	32.0	4.3
32–59	16.20	75.5	19.0	7.5
59–103	12.45	74.7	16.3	11.6

Source: own study.

The data in Table 3 show that the amount of absorbed bases is 18–16 mEq in upper horizons. It increases with depth together with a predominance of calcium. Its content reaches 63–77% of the total absorbed bases.

Figure 3 shows quantitative indicators for the most important physical and water – physical properties of the light chestnut soil. The specific gravity ranges from 2.63 to 2.78 $g{\cdot}cm^{-3}$ and gradually increases with depth.

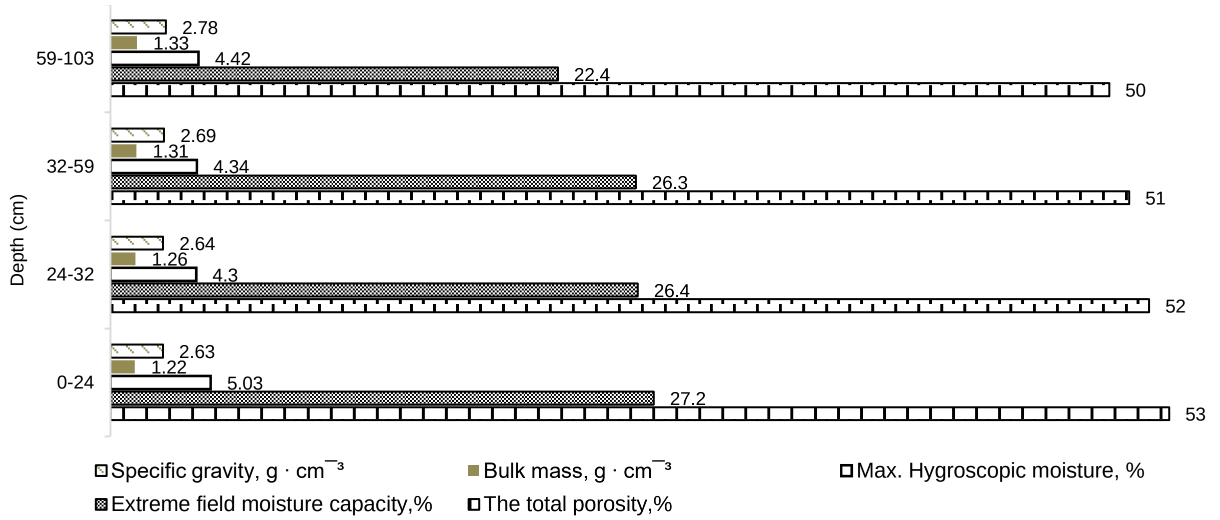

Fig. 3. Physical and water-physical properties of light chestnut soil; source: own study

The bulk mass of upper horizons is relatively small 1.22–1.33 g·cm^{-3}. Its sharp increase is observed only from the depth of one meter. In this regard, the overall porosity of upper horizons is rather high 50–53%. Compaction begins with a layer deeper than 80 cm.

The maximum field moisture capacity in the upper twenty-centimeter layer of the soil is 27.3%, with a depth its value decreases to 22.4%. The maximum hygroscopicity due to the heavy particle size distribution reaches 5.03% in upper horizons and decreases with depth to 4.42%.

Water permeability data allow us to note that soils, despite the heavy particle size distribution, have a satisfactory absorption rate, the average coefficient of water permeability on virgin soil is 0.9 mm·min^{-1}, which is facilitated by well-defined macroaggregation and high porosity.

The humus content in the arable horizon is 1.84%, which gradually decreases with depth. The content of total nitrogen is 0.19% of phosphorus average – 0.24%. According to the availability of accessible nutrients, the soils at the experimental plot are characterized as medium-sized with easily hydrolysed nitrogen of 87 mg·kg^{-1} and high potassium 435.4 mg·kg^{-1}. According to the content of mobile phosphorus, it belongs to the group of low-secured soil of 26 mg·kg^{-1}.

Physicochemical parameters were determined for the light chestnut soil sampled from the Almalybak Village, Almaty Region. We found that the decrease in soil humus content is explained by its mineralization by soil invertebrates, as well as the toxicity of the studied pesticide – tebuconazole (Tab. 4).

Table 4. The effect of pesticide (tebuconazole) on the content of humus in light chestnut soil, %

Duration (day)	Soil pesticide content				
	control	1 MPC	10 MPC	100 MPC	$LDS_{0.05}$
7	2.88	3.02	3.10	3.06	0.34
30	2.84	3.14	3.42	3.15	0.32
180	3.19	3.26	3.23	0.29	0.35
$LDS_{0.05}$	0.26	0.29	0.40		

Explanations: MPC = maximum permissible concentration, $LDS_{0.05}$ = least significant difference at 5%.
Source: own study.

Contamination of light chestnut soil with pesticides in several experimental variants influenced even such a stable indicator as the content of humus in the soil. Moreover, the values of the soil humus content in some instances decreased from 4% to 2.7%. The decrease in humus content depends not only on the activity of soil invertebrates, but also on the action of oppressed representatives of soil invertebrates at the saprotrophic level, the preparation of organic residues in the soil, and the activity of destructors.

The study established the effect of pesticides on dehydrogenase, invertase, and urease. In most cases, pesticides inhibited the action of enzymes. The least significant difference (*LDS*) is a value that indicates the border of possible random deviations in the experiment; this is the minimum difference in yields between the average, which in this experiment 5% ($LDS_{0.05}$) has been recognized as significant. The average values calculated using the $LDS_{0.05}$ analysis of variance revealed that when contaminated by 1 MPC and 10 MPC, the dehydrogenase activity in light chestnut soil was significantly higher than in the control soil (without contamination), and at a dose of 100 MPC it was lower. This dependence was observed on the seventh day from the onset of pollution. On the thirtieth day from the onset of pollution, the following dependence was observed: the dehydrogenase activity in soil samples was significantly higher at a dose of 1 MPC and 10 MPC, as well as 100 MPC. Almost the same dependence was observed with the action of tebuconazole.

We have carried out research to determine the dynamics of the species composition on bogara (light chestnut soils with a humus content in the arable layer of 2.70–2.04%) depending on the factors studied (predecessors, tillage). The correlation between the humus content in the soil and the composition of the mesofauna has been shown in Table 5.

The analysis of the mesofauna on the studied experimental variants (light chestnut) showed that common species are insect larvae from *Carabidae*, *Scarabaeidae*, and *Formicidae* families, since these species show significant plasticity (ability to inhabit wide variety of biotopes). The

Table 5. The content of humus and composition of mesofauna in light chestnut soils at the experimental site (under crops of winter wheat)

Depth (cm)	Humus content (%)	Soil invertebrates	
		number of groups	number of invertebrates ind.·m^{-2}
0–24	2.78(±0.67)	5	10
24–32	2.08(±0.61)	8	16
32–59	1.44(±0.53)	2	4
59–103	1.20(±0.55)	0	0

Source: own study.

dominant species are *Formicidae* and *Scarabaeidae* insect larvae.

It was established that the quantitative and qualitative composition of the soil mesofauna is associated with a specific soil type. It turned out that the larvae are from *Chrisomelidae*, *Elateridae* and *Chloropidae* families. *Pyraustidae* are associated with more humus-rich and moisture-rich soils (light chestnut soils). Along with other soil characteristics, mesofauna may well be used as a bioindicator. It has been established that the species composition of soil organisms can be used as a criterion for a qualitative assessment of the impact of pesticides on soils.

Based on the assessment of sensitivity of *Formicidae, Scarabaeidae*, *Chrisomelida*, *and Pyraustidae* invertebrates to various chemical elements from pesticides, the results, in contrast to abundance indicators, suggest that the species composition of soil invertebrates can be used as a criterion for a qualitative assessment of the impact of pesticides on the soil. It was also revealed that soil pollution with pesticides leads to changes in the functioning of soil invertebrates. Soil pollution with pesticides leads to a change in the structure and composition of soil invertebrate complexes. This is manifested in a decrease in the species diversity and a change in the occurrence of species. When conducting the research, the “concentration of dominance” was observed, i.e. preservation in the soil of only some types of highly prevalent mesofauna (*Formicidae*, *Scarabaeidae*). We found that under the influence of the same pesticides, the similarity of mushroom complexes in different zonal soil types increased. This indicated a danger of destroying the primary and the formation of microorganism communities atypical for natural soil ecological conditions under the influence of high pollution levels.

We found that pollution with chemicals affected the activity of biochemical processes in the soil. They alter its enzymatic activity. According to the degree of resistance to pollution, the enzymes studied in light chestnut soil show the following ranking: dehydrogenase > invertase > urease.

Pesticides inhibit the activity of soil invertebrates. Contamination of light chestnut soils with pesticides in several experimental variants has influenced even such a stable indicator as the content of humus in the soil. In our opinion, the decrease in humus content depends on the action of oppressed representatives of saprotrophic level soil invertebrates. These include representatives of the *Lumbricidae* family.

Our results indicate that, according to the number of individual groups of mesofauna (phytophagous, zoophagous, saprophagous), it is impossible to identify significant differences in the impact of pesticides on the soil at concentrations several times higher than the MPC. Significant changes are detected only when the concentration of the pollutant is two orders of magnitude higher than the MPC.

CONCLUSIONS

1. Pesticide contamination leads to a decrease in the biological activity of light chestnut soil. This results in a change of the following indicators: increase in pH from 7.45 to 7.6; changes in the absolute population density of soil animals: *Formicidae* from 16.5 to 0.85 ind.·m^{-2}, *Scarabaeidae* from 13.5 to 0.7 ind.·m^{-2}, and *Lumbricide* from 22.3 to 0.9 ind.·m^{-2}; change in soil enzyme parameters, i.e. dehydrogenases from 7.3 to 8.1%·g^{-1} and invertase from 15.1 to 23.5%·g^{-1}.

2. Based on the studies, it has been established that the light chestnut soil of the foothill zone is well provided with organic matter and the initial humus content in the soil at the beginning of the studies has been quite high both in the arable (1.84–2.21%) and in the subsoil layers (4.41–4.50%) for all options and crop rotation fields.

3. It has been established that the contamination with chemicals affects the activity of biochemical processes in the soil. They alter its enzymatic activity. According to the degree of resistance to pollution, the enzymes studied in the light chestnut soil show the following ranking: dehydrogenase > invertase > urease.

4. The toxic effect of pesticides on the ecosystem biological activity manifests itself shortly after it has been polluted, when the prevalence of mesofauna in the soil decreases significantly. These include representatives of the families *Chrisomelidae*, *Elateridae*, *Chloropidae*.

5. Pesticides inhibit the activity of soil invertebrates. The contamination of light chestnut soils with pesticides in several experimental variants influenced even such a stable indicator as the content of humus. In our opinion, the decrease in the humus content depends on the action of *Lumbricidae*, oppressed representatives of soil invertebrates at the saprotrophic level.

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