

## NEW STRATEGIES FOR BIOREMEDIATION OF SOIL CONTAMINATED BY OBSOLETE PESTICIDES IN THE REPUBLIC OF MOLDOVA

**RASTIMESINA Inna, POSTOLACHI Olga, CINCILEI Angela, TOLOCICHINA Svetlana, STREAPAN Nina, MAMALIGA Vera**

**Abstract.** This paper is focused on the elaboration of the new approaches to activate indigenous soil microflora for the remediation of long-term pesticides contaminated soil collected nearby the former storehouse of persistent organic pesticides: DDTs and trifluralin. The bioremediation of the contaminated complex was carried out in two main directions: strictly oxic conditions and alternating anoxic and oxic conditions. Bioremediation procedures have had a beneficial effect, resulting in the reduction of the pesticides content and mineralization of DDT.

**Keywords:** bioremediation, soil, pesticides, DDT, trifluralin.

**Rezumat. Noi strategii de bioremediere a solului îndelung contaminat cu pesticide în Republica Moldova.** Articolul este axat pe elaborarea unor procedee noi de activizare a microflorei indigene pentru remedierea solului, îndelung contaminat cu pesticide, din apropierea fostului depozit de poluanți organici persistenti: DDTs și trifluralina. Bioremedierea complexului contaminant a fost realizată pe două căi principale: crearea condițiilor strict oxice și alternarea condițiilor anoxice cu cele oxice. Procedurile de bioremediere au avut efect benefic, soldat prin reducerea conținutului de pesticide și mineralizarea DDT-ului.

**Cuvinte cheie:** bioremediere, sol, pesticide, DDT, trifluralina.

### INTRODUCTION

Pesticides are the only toxic substances released intentionally into our environment to kill living things. This includes substances that kill weeds (herbicides), insects (insecticides), fungi (fungicides), rodents (rodenticides), and others. The use of toxic pesticides to manage pest problems has become a common practice around the world (<http://www.toxicsaction.org/problems-and-solutions/pesticides>).

The Republic of Moldova has never produce pesticides, but the country still has frequent pollution accidents in the small spaces of the natural environment (water, soil). Despite the restrictions, considerably reducing the volume of pesticide in use, the problem related to environmental pollution in Republic of Moldova, including accidental pollution, remains acute. Currently over a thousand locations in the country - the former territories of pesticide deposits, reached the deplorable state - is a continuing source of pollution and threat to the environment and to human health in adjacent areas.

The management of domestic and hazardous wastes is considered as one of the most urgent environmental problems in Moldova. Currently, in Moldova, approximately 3,000 tons of obsolete pesticides are stored in various former collective agricultural warehouses or disposed in uncontrolled dumps (NATIONAL IMPLEMENTATION PLAN, 2004). The spatial analysis showed a strong pollution impact to surrounding agricultural territory near old pesticide storage (BOGDEVICH & CADOCINICOV, 2007; 2009). Huge areas of contaminated soil around old storehouses are a continuous danger for the environment and public health (The Eliminators in Moldova, 2011). However, the information about the actual condition of soil after the repacking on former storages is not sufficient at present. This investigation is important also for the assessment of the remediation technologies that can be used for future soil detoxification.

There are many methods available for soil remediation like excavation, In-situ vitrification, soil dressing, washing and bioremediation. Bioremediation methods have drawn the attention of the researchers as chemical detoxification methods failed to handle the issue of soil remediation economically. Bioremediation, including stimulation of native microflora, bioaugmentation, phytoremediation, and rhizoremediation are methods or procedures for cleaning soil contaminated with persistent organic pollutants that have already become common. The activation of native microorganisms destructive abilities by introducing additional sources of carbon, nitrogen, phosphorus, hydrogen peroxide, in aeration, maintaining high humidity, is one of the technologies using microorganisms to degrade pesticides, petroleum products, detergents, etc., in soil and water (PHILLIPS et al., 2004; DENYS et al., 2007; AYOTAMUNO et al., 2009).

Our goal was to identify more efficient approaches to activate soil microorganisms' biodestructive capacity for remediation of long-term pesticides contaminated soil.

### MATERIAL AND METHODS

Soil samples were collected from the site nearby the former storehouse of persistent organic pesticides (POPs) located in the central part of the Republic of Moldova, Chișinău municipality, Sangera village. Collected soil was cleared of roots and other impurities, sieved (mesh number 2) to 2-3 mm, air-dried at 22-23°C and analysed. Soil pH, moisture content (SMC), water-holding capacity (WHC) and soil organic matter content were determined using standard methods (ARINUSHKINA, 1970; KOZLOVA, 2009).

The extraction of DDTs and trifluralin from soil has been performed in four repetitions per option according (KLISENKO & ALEXANDROVA, 1983). The determination of pesticide residues in soil was confirmed by gas chromatography with mass spectrometry GC/MS multiresidue method, at the gas chromatograph "Agilent Technologies" 6890N coupled with MSD mass selective detector "Agilent Technologies" 5973.

The bioremediation was established in experimental plastic jars, each containing 1,000 g of contaminated soil (Table 1). The duration of the experiment was 135 days. The experiment was carried out in two main directions: (1) strictly oxic conditions, the constant humidity were maintained at 60% of WHC, and (2) alternating anoxic and oxic conditions, each cycle consisting of two phases – anaerobic and aerobic. There were two variants that served as a control: initial air-dried soil and variant 1 – without stirring and amendments, the soil moisture maintained at 60% of WHC.

Table 1. Treatment protocol for bioremediation of soil in laboratory conditions.

Variant	Bioremediation factors						
	Biostimulation					Bio-augmentation	Phyto-remediation
	Soil moistening, % of WHC	Periodic tilling	Amendments				
			Peptone	K <sub>2</sub> HPO <sub>4</sub> + (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	Ammophoska NPK 15:15:15	<i>Rh. meliloti</i>	<i>M. sativa L.</i>
Oxic conditions							
Initial	–	–	–	–	–	–	–
1	60	–	–	–	–	–	–
2	60	+	–	–	+	–	–
3	60	+	–	–	–	–	+
4	60	+	–	+	–	+	+
Anoxic/oxic conditions							
5	80/60	– / +	+	+	–	–	–
6	80/60	– / +	+	+	–	–	+

**Section 1:** strictly aerobic conditions. The constant humidity was maintained at 60% of WHC. There were variants:

1. Without stirring, without amendments, soil moistened to maintain 60% of WHC.
2. At the beginning of experiment this variant was amended with ammophoska, 0.49 g/kg of soil, aerated by tilling twice a week.
3. Without amendments, aeration by tilling weekly. For phytoremediation purpose, it was chosen alfalfa (*Medicago sativa L.*). Periodically plants were cut, mixed with soil and new seeds were planted. Aeration by tilling weekly.
4. At the beginning of the experiment, this variant was amended with potassium and ammonium phosphates (K<sub>2</sub>HPO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>) at a concentration of 1.0% by weight of soil each. Phytoremediation – alfalfa (*M. sativa L.*). Periodically plants were cut, mixed with soil and new seeds were planted. Soil bacterium *Rhizobium meliloti* was introduced to soil once at the same time when the seeds were planted. Aeration by tilling weekly.

**Section 2:** alternating anoxic and oxic conditions, each cycle consisting of two phases – anaerobic (for 14 days) and aerobic (for 7 days). Anaerobic conditions were created by saturating the contaminated soil with water (up to 80% of WHC) in the dark plastic jars sealed with Parafilm, and stored in the dark at 20-22°C. At the beginning of the aerobic phase Parafilm was removed, soil mixed with a metal spatula and gradually brought soil moisture up to 60% of WHC. At the beginning of each anaerobic phase peptone and phosphates salts were added at a concentration of 0.5% and 0.2% respectively, soil humidity was maintained at 80% of WHC.

5. At the beginning of experiment, there were added potassium and ammonium phosphates (K<sub>2</sub>HPO<sub>4</sub> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>) in concentration of 1.0% by weight each, and peptone in concentration of 0.5%.

6. Alternating aerobic-anaerobic conditions as in variant 5 for 63 days. Then, during the last aerobic phase, the soil humidity was brought up to 60% of WHC, sawdust was added to soil in amount of 1/3 of the volume of soil. Alfalfa was planted as for variant 3 for 72 days.

## RESULTS AND DISCUSSIONS

At the start of the experiment, the soil pH was 8.0 and the air-dry soil moisture content was 1.84%. Water holding capacity was 33.60% and soil organic matter content was 2.06%. Soil type was determined as carbonated chernozem (Table 2).

Table 2. Characterization of experimental soil samples (Sangera storehouse, mun. Chişinău).

Soil type	pH	SMG, %	VHC, %	Organic Matter, %
Carbonated chernozem	8.0	1.84	33.6	2.06

The total content of pesticides exceeded 21.00 mg/kg soil, the rate of POPs – DDTs content (total amount of DDT and its metabolites DDE and DDD) was estimated to 1.48 mg/kg. The major component of pollution was halogenated herbicide trifluralin; its concentration was 19.52 mg/kg (Table 3). According to the Hygienic norms, established for the Republic of Moldova, DDTs and trifluralin Maximum Residue Limits (MRL) in soil is 0.1 mg/kg (NORMATIVELE IGIENICE, 2003). Thus, soil pollution by DDTs and trifluralin exceeded the MRL about 15 and 200 times respectively. Pesticides contaminated soil nearby the storehouse area for a long time and pollution was complex, so soil from that site was selected for modelling bioremediation project.

Table 3. The level of soil contamination with POPs.

Contamination, mg/kg						
Trifluralin	$\Sigma$ DDTs	DDT		o,p'-DDD	DDE	
		o,p'-DDT	p,p'-DDT		o,p'-DDE	p,p'-DDE
19.52 $\pm$ 0.22	1.48	0.21 $\pm$ 0.01	0.11 $\pm$ 0.005	0.27 $\pm$ 0.027	0.29 $\pm$ 0.020	0.60 $\pm$ 0.012

Chromatographic analyses of soil extracts at the end of the experiment demonstrated the effectiveness of bioremediation processes in decreasing concentrations of organochlorine pesticides and trifluralin in the contaminated soil. The results of bioremediation and phytoremediation procedures are displayed in figure 1.

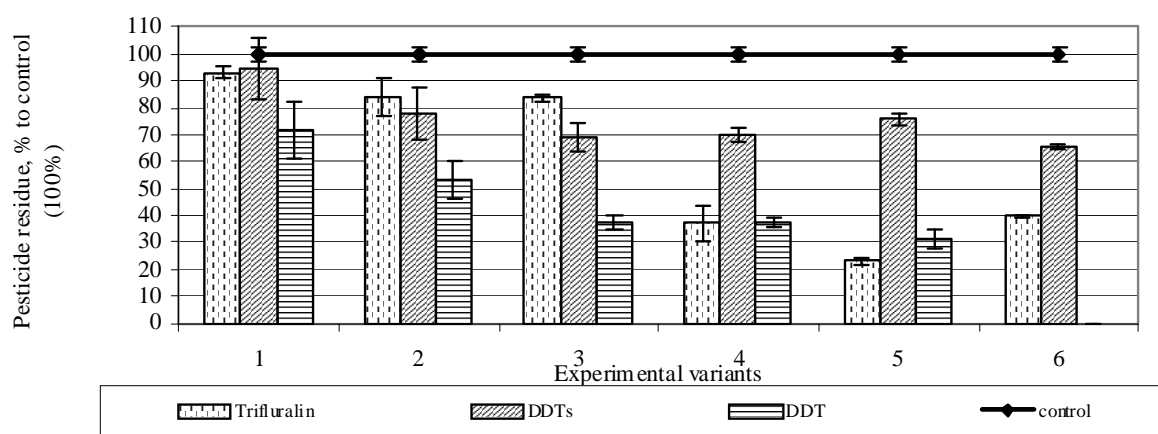


Figure 1. DDTs, DDT and trifluralin degradation.

Regular soil moistening without stirring and amendments contributed to the mineralization of 28% of DDT and slightly influenced trifluralin degradation. Bioremediation treatments setting in oxic conditions reduced the total DDTs' content up to 31% and the content of trifluralin up to 63% in 135 days. The most significant results were obtained using bioaugmentation as a factor of remediation (Fig. 1). While DDT and total DDTs removal was at the same rate as in variant 3 (phytoremediation without amendments), trifluralin concentration decreased more than 2 times. Recently, there are also reports that microorganisms associated with plants, especially rhizobacteria, induced the degradation of xenobiotics, such as phenanthrene and simazine herbicide (TAKASHI & RYOTA, 2004; TURKOVSKAYA et al., 2007).

The amendments of the soil with phosphates and organic compounds in anaerobic/aerobic conditions favoured the reductive cleavage of organochlorine pesticide DDT and intensive accumulation of its metabolites. The alternation of anoxic and oxic treatment combined with the stimulation of indigenous microflora by phosphates and peptone amended to soil reduced the DDT concentration by 3-3.5 times, and the total concentration of DDTs by 35%. The prolongation of the experiment in aerobic conditions enhanced DDT metabolization by soil microflora and resulted in complete mineralization of DDT.

Cycled anaerobic and aerobic treatment provided the degradation of 60-77% of the initial rate of trifluralin. As a result of phosphates and peptone application in anaerobic/aerobic conditions maintained for 135 days (6 full cycles), trifluralin content decreased by more than 4 times. Trifluralin removal in variant 6 was not so significant, and, thus, it can be suggested to increase the duration of anaerobic/aerobic phase (from 3 cycles to 6 or more).

Additionally, two consortia of microorganisms adapted to high concentrations of organochlorine toxicants were isolated from the contaminated soil. By analyzing soil microbiota long-term adapted to toxic presence of pollutants, there were selected 4 strains with ability to degrade the organochlorine compounds.

## CONCLUSIONS

Thus, by using different bioremediation factors and treatments, we elaborated two strategies for the remediation of a long time and complex contamination with persistent organic pesticides DDT and trifluralin. Bioaugmentation of rhizobacteria associated with alfalfa in oxic conditions enhanced the trifluralin degradation.

Alternation of anoxic and oxic treatment combined with stimulation of indigenous microflora by phosphates and peptone resulted in complete mineralization of DDT.

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**Rastimesina Inna, Postolachi Olga, Tolocichina Svetlana, Streapan Nina, Mamaliga Vera**  
 Institute of Microbiology and Biotechnology,  
 Academy of Sciences of Moldova,  
 1 Academy str., MD 2028, Chişinău, Republic of Moldova.  
 E-mail: rastimesina@gmail.com, oleseap@yahoo.com

**Cincilei Angela**  
 The State Center for Certification and Registration of Phyto-sanitary Means and Fertilizers,  
 Ministry of Agriculture, 16 A Sarmizegestusa str., MD 2032, Chişinău, Republic of Moldova.  
 E-mail: angela\_cincilei@yahoo.fr

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