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Overcoming of Soil Contamination with Pesticides in Forest Nurseries Using the Activity of Microorganisms

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SUMMARY

The use of pesticides during cultivation of pine seedlings in forest nurseries results in the formation two phenotypes of teratomorph seedlings – conditionally normal and abnormal. Growing forest cultures from teratomorph seedlings leads to their low survival rate. It is known that pesticides and their metabolic products can remain in soil for many years. It is therefore impossible to rely only on natural degradation of pesticides in soil. A promising way of removing pesticides from soil is their microbiological decomposition. This method is preferable because there is a meliorative organic substance not far from forest nurseries – i.e. forest litter rich in microorganisms. The purpose of these experiments was to examine the influence of forest litter applied on pesticide decomposition in soil and morphology of pine seedlings. The rates of forest litter that were most effective in decomposition of pesticides and the activity of microbial communities in litter, depending on forest stand structure, were determined. Estimation of that action was based on the morphology of seedlings (rate of pine seedlings with normal, conditionally normal and abnormal phenotypes), intensity of CO_2 emission from soil and catalase activity, which correlates with the number of soil microorganisms. The results of these experiments showed the most effective activity of forest litter at the application rate of 20 kg/m^2. The number of seedlings with normal phenotype rose from 32% up to 40%. Besides, it was noted that saprophytes were most effective in pine forest litter, which is characterized by a more acid reaction of soil solution, while most others were rich in fungi. The highest number of normal phenotype seedlings, intensity of CO_2 emission and activity of soil catalase were correlated with the microbiological activity of the applied pine forest litter.

Keywords: Pine seedlings; Pesticides; Forest nurseries; Microbiological decomposition; Bioremediation

INTRODUCTION

Numerous scientific publications devoted to pesticides worldwide have reported their negative side-effects over the past decades. So, it is important to study the negative sides of pesticide use, as a number of outstanding researchers have repeatedly said. Among the many negative side-effects of pesticides, two deserve some attention. The first one is the negative influence on plants that they are expected to protect and, the second one is environmental contamination, including that of soil.

Our long-term researches of the reaction of Scots pine (*Pinus sylvestris*) to a complex of pesticides included in cultivation of pine seedlings in forest nurseries, have shown first of all their influence on seedling morphology and contamination of soil (Freiberg et al., 2004). The second negative effect is contamination of soil by pesticides and their metabolites. A specificity of pesticidal contamination is that the morphology of soil, its chemical and water-physical properties do not change, but the soil gets "selective fertility" (Lebedeva et al., 1990), i.e. starts influencing organisms as depending on their genetic nature.

Besides physical and chemical methods in determining soil contamination, great value is given to methods of bioindication (Lunev, 1989). In some cases it is important to know not so much the residual contents and quantities of pesticides, but their phytotoxity. Thus, a presence of teratomorph pine seedlings is also a bioindicator of suitability of a place for growing further pine crops.

It is known from publications connected to contamination of the environment that natural purification of soil from pesticidal contaminants takes significant time – up to 10 or more years after termination of their use (Tsukerman and Chavar, 1989; Ivanov, 2001). Thus, fast selfpurification of soil from pesticides is impossible. Now the problem of prevention against the damaging action of pesticides is solved in three ways: the use of adsorbents, antidots and microbiological methods (Pitina et al., 1986). Microbiological decomposition is a promising way of removing pesticides from soil (Babieva and Zenova, 1989). The use of pure cultures of microorganisms is a difficult enough task. Data are being collected about possibilities of microbiological transformation of pesticides, which correspond to opinions about the cooperative action of microorganisms. The method of purification of soils from pesticides is preferable because there is a meliorative organic substance not far from forest nurseries – i.e. forest-litter, which is rich in microorganisms. Forest litter is filled with various microorganisms possessing a wide selection of enzymes in aggregate capable to transform organic substance (Vedrova, 1997), which will promote decomposition of pesticides. This process is connected to and controlled by hydrothermal conditions, weight of the decaying vegetative remains and other factors. The first experiment in a forest nursery in which pesticides had been actively applied has given positive results. Application of forest litter (at a rate of 10 kg/m^2) from a mixed stand, consisting of pine and birch wood, has provided an output of 23% of normal phenotype seedlings, i.e. almost a fourth part of all plants in the experiment.

MATERIAL AND METHODS

Research was carried out in a forest nursery, on a soddy-podsolic loamy soil, and the density of its arable soil horizon was 1.03 g/sm^3, pH of a salt extract was 4.9, humus content was 4.23%, and available potassium and phosphorus contents were 1.6 and 5.2 mg per 100 g of soil, respectively, i.e. the level of soil fertility did not preclude standard pine seedlings cultivation. However, the soil was characterized by pesticide contamination because a large number of them (2,4-D, Roundup and others) had been used in the nursery for more than 20 years. This is evident from the appearance of a great many seedlings with teratomorph phenotypes – i.e. conditionally normal and abnormal.

The purpose of these experiments was to examine the effect of forest litter on decomposition of pesticides and morphology of pine seedlings after applying the litter to soil. The rates of forest litter that were most effective for decomposition of pesticides and the activity of microbial communities, depending on forest stand structure, were determined. Forest litter was not applied in the control plot. The estimation of action was carried out based on the morphological condition of seedlings (i.e. amount of pine seedlings with normal, conditionally normal and abnormal phenotypes), the intensity of CO_2 emission from soil and the activity of catalase (1.11.1.5), which correlates with the number soil microorganisms (Kurbatov, 1962) and is a nonspecific indicator of contamination (Kovalenko and Babushkina, 2003).

The experiments were conducted under field conditions. The sowing of seeds and preparation of soil were carried out according to standard procedures in forest nurseries. Seedlings were grown for two years. In mid-September of the second year, they were dug out and sorted according to phenotype criteria developed earlier (Freiberg et al., 2004).

RESULTS AND DISCUSSION

In preceeding studies of seedlings exposed to pesticides it had been found that changes in morphogenesis included two phenotypes of teratomorph seedlings – conditionally normal and abnormal. The first is characterized by a disturbed correlation of stem and needles, the second by variable number of additional shoots (Figure 1). Any teratism of plants, as it is known from scientific publications (Feodorov, 1958), is caused by change in their metabolism. Our observations have shown that the same occurs with seedlings. Research of some physiological and biochemical parameters in teratomorph seedlings, which are important for viability of plants during transplantation from nursery to forest, has shown their difference from seedlings of a normal phenotype (Figure 2).

Figure 1. Phenotypes of 2-year old pine seedlings: a – normal; b – conditionally normal; c – abnormal

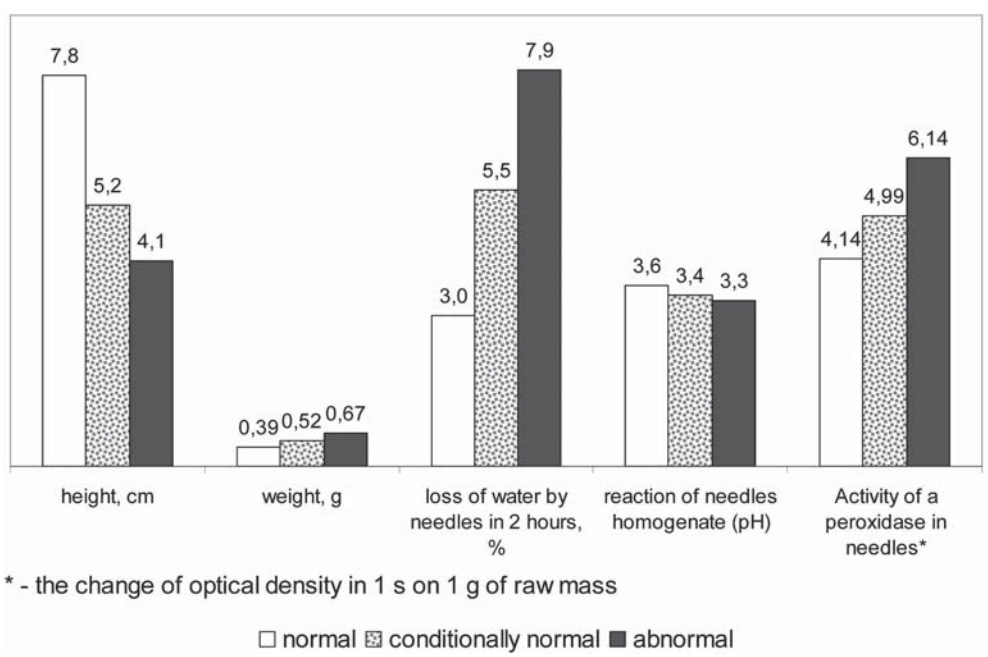

Figure 2. Growth and some physiological and biochemical parameters in 2-year old pine seedlings.

Our experiments of overcoming the abovementioned teratogenesis of pine seedlings by applying forest litter were sufficiently effective. The results of the experiments presented in Table 1 show that the most effective action was achieved by applying forest litter at a rate of 20 kg/m^2. The amount of seedlings with normal phenotype in the case of forest litter application at the rate of 20 kg/m^2 changed from 32% up to 40%. Data on the intensity of CO_2 emission from soil and data about the activity of catalase confirmed a high contribution of microorganism activity in this variant of experiment.

Table 1. Distribution of 2-year old pine seedlings per phenotype and biological activity of soil

Doses of forest-litter, kg/m^2	Intensity of CO_2emission, mg CO_2/100 g soil		Catalase activity *, ml O_2/g of soil per day	Distribution of pine seedling phenotypes, %				Number of seedlings per 1 m in lenght
	first year	second year		normal	conditionally normal	abnormal	total teratomorph	
Experiment 1. Application of forest litter and pine sowing in spring of 2000								
10	2.25	1.59	1.0	15.1	65.7	19.2	84.9	97
20	4.75	1.73	1.3	40.4	38.8	20.8	59.6	103
Control	0.88	0.67	1.1	0.3	88.7	11.0	99.7	97
Experiment 2. Application of forest litter and pine sowing in spring of 2003								
10	1.74	1.08	1.5	22.2	72.7	5.1	77.8	138
20	3.31	1.56	1.9	32.9	60.7	6.4	67.1	109
Control	0.10	0.76	0.7	1.0	89.3	9.7	99.0	109

*Catalase activity in soil was determined in the second year of seedling growth

The activity of microorganisms applied with forest litter from forest stands different in structure on pesticide decomposition is shown in Table 2. The data in tables show that the addition of saprophyte microorganisms from forests, regardless of their structure, into soil contaminated by pesticides provides a positive influence on seedlings. Besides, saprophytes from pine wood forest litter were found to be the most effective as that litter is known to be characterized by a more acid reaction in soil solution, while the others were rich in fungi. pH values of the forest litter from pine, birch and mixed stands were 5.54, 4.23, and 4.84, respectively. As it is known from publications (Rode and Smirnov, 1972), coniferous litter decays mainly through the activity of fungi, which are the most active destroyers of hard decomposed organic residues. Fungi have a powerful enzymatic structure, different biochemical functions and more economical metabolism, and therefore they consume not only accessible substances, but those that are more restrictively available as well.

Table 2. Distribution of 2-year old pine seedlings per phenotype and biological activity of soil in experiment variants with forest litter from forest stands of different structure

Type of forest litter, forest stand	Intensity of CO_2 emission, mg CO_2/100 g soil	Catalase activity*, ml O_2/g of soil per day	Distribution of pine seedlings per phenotype, %			
			normal	conditionally normal	abnormal	total teratomorph
Birch	0.55	1.0	50.7	32.0	17.3	49.3
Pine	0.73	1.1	64.3	25.9	9.8	35.7
Mixed	0.67	1.0	56.4	25.9	17.7	43.6
Control	0.43	0.8	1.9	79.2	18.9	98.1

The experimental variants presented in Table 2 show the greatest number of normal phenotype seedlings, intensity of CO_2 emission and activity of soil catalase as correlated with the microbiological activity of pine forest litter. As a confirmation, data on the intensity of CO_2 emission and catalase activity in soil in the experiments conducted in July, 2009 are shown in Table 3.

Table 3. Biological activity of soil in variants of experiment with application of forest litter from forest stands of different structure

Type of forest litter, forest stand	Intensity of CO_2emission, mg CO_2/100 g soil	Catalase activity*, ml O_2/g of soil per day
Birch	0.56	2.2
Pine	0.71	2.5
Mixed	0.80	2.3
Control	0.52	2.0

In further research on these and other experimental plots, the quantity of functional groups of microorganisms and ecological conditions in soil in each of experimental variant will be established.

Thus, in the first experiments devoted to the cooperative action of microorganisms towards elimination of pesticidal contamination from soil in forest nurseries, the optimum rates of forest litter from stands different in structure in soil bioremediation were obtained. Also, it is possible to assume with high probability that significant efficiency in soil purification from pesticidal contamination has been achieved by applying forest litter from pine stands owing to the activity of saprophyte fungi.

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Rešavanje kontaminacije zemljišta pesticidima u šumskim rasadnicima korišćenjem delovanja mikroorganizama

REZIME

Korišćenje pesticida u gajenju borovih izdanaka u šumskim rasadnicima dovodi do formiranja dva fenotipa teratomorfnih izdanaka, uslovno normalnih i abnormalnih. Uzgajanje šumskih kultura iz teratomorfnih izdanaka ima za rezultat nizak nivo njihovog preživljavanja. Poznato je da se pesticidi i njihovi produkti metabolizma mogu zadržati u zemljištu veći broj godina. Otuda se ne možemo osloniti samo na prirodan proces razgradnje pesticida u zemljištu. Mikrobiološka razgradnja predstavlja perspektivan način uklanjanja pesticida iz zemljišta. Toj metodi se daje prednost zbog postojanja meliorativne organske materije koja se nalazi u blizini šumskih rasadnika, tj. šumskog otpada koji je bogat mikroorganizmima. Cilj ovih eksperimenata bio je da se ispita uticaj šumskog otpada na razgradnju pesticida i morfologiju borovih izdanaka. Određene su količine šumskog otpada koje su najdelotvornije za razgradnju pesticida i delovanje mikrobioloških zajednica u otpadu u zavisnosti od strukture šumskih zasada. Ocenjivanje tog delovanja zasnovano je na morfološkom stanju izdanaka (učestalost borovih izdanaka normalnog, uslovno normalnog i abnormalnog fenotipa), intenzitetu emisije CO_2 iz zemljišta i aktivnosti katalaze, koja je u korelaciji sa brojnošću mikroorganizama. Rezultati ovih eksperimenata pokazali su da je najefikasnije delovanje šumskog otpada u količini primene od 20 kg/m^2. Brojnost izdanaka sa normalnim fenotipom povećala se sa 32% na 40%. Pored toga, primećeno je da su saprofitni mikroorganizmi najefikasniji u otpadu iz borove šume, koji karakteriše veća kiselost zemljišnog rastvora, dok su drugi šumski otpadi bili bogati gljivama. Najveća brojnost izdanaka sa normalnim fenotipom, kao i intenziteta emisije CO_2 i aktivnosti katalaze u zemljištu bili su u korelaciji sa mikrobiološkim delovanjem primenjenog otpada iz borove šume.

Ključne reči: Borovi izdanci; pesticidi; šumski rasadnici; mikrobiološka razgradnja; bioremedijacija