APPROACHES FOR BIOREMEDIATION OF PESTICIDE CONTAMINATED SOIL: COMPLEX POLLUTION PROBLEMS

PROCEDEE DE BIOREMEDIERE A SOLULUI POLUAT CU PESTICIDE: PROBLEMELE POLUÃRII COMPLEXE

***RASTIMESINA Inna*[1], *CINCILEI A.*[2], *POSTOLACHI O.*[1], *TOLOCICHINA S.*[1], *MAMALIGA V.*[1], *STREAPAN N.*[1]**

e-mail: oleseap@yahoo.com

Abstract. *A strongly pollution with trifluralin and DDT of soil samples collected nearby an old pesticides storehouse was revealed. Two main approaches of treatment were used to remediate this complex contamination – creation strictly anoxic conditions, and alternating anoxic and oxic conditions. In both cases, trifluralin content in soil decreased by more than 4 times and degradation rate reached 95-96%. The cycled anoxic and oxic treatment of soil amended with phosphates and peptone, promoted the DDT and DDTs degradation in compare with the anoxic treatment. The prolongation of the experiment in aerobic conditions resulted in complete mineralization of DDT.*
Keywords: *bioremediation, soil, pesticides, trifluralin, DDT.*

Rezumat. *A fost depistat că solul din apropierea unui depozit vechi de pesticide este puternic poluat cu trifluralina si DDT. Pentru remedierea acestei contaminari complexe, s-a recurs la doua procedee fundamentale de tratare – crearea condițiilor strict anaerobe si alternarea condițiilor anaerob-aerobe. În ambele cazuri continutul trifluralinei a scazut cu mai mult de 4 ori, iar procentul degradării atingea 95-96%. Alternarea conditiilor anaerob-aerobe cu adăugarea în sol a fosfaților şi peptonei, a favorizat degradarea DDT şi DDTs, comparativ cu tratamentul anoxic. Continuarea experimentului în condiții aerobe s-a soldat cu mineralizarea completă a DDT.*
Cuvinte cheie: *bioremediere, sol, pesticide, trifluralina, DDT.*

INTRODUCTION

Recent years the using of pesticides in the national economy of Republic of Moldova is significantly reduced. However the import, storage and application of harmful substances still is a current issue (National Report, 2011). According to the National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants, during the years 1991-2003 about 60% of deposits were destroyed and liquidated and only 20% of the remaining ones are in a satisfactory condition (National Implementation Plan, 2004; National Report, 2011). Considerable amounts of dangerous chemicals and pesticides have been abandoned in the fields and the devastated deposits cause a negative impact on public health and the

[1] Institute of Microbiology and Biotechnology, ASM, Chisinau, Republic of Moldova
[2] The State Center for Certification and Registration of Phytosanitary Means and Fertilizers, Ministry of Agriculture, Chisinau, Republic of Moldova

environment, since some of them are located near residential areas or near water resources.

Bioremediation of contaminated natural environments, soil in particular, is an innovative technology, highly topical, that uses biological systems in the pollutant elimination, promotes rehabilitation of contaminated sites and preservation of soil suitable conditions for further cultivation of plants (Matsumoto, 2009). The most efficient way for decomposition of organochlorine pesticides POPs is a reductive bioremediation, which promotes dehalogenation of organochlorine compounds. Creation of anaerobic conditions and supplementation with specific nutrients for anaerobic/facultative anaerobic soil microorganisms leads to efficient removal of residues of persistent compounds (Sasek et al., 2003; Phillips et al., 2004; Kantachote et al., 2004; Reference Guide, 2004). The bioremediation methods permit to value in particularly the amazing ability of microorganisms for adapting to changing environmental conditions and to decompose a wide range of xenobiotic compounds, including pesticides.

The purpose of our research was to identify the most effective methods for activation of biodestructive capacity of indigenous microorganisms to remediate the soil contaminated for a long time with complex pesticide mixture.

MATERIAL AND METHODS

Soil samples were collected near the storehouse of persistent organic pollutants, located in the center of the Republic of Moldova, Chisinau city, Singera village. Previously the collected soil samples were cleaned of roots and other impurities, sieved (mesh No. 2) and air-dried at 22-23^{0}C. Soil moisture content (SMC), water-holding capacity (WHC), soil pH and 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 and Alexandrova, 1983). The determination of pesticide residues (PR) 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. Percentage of degradation was calculated according to Bento (Bento et al., 2003) using the expression: % of degradation = [(PR control – PR experience) / PR control] $\times$ 100.

The bioremediation was established in plastic jars, each containing 1,000 g of contaminated soil. The experiment was carried out in two main directions: strictly anaerobic conditions (Section 1) and alternating of anoxic/oxic conditions (Section 2).

Section 1: Creating of strictly anaerobic conditions by saturating the polluted soil with water (up to 80% of WHC) in the dark plastic jars sealed with Parafilm, in the dark and at temperature 22-24^{0}C. The experiment duration – 112 days.

1. The soil was saturated with water (80% of WHC);
2. Amendment of soil with zero-valent Fe 0.5% w/w, twice during the experiment;
3. The soil was amended with mono- and dipotassium phosphate (1.0%) and peptone (0.5%) at the begining of the experiment;
4. The soil was amended with dipotassium and diammonium phosphate (1.0%) and peptone (0.5%) at the begining of the experiment.

Section 2: alternating of anoxic and oxic conditions. Each cycle consists of two phases – anaerobic (for 21 days) and aerobic (for 7 days). Anaerobic conditions were created by saturating the contaminated soil with water (80% of WHC) in the dark plastic jars sealed with Parafilm, and stored in the dark at 22-24°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 start of each anaerobic phase mineral and organic (peptone) amendments were added to the soil, soil humidity was maintained at 80% of WHC.

5. The soil was amended with mono- and dipotassium phosphate (1.0%) and peptone (0.5%) at the begining of the experiment, the experiment duration – 112 days;
6. At the beginning of the experiment, there were added dipotassium and diammonium phosphate in concentration of 1.0% by weight each, and peptone in concentration of 0.5%, the experiment duration – 135 days;
7. Alternating anoxic and oxic conditions as in the variant 6 for 63 days, than passing to the aerobic phase followed by stimulation of microorganisms by applying sawdust, phytobioremediation with oat (*Avena sativa L.*), monthly plants were cut, mixed with the soil and new seeds were planted, for 72 days.
8. The soil was amended with dipotassium and diammonium phosphate (0.5%) and peptone (1.0%) at the begining of the experiment, the experiment duration – 112 days.

As a control were used: (1) contaminated soil befor remediation, (2) variant 1 without variable factors of remediation.

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.

The total content of organochlorine compounds was 21.00 mg/kg soil, which demonstrates that the level of pollution near the deposit exceeds the national standard. Soil pollution by trifluralin exceeded 195 times the Maximum Residue Limit (MRL) and made up 19.52 ± 0.22 mg / kg soil. ∑ DDTs (DDT, DDE, DDD) was 14.8 MRL (Normativele igienice, 2003). These data clearly indicates that the studied site was long-term and complex polluted and the major component is presented by the fluorine organic herbicide trifluralin and the minor component by organochlorine insecticide DDT and its metabolites.

After the soil bioremediation experiment in laboratory conditions was completed, soil samples were analyzed by GC/MS and the percentage of pesticides degradation was calculated.

The decrease of trifluralin concentration in polluted soil to 24.6% was managed merely by creating anaerobic conditions by saturation of soil with water up to 80% of WHC (trifluralin sanitary and toxicological MRL – 0.1 mg / kg soil). The decomposition of trifluralin in the Section 1 was favoured by anoxic conditions, and, in particular, by the soil amendment with peptone and dibasic phosphates of potassium and ammonium. These conditions ensured the mineralization of 76.6% and 95.8% of herbicide in the experimental variants 3 and 4 (tab.1).

In the Section 2 alternation of anoxic / oxic conditions along with the amendment of phosphates of potassium and ammonium, and peptone (variants 5-8) has facilitated the trifluralin degradation (tab. 1). The conditions created in the experimental variant 8 have allowed the mineralization of 95% of trifluralin. Thus, the most important factor for trifluralin biodegradation in carbonated chernozem is the creation of anoxic / oxic conditions, favourable for the development of anaerobic / facultative anaerobic microflora.

Table 1

The impact of bioremediation processes on general amount of trifluralin in polluted soil

Experimental variants	Trifluralin	
	mg/kg	% of degradation
Control	19.52 ± 0.22	-
1	14.71 ± 0.18	24.6
2	14.87 ± 0.85	23.8
3	4.57 ± 0.53	76.6
4	0.81 ± 0.03	95.8
5	4.76 ± 0.26	75.6
6	4.50 ± 0.37	76.9
7	7.76 ± 0.13	60.2
8	0.95 ± 0.19	95.1

It is known, that organochlorine pesticides are highly persistent, having at the same time relatively low environment mobility. The type and duration of degradation are determined by the chemical structure and stability of the pesticide. The ratio between DDT and its metabolites allows to roughly estimate the time of occurrence and degradation rate of pesticide residues in polluted soil. Thus, the ratio **(DDE + DDD) / DDT > 1** indicates an old pollution with DDT, exposed to active microbial transformation, and the ratio **(DDE + DDD) / DDT < 1** testifies about a recent pollution and low degradative activity of soil microorganisms (Galiulin and Galiulina, 2008; Kulikova-Khlebnikova, 2011). After the ratio between the soil concentration of DDT metabolites and DDT has been calculated, according to indicated expression, the value 3.92 > 1 was obtained, which indicates an old pollution and the pesticide degradation by the microbial biota.

Microbial degradation of POPs depends on several factors: temperature, pH, redox potential, humidity, aeration, the „special” microorganisms etc. (Phillips, 2004). Practically the optimal combination of these factors absents in the environment, therefore the transformation of pesticides is limited in its initial stages. The most effective bioremediation methods targeting DDTs contaminants, according our experimental data, was amendment of soil with peptone and dibasic phosphate of potassium and ammonium (variants 4 and 8), that have led to a decrease of ΣDDTs up to 64.6 and 61.3% respectively, comparing with non remediated control (tab.2).

Table 2

Residue of DDTs metabolites and DDT in soil befory and after bioremediation procedures

Experimental variants	DDT		DDTs	
	mg/kg	% of degradation	mg/kg	% of degradation
Initial (control)	0.32 ± 0.02	-	1.48 ± 0.07	-
1	0.23 ± 0.02	28.1	1.07 ± 0.10	27.7
2	0.23 ± 0.01	28.1	1.10 ± 0.09	25.7
3	0.14 ± 0.02	56.3	1.15 ± 0.12	22.3
4	0.21 ± 0.04	32.9	0.52 ± 0.11	64.6
5	0.10 ± 0.01	68.8	1.01 ± 0.09	31.8
6	0.09 ± 0.01	72.2	1.12 ± 0.03	24.4
7	0.00	100.0	0.97 ± 0.02	34.7
8	0.22 ± 0.04	31.4	0.57 ± 0.12	61.3

Amendment of soil with phosphates and peptone in Section 2 ensures a deep reductive cleavage of the DDT (up to 0.09 mg / kg soil) and further phytoremediation favours total degradation of this pesticide in the variant 7. An important increase of the metabolite DDD and an essential decrease of DDT – up to 68.7 and 72.2% – were observed in bioremediation variants 5 and 6, due to the alternation of anaerobic / aerobic cycles and soil supplementation with peptone and phosphates (tab.2). A deep reductive cleavage of investigated organochlorine pesticides occurred under these conditions, manifested by the disappearance of DDT and intensive accumulation of degradation products, especially *o,p′*-DDD.

CONCLUSIONS

Thus, the eight procedures of bioremediation of complex soil pollution with trifluralin and organochlorine compounds POPs have been examined, and the decrease of their amount in all experimental variants was determined, that indicated a good selection of remediation procedures tested.

The main factor for the trifluralin decomposition in carbonated chernozem is soil microbiota, especially the anaerobic / facultative anaerobic, and its stimulation with nutrient supplements substantially improves decomposition of the trifluralin herbicide in soil. The degree of trifluralin mineralization after an application of the anoxic treatment was at the same level as after a combination of the anoxic / oxic cycled condition and stimulation of indigenous microflora with phosphates and peptone. In both cases the concentration of trifluralin decreased by more than 4 times and degradation rate reached 95-96%.

Amendment of soil with phosphates and peptone under anaerobic conditions provides a deep reductive cleavage of organochlorine pesticides DDT and intensive accumulation of degradation products, especially of *o,p′*-DDD. The alternation of anaerobic-aerobic conditions favour degradation of DDT and DDTs metabolites, compared with an anoxic treatment. To reduce the amount of extremely persistent metabolites DDD and DDE the prolongation of the experiments for bioremediation of the contaminated soil is necessary.

REFERENCES

1. **Arinushkina E.V., 1970** – *Guidance on chemical analysis of soils.* Editura MGU, Moscow, p. 488.
2. **Bento F.M., Camargo F.A.O., Okeke B., Frankenberger Jr. W.T., 2003** – *Bioremediation of soil contaminated by diesel oil.* Brazilian Journal of Microbiology, 34 (1), pp. 65-68.
3. **Galiulin R.V., Galiulina R.A., 2008** – *Ekologo-geokhimicheskaya otsenka «otpechatkov» stoykikh khlororganicheskikh pestitsidov v sisteme pochva – poverkhnostnaya voda.* Agrokhimiya, No. 1, pp. 52-56.
4. **Kantachote D., Singleton I., Naidu R., Mcclure N., Megharaj M., 2004** – *Sodium Application Enhances DDT Transformation in a Long-Term Contaminated Soil.* Water, Air, and Soil Pollution, 154 (1-4), pp. 115-125.
5. **Klisenko M.A., Alexandrova L.G., 1983** – *Determination of pesticide residues.* Kundiev Yu.I., eds., Editura Health, Kiev, p. 174.
6. **Kozlova A.A., 2009** – *The educational practice on soil physics: methodical handbook.* Editura Irkutsk State University Press, Irkutsk, pp. 52-53.
7. **Kulikova-Khlebnikova E.N., Robertus Yu.V., Kivatskaya A.V., 2011** – *Osobennosti metabolizma khlororganicheskikh pestitsidov v ob"yektakh okruzhayushchey sredy v usloviyakh Gornogo Altaya.* Vestnik Altayskogo gosudarstvennogo agrarnogo universiteta, 10 (84), pp. 50-53.
8. **Matsumoto E., Kawanaka Z., Zun S., 2009** – *Bioremediation of the organochlorine pesticides, dieldrin and endrin, and their occurrence in the environment.* Appl.Microbiol.Biotechnol., 84, pp. 205-216.
9. **Phillips T., Lee H., Trevors J.T., Seech A.G., 2004** – *Mineralization of hexachlorocyclohexane in soil during solid-phase bioremediation.* J.Ind.Microbiol.Biotechnol., no. 31, pp. 216-222.
10. **Sasek V., Glaser J.A., Baveye P., 2003** – *The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions.* Proceedings of the NATO Advanced Research Workshop on the Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions, Prague, Czech Republic, 14-19 June 2000. Editura Springer, Nature, p. 417.
11. **Takashi O., Ryota Y., 2004** – *Improvement of simazine degradation by inoculation of corn and soybean plants with rhizobacteria.* Soil Sci.and Plant Nutr., 50 (8), pp. 1295-1299.
12. *****, 2003** – *Normativele igienice privind reziduurile preparatelor de uz fitosanitar în obiectele mediului înconjurător din 21.08.2003.* Editura Ministerul Sănătății, Chişinău, Monitorul Oficial 248-253/359, 19.12.2003.
13. *****, 2004** – *Republic of Moldova. National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants. Ministry of Ecology and Natural Resources.* World Bank, Editura Stiinta, Chisinau, p. 80. http://www.pops.int/documents/implementation/nips/submissions/moldova.pdf (accessed March 29, 2014).
14. *****, 2011** – *State of the Environment in the Republic of Moldova*, 2007-2010 (National Report – Synthesis). Editura “Nova Imprim” SRL, Chisinau, p. 88. ISBN 978-9975-4224-3-7.