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BIOREMEDIATION AND PHYTOREMEDIATION OF PESTICIDE CONTAMINATED SOIL: MICROBIOLOGICAL STUDY

BIOREMEDIAREA ŞI FITOREMEDIAREA SOLULUI CONTAMINAT CU PESTICIDE: STUDIU MICROBIOLOGIC

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Abstract. *The paper is focused on screening of the approaches to remediate the soil, complex contaminated by obsolete persistent organic pesticides: DDTs and trifluralin. The aim was to increase the biodegradation rates by activation of indigenous soil microflora with mineral and organic amendments. The experiment was carried out in oxic conditions, and cycled anoxic and oxic conditions, with phytoremediation applied afterwards. The effectiveness of the bioremediation procedures for soil microbiota along with degradation of pesticides was discussed. Microbiological analysis of soil at the final of experiments demonstrated the necessity of phytoremediation for soil microflora recovery.*

Key words: contaminated soil, pesticides, DDT, trifluralin, microflora

Rezumat. *Lucrarea este axată pe screening*-ul *procedeelor de remediere a solului, contaminat timp îndelungat cu complex de pesticide organice persistente: DDT-uri şi trifluralină. Scopul a fost creşterea ratelor de biodegradare prin activarea microflorei indigene a solului cu amendamente minerale şi organice. Experimentul a fost realizat în condiții oxice şi în condiții ciclice anoxice-oxice, cu aplicarea ulterioară a fitoremedierii. E fost arătată eficacitatea procedurilor de bioremediere pentru microbiota solului, paralel cu degradarea pesticidelor. Analiza microbiologică a solului, la finalul experimentelor, a demonstrat necesitatea fitoremedierii, pentru recuperarea microflorei solului.*

Cuvinte cheie: sol contaminat, pesticide, DDT, trifluralină, microfloră

INTRODUCTION

In recent decades, in Republic of Moldova, the background pollution of soil has become less significant due to the current reduction of the main sources of diffuse pollution. The quantities of fertilizers and pesticides used in agriculture were significantly reduced, and there is no current problem of pollution with nitrates and heavy metals (Anuarul IPM – 2019, 2020). Compared with previous years, Σ DDT content was significantly reduced, the content of Σ HCH and Σ PCBs remained the same. But the problem of local pollution of soils with different wastes and harmful substances is becoming more acute (Anuarul IPM –

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2019, 2020; State of the Environment..., 2011). A large number of locations are contaminated with several Persistent Organic Pollutants (POPs), which raises the question of potential synergistic effects of impact on the population and the natural environment, 112 locations polluted with two compounds, 43 locations – three compounds and 13 locations – four compounds (State of the Environment..., 2011). In most cases the mixture of detected pollutants includes DDT metabolites, isomers of HCH and toxaphene (Pleşca *et al.*, 2010).

Destroyed storehouse for pesticides and organic fertilizers located near Sangera village, Chisinau municipality, the Republic of Moldova, has been selected using Management of POPs (http://pops.mediu.gov.md/). The site was long-term and complex polluted by pesticides, the total content of which was 21 mg/kg dry soil; the major component of contamination was dinitroaniline herbicide trifluralin and the minor component was organochlorine insecticide DDT and its metabolites DDE and DDD (Rastimețina *et al.*, 2016). Both compounds belong to organic halogenated pesticides, but the approaches for bioremediation of contaminated soil differed depend of ways in their transformation (Kantachote *et al.*, 2004; Trifluralin, 2007; Wang and Arnold, 2003).

Dichlorodiphenyltrichloroethane (DDT) was one of the most widely used organochlorine pesticides. In 2001, the governing council of the United Nations environment program issued a treaty to eliminate or restrict the production and use of persistent organic pollutants (POPs). Although the use of DDT has been banned for wide agricultural use, part of DDT was released to environment and this trend continues, because it was still used as an anti-malaria agent or a raw and processed material for dicofol production (Guatam and Sumathi, 2006). A large amount of DDT still remains in soils. In some area, the DDT concentration found in soil markedly exceeded the level set by the national soil quality standards (Zao *et al.*, 2010).

Trifluralin is a synthetic fluorinated dinitroaniline herbicide, which is used in the control of annual grasses and broad-leaved weeds in agriculture, horticulture, viticulture, amenity and home gardens. Trifluralin can exert a phytotoxic action on some cereals, such as wheat, oats, rice, and corn. The toxic effect of this herbicide lies in the inhibition of the growth of roots, namely, in violation of the cell division processes in the roots of plants. In the plants subjected to this treatment, secondary roots are not developed, the growth of the shoot stops, and the plant died (Antonious, 2012).

Trifluralin was first registered in 1963 and is marketed in a number of names, such as Treflan, Triflurex, Triflusan and others (Trifluralin, 2007). It is currently widely registered for use throughout the world although the use within the EU was banned from 2008. In 2012, trifluralin was excluded from the State Register of plant protection products and fertilizers permitted for use in the territory of the Republic of Moldova; however, residues of the pesticide are registered in soils of Moldova to the present day (Rastimesina *et al.*, 2016).

Our goal was to estimate the effect of bioremediation and phytoremediation procedures using the stimulation of anaerobic or facultative anaerobic microflora of soil contaminated with organic halogenated pesticides.

MATERIALS AND METHODS

Samples of polluted soil were collected nearby the destroyed storehouse for pesticides and organic fertilizers, were cleaned of roots and other impurities, sieved (mesh No. 2) and air-dried at 22-23^{0}C.

The extraction of pesticide residues from soil has been performed in four repetitions per option according handbook of methods (Metode de determinare…, 2000). 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. Percentage of degradation was calculated using the expression:

% of degradation = [(PR control – PR experience) / PR control] × 100; where PR is pesticide residue (Bento *et al.,* 2003).

The bioremediation was established in the dark plastic jars, each containing 1,000 g of polluted soil. Contaminated soil without remediation was used as a control.

The experiment was designed in oxic, anoxic and cycled anoxic / oxic conditions. At the set up of the experiment in oxic conditions, the soil was amended with water, 60% of water-holding capacity (WHC) (Options 1-6), mineral fertilizer ammophoska (Option 2, 5, and 6), there were planted cucumbers *Cucumis sativus* L. (Option 3), alfalfa *Medicago sativa* L. (Option 4 and 5), and oat *Avena sativa* L. (Option 6). The duration of experiment was 112 days.

Anaerobic conditions were created by saturating the contaminated soil with water, 80% of WHC, in the plastic jars sealed with Parafilm, and stored in the dark at 22-24°C (Options 7-12). At the set up of the experiment in anoxic (Options 7, 8, and 10) and cycled anoxic / oxic conditions (Options 9, 11, and 12), the soil was amended with 0.5% peptone (Options 8-12), 1.0% mono- and dipotassium phosphates (Option 8 and 9), 1.0% mono- and disodium phosphates (Option 10 and 11), and 1.0% dipotassium and diammonium phosphates (Option 12). At the beginning of the aerobic phase, lasted for 7 days, Parafilm has been removed, and the soil mixed with a metal spatula and soil moisture was gradually brought up to 60% of WHC. At the start of each anaerobic phase, lasted for 21 days, amendments (0.2% each) were added to the soil, and soil humidity was maintained at 80% of WHC. The anoxic and cycled anoxic / oxic conditions continued for 112 days. For Options 10-12 these conditions lasted for 63 days, than it was passing to the aerobic phase by applying sawdust, and phytoremediation with oat (*Avena sativa L.*), for 72 days. Monthly plants were cut, mixed with the soil and new seeds were planted.

At the end of the experiment, the systematic groups of microorganisms (micromycetes, bacteria, actinomycetes) present in the soil were observed through inoculations on solid selective media.

RESULTS AND DISCUSSIONS

The soil was polluted by trifluralin 30.00 ± 0.34 mg/kg soil, ∑ DDTs (DDT, DDE, DDD) was 1.80 ± 0.07 mg/kg dry soil. The total content of organochlorine compounds was 21.00 mg/kg dry soil, which demonstrates that the level of pollution near the storehouse exceeded the maximum residue limits (MRL) (0.1 mg/kg dry soil for trifluralin and for each metabolite of DDT) (Rastimesina *et al.*, 2016).

Natural attenuation (Option 1), or maintenance of soil moisture at 60% of WHC without tilling, any amendments, and other treatments, for 112 days, led to degradation of about 7% trifluralin and 5% DDTs (fig. 1 and 2).

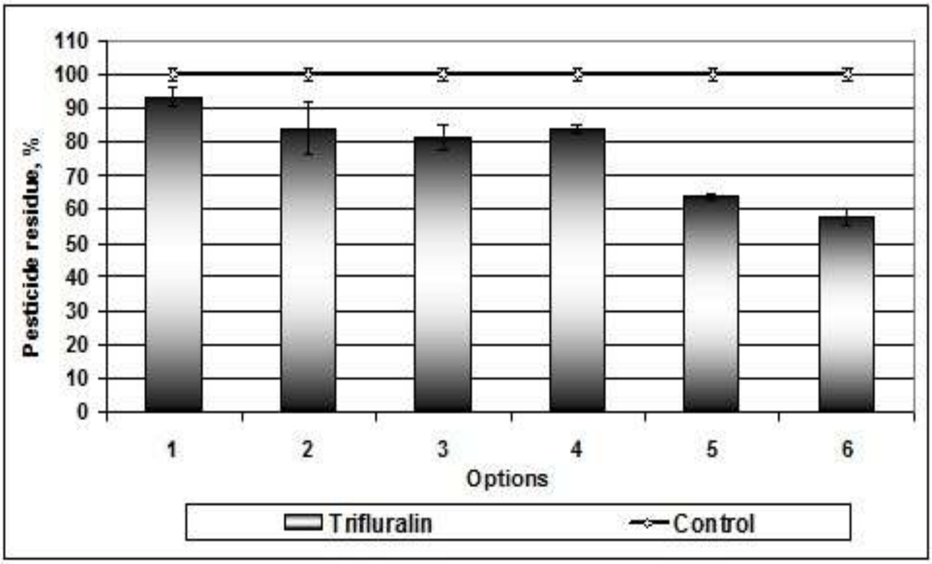

Fig. 1 The impact of bioremediation procedures in oxic conditions on trifluralin degradation in soil

Conform investigations, the biodegradation of the organofluorine herbicide – trifluralin best occurs under anaerobic conditions, and the presence of oxygen can inhibit this process (Tor *et al.*, 2000; Wang and Arnold, 2003). In our experiments on biodegradation of trifluralin in aerobic conditions the complete mineralization had not been achieved. In spite of this, the combination of bio- and phytoremediation, in experimental Options 5 and 6 led to the considerable decrease in the herbicide concentration without creating anaerobic conditions, up to 36-42% (fig. 1).

Tilling and amendments with mineral fertilizer performed in the Option 2 favored the decomposition of organochlorine pesticides by 22% compared to the control – and by 18% – compared to the Option 1. The cultivation of phytoremediation plants accompanied by the periodic tilling of the contaminated soil led to the decrease of ∑DDTs by 31-33% compared to the control (fig. 2).

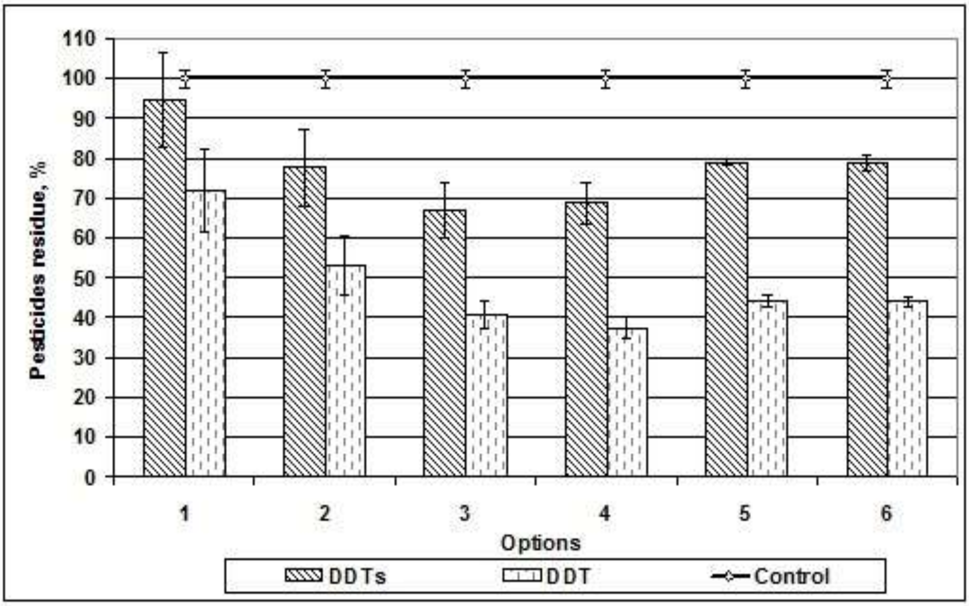

Fig. 2 The impact of bioremediation procedures in oxic conditions on DDTs and DDT degradation in soil

Creating anaerobic conditions by saturation of soil with water up to 80% of WHC allowed increasing the degradation of trifluralin to 25% and DDTs – up to 28% of the initial concentration (fig. 3 and 4).

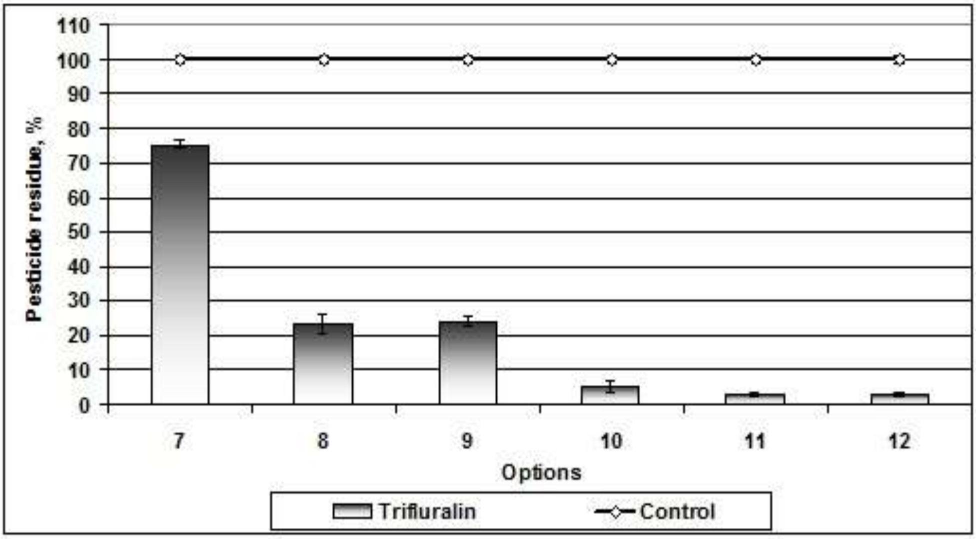

Fig. 3 The impact of bioremediation procedures in anoxic and anoxic / oxic conditions on trifluralin degradation in soil

The improving in efficiency of pesticides degradation in soil could be achieved using anoxic and cycled anoxic/oxic treatment, combined with stimulating the indigenous microflora with mineral and organic amendments (phosphates and peptone); trifluralin content in soil decreased by more than 4 times. Combination of bio- and phytoremediation techniques in the Options 11 and 12 allowed the mineralization of 98% trifluralin (fig. 3).

Amount of DDTs, decomposed in anoxic and cycled anoxic/oxic conditions without phytoremediation was not significant, degradation made less than one third of the initial concentration – 27-29.0%. Soil amendments with phosphates and peptone in cycled anoxic/oxic conditions favors the degradation of DDT (up to 0.09 mg/kg dry soil), compared with anoxic treatment. Phytoremediation provided a decrease in DDTs concentration to 69-73% (fig. 4).

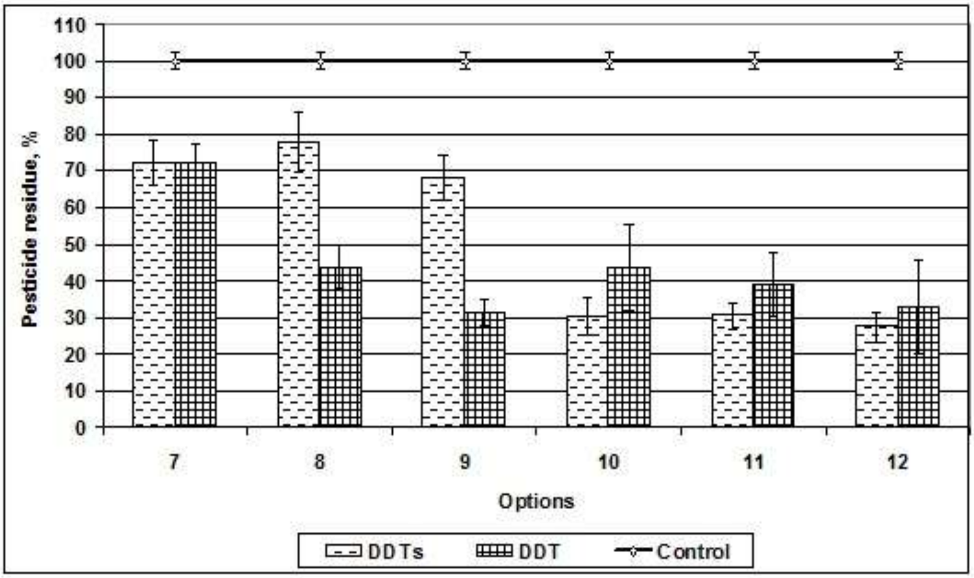

Fig. 4 The impact of bioremediation procedures in anoxic and anoxic / oxic conditions on DDTs and DDT degradation in soil

At the end of the experiment, the microbiological activity of the soil was determined depending on the bioremediation measures taken. Our experiments did not aim at characterizing all groups of microorganisms in the soil, but only at studying the indigenous soil microflora, which is involved in nitrogen transformation processes, and survived the conditions of prolonged toxic stress.

The analysis showed that in the control variant (Option 1), where the only remedial factor was maintaining sufficient soil moisture – 60% CRA, the diversity of microorganisms was low and corresponded to the carbonate chernozem. Under the long-term influence of toxicants there is a restructuring of soil microbial cenosis in the direction of reducing microbial diversity, but with the emergence of more resistant species.

Carrying out various bioremediation measures, in most cases, considerably facilitates the activation of the soil microbiota. In experimental Options 5 and 6, maintained in aerobic conditions using alfalfa and oat as phytoremediation plants, significant stimulation of bacterial growth was observed.

For the microbiological characterization we used the method of calculating the total number of microorganisms (fig. 5, fig. 6).

The ability to assimilate xenobiotic compounds in anaerobic conditions has been detected in a wide variety of microorganisms: anaerobic, facultative anaerobic, including bacteria of the genera *Pseudomonas* and *Bacillus*. In the experimental variant with alternating anaerobic-aerobic conditions, the total number of studied microorganisms decreased practically 4 times compared to the control; and the transfer of the variant in aerobic state by the introduction of the sawdust and the subsequent phytoremediation, cardinally modified the microbial cenosis and led to the increase of the number of microorganisms from all the physiological study groups (fig. 6).

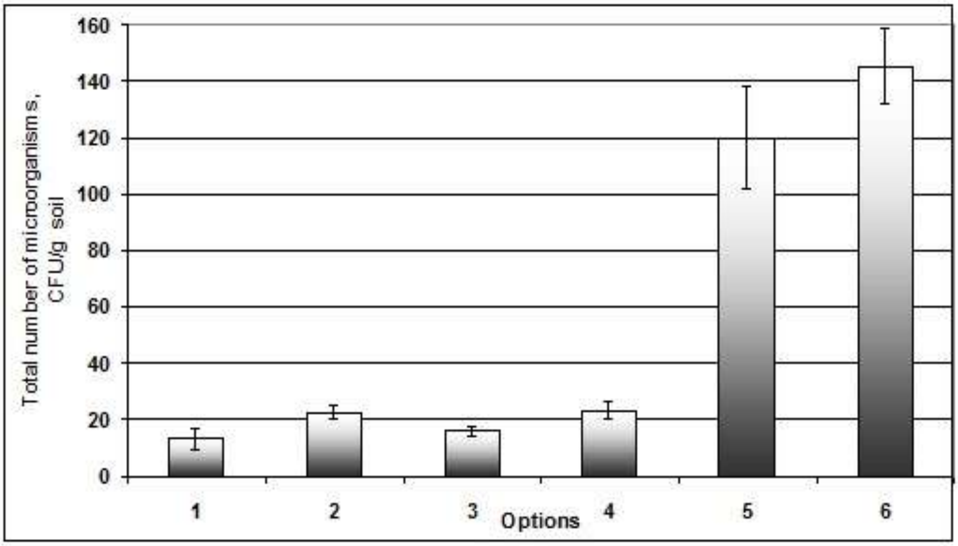

Fig. 5 Total number of microorganisms after the bioremediation procedures in oxic conditions

The increase in the number of microorganisms in all experimental variants, compared to the control, demonstrated the high effectiveness of treatments used. These include both microorganisms that have survived and / or adapted to the continuous action of pollutants, as well as forms at rest. In the control, naturally attenuated, the number of bacteria and micromycetes was much smaller and represented by microorganisms that adapted to the action of pesticides. In the experimental variants, the number of microorganisms was tens and even hundreds of times higher than in the control. The formation of a larger pool of microorganisms indicates the recovery of the soil.

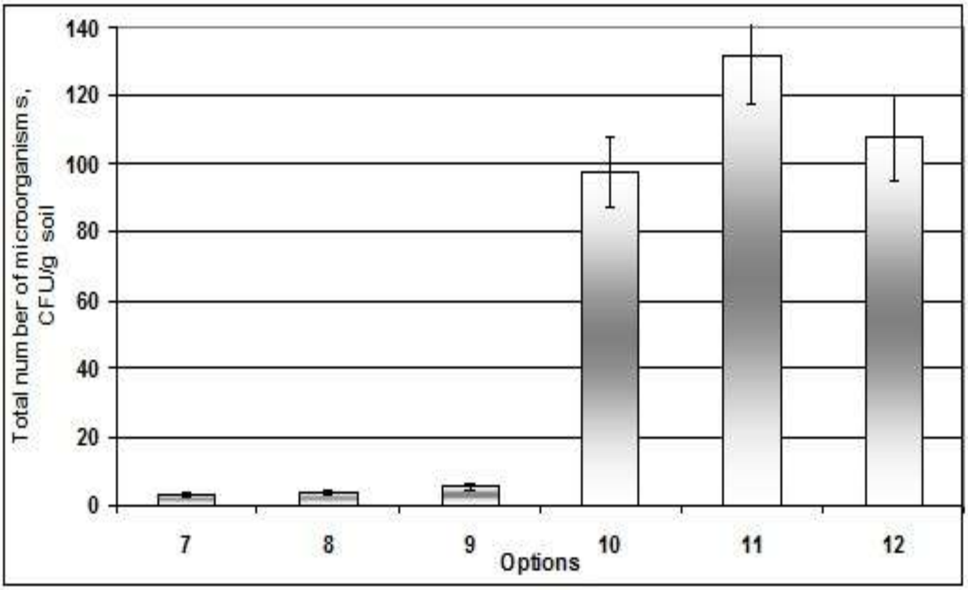

Fig. 6 Total number of microorganisms after the bioremediation procedures in anoxic and anoxic / oxic conditions

The number of microorganisms varies depending on the experimental variant. The most favorable conditions for the vital activity of microorganisms were created in the options maintained in cycled anaerobic / aerobic conditions, followed by phytoremediation.

Thus, the activation of microbial cenosis, the good growth of plants, along with the significant decrease of DDTs concentrations and practically the total elimination of trifluralin from the soil, prove the effectiveness of the bioremediation techniques used.

CONCLUSIONS

The main factor for the trifluralin decomposition in soil is anaerobic/facultative anaerobic soil microbiota, and its stimulation with nutrient supplements substantially improves trifluralin degradation.

Stimulation the indigenous microflora with phosphates and peptone in anoxic or anoxic/oxic conditions, followed by phytoremediation, can be used as a basic principle method in bioremediation of complex pollution.

In the case of integrated, complex pollution, phytoremediation, as an additional way of remediation, was important for the DDT degradation and the recovery of soil microflora.

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