

## DDT CONTENT IN POLISH SOILS – CURRENT STATE AND ATTEMPTS OF RHIZO-BIOREMEDIATION

### Summary

Soil and some plant products are still found to be contaminated by DDT residues or metabolites 30 years after its total ban. In order to ensure high quality of organic products and their compliance with the EU rules, following a monitoring carried out to assess DDT and its metabolites residues level in different soils, we started a research program to test some methods of soil remediation, using fungal and bacteria consortia in association with *Cucurbita pepo* L. var. *Zucchini* - *giromontina* plants. Results of the monitoring program showed that about 80% of the soil samples collected from eight Voivodeships contained DDT residues. The consortia utilized in the bioremediation trials showed to favor DDT uptake by the plants and its translocation to above ground organs. Rhizoremediation strategy seems thus to better sustain the uptake and accumulation of DDT residues by plants.

**Key words:** *Cucurbita pepo*, *Zucchini*, DDT, bioremediation, rhizoremediation, organic farming

## ZAWARTOŚĆ DDT W POLSKICH GLEBACH – STAN OBECNY I PRÓBA JEGO BIOREMEDIACJI

### Streszczenie

Gleba i niektóre produkty roślinne nadal mogą być zanieczyszczone pozostałościami lub metabolitami DDT po upływie 30 lat od całkowitego zakazu jego stosowania. Aby zapewnić wysoką jakość produktów ekologicznych i ich zgodność z przepisami UE, po przeprowadzeniu monitoringu w celu oceny zawartości pozostałości DDT i jej metabolitów w różnych glebach, rozpoczęliśmy program badawczy w celu przetestowania niektórych metod ich ekstrakcji z gleby przy zastosowaniu konsorcjów mikroorganizmów (bakterie i grzyby mikoryzowe) w połączeniu z uprawą roślin cukinii odm. *Soraya*. Wyniki monitoringu wykazały, że około 80% próbek gleby pobranych na terenie ośmiu województw zawierało pozostałości DDT. Konsorcja mikroorganizmów wykorzystywane w próbach bioremediacyjnych wykazały korzystny wpływ na pobieranie DDT przez rośliny i ich przemieszczanie się do części nadziemnych. Strategia rizomediacji wydaje się wspomagać pobieranie i akumulację pozostałości DDT przez rośliny.

**Słowa kluczowe:** *Cucurbita pepo*, *cukinia*, DDT, bioremediacja, rizomediacja, rolnictwo ekologiczne

### 1. Introduction

Dichloro diphenyl trichloroethane (DDT) was commonly used in Poland as a plant protection product during the '50-70s of the last century. However, with the introduction on the market of new, relatively less risky, active substances (e.g. organophosphorus and other organic compounds), the use of highly toxic chloro-organic substances, especially those containing DDT, started to be banned. Even though almost 30 years passed since that moment, soil and some plant products are still found to be contaminated with residues of DDT and its metabolites [5]. Beside the environmental hazard as both water, soil and various sediments are prone to bioaccumulation of these substances, plants can also accumulate compounds belonging to the group of chlorinated pesticides [6]. Marketing of agricultural products for food or animal feed is allowed if the residues content is within the maximum residue level (MRL) set up by the EU Regulation 396/2005. However, in case of organic production, regulated by EU Regulations 374/2008 and 899/2009, the finding of accidental presence of DDT residues in soil, eventually contaminating organic products, can result in the loss of organic certification.

The finding, during the normal controls carried out by certifying bodies, of some samples of organic products con-

taining DDT residues in Poland has prompted the need to assess the current status of DDT pollution in agricultural soils and to search methods that could reduce the risk of plant contamination. A monitoring was carried out sampling soils of different physico-chemical characteristics and trials applying different remediation strategies were performed. The present paper is reporting the results of the monitoring and of the preliminary tests of remediation.

### 2. Material and methods

In order to assess the occurrence of DDT and its metabolites in the soil and in growing plants, 53 sites, in 8 Voivodeships, characterized by organic certified fields and crops were monitored, sampling soil and the growing plants. Soil samples were collected according to the methodology given in the Regulation of the Minister of Agriculture and Rural Development of 27 November 2013 "on the sampling of plants, plant products or objects for testing for residues of plant protection products" (OJ 2013, p. 1549). Various vegetative organs (aboveground and root parts) were harvested from the crops, collecting at least 300 g, during different periods of the growth season, from controlled or field trials (see below).

Trials on soil remediation were carried out in the greenhouse of the Plant Protection Department of the Research Institute of Horticulture in Skierniewice. Soil contaminated with DDT or its metabolites was collected from organic plantations and further added of 10 mg of analytical grade p,p'-DDT, which brought the total concentration up to 0.826 mg/kg. The prepared soil was inoculated with selected microorganisms' consortia (Table 1) when preparing the potting mixture, thoroughly mixed to it. In case of the EmFarma and EmFarma Plus consortia, the soil was further inoculated two weeks later. The contaminated soil was used to fill plastic pots used for bio- and rhizoremediation experiments. For the latter, seeds of zucchini cv. Soraya were sown in the pots. Samples of soil and plant materials from both trials were collected at the end of the growing season, about 53 days after sowing, and immediately processed for analysis.

Residues determination of DDT and its isomers and metabolites (p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, o,p'-DDE, p,p'-DDE) in soil and plant material was made by gas chromatography (Agilent Technologies 6890N), using a Zebtron™ ZB-MultiResidue™-1 chromatographic column, with mass detector (5975B Inert XL MSD). Extraction of the compounds was carried out according to the QuEChERS method (EN 15662:2008). In brief, after sample comminution and homogenization in the presence of dry ice, 10g (soil, fruits, vegetables) or 5 g (roots, stems, leaves) aliquots were taken for extraction. After addition of an extraction solution (10 ml water, 10 ml acetonitrile, 4 g magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate), the sample was shaken intensively and centrifuged for phase separation. An aliquot of the organic phase

was cleaned-up by dispersive solid phase extraction with 25 mg of amino sorbent (PSA) and 150 mg of magnesium sulfate for removal of residual water. Triphenyl phosphate (TPP) solution was used as internal standard. All data were determined using certified analytical standards, taking into account specific matrix effects, corrected by internal standard. Data for plant materials were adjusted for fresh mass, while those for soil were adjusted to dry mass (soil drying was obtained with heating 24 h at 80°C).

Typical chromatograms of DDT isomers and metabolites at the level of 0.1 and 0.01 mg/kg are shown in Fig. 1.

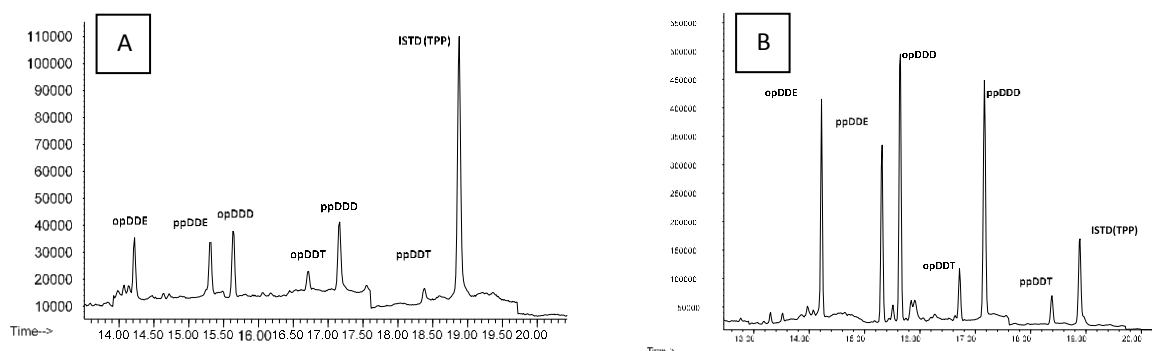
The monitoring of DDT content in the several soils sampled, which resulted to be mainly Luvisols, showed that more than 80% of the samples (i.e. 43 out of 53 sites monitored) contained DDT or its isomers and metabolites in trace amounts (Table 2), thus confirming early reports about the time persistence of these compounds [8]. The majority of sample had a content at the level of µg/kg, with only four samples having a higher residues level - from 0.101 to 0.268 mg/kg. Even though the ratio between the different isomers and metabolites differed among samples, DDM was never detected (Table 2). Differences in the level of DDT soil detection may be due to the type of soil, the crops normally grown on the field (i.e. the need of insecticides for crop protection) and the frequency of treatments using DDT [4].

Despite the quite wide detection of DDT in the soil of the monitored sites, only four root samples of the plant grown in these sites were found to contain traces of DDT (Table 2). However, it should be underlined that the root system of three of these plants (cabbage, celery and leek) is normally not used for human consumption and that no residues were detected in other plant parts (data not shown).

Table 1. List of microorganisms' consortia used in the trials  
Tab. 1. Wykaz zastosowanych konsorcjów mikroorganizmów

Product name / nazwa produktu	Dose equivalent / dawka
Micosat F (CCS Aosta) (a consortium of mycorrhizal fungi and bacteria)	60 kg/ha
Micosat Fito (CCS Aosta) (a consortium of mycorrhizal fungi, yeasts and bacteria)	60 kg/ha
EmFarma (ProBiotics Polska Sp. z o.o) (Culture of Live Microorganisms SCD ProBio ORIGINAL®)	1% of a 5% inoculum solution
EmFarma Plus (ProBiotics Polska Sp. z o.o) (Culture of Live Microorganisms SCD ProBio ORIGINAL® +Phototropic bacteria)	1% of a 5% inoculum solution

Source: own work / Źródło: opracowanie własne



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Figure 1. Chromatogram of DDT isomers and metabolites at the level of 0.1 mg/kg (A) or 1 mg/kg in soil matrix (B)  
Rys. 1. Chromatogram izomerów i metabolitów DDT w stężeniu 0,1 mg/kg (A) i 1 mg/kg (B) w glebie

Table 2. Amount of DDT residues in the soil of eleven sites and on plants collected from these sites  
 Tab. 2. Poziom pozostałości DDT w glebie i w roślinach na niej uprawianych w jedenastu lokalizacjach

Sites of soil sampling and plant samples found with residues	Soil type	Content of DDT and its metabolites						Total DDT content [mg/kg]
		o,p- DDE	p,p- DDE	o,p- DDD	p,p-DDD	o,p-DDT	p,p-DDT	
		LOD (limit of determination) 0.002 mg/kg						
Brzostówka 1	Sandy clay loam	nd	0,011	nd	0,0055	nd	0,0091	0,028
cabbage, roots		nd	0,005	nd	nd	nd	nd	0,006
celery, roots		nd	0,023	nd	0,0039	nd	0,0083	0,038
leek, roots		nd	0,006	nd	nd	nd	nd	0,007
Brzostówka 2	Sandy clay loam	nd	0,0054	nd	0,0028	nd	nd	0,009
Cetyń	Sandy clay	nd	0,017	nd	nd	nd	0,021	0,040
Dąbrowiec n/Żdzary	Sandy clay	nd	nd	nd	nd	nd	nd	nd
Dębowa Góra	Clay loam	nd	0,037	nd	0,025	nd	0,126	0,194
Jastrzębna n/Sztabina	Sandy clay	nd	0,0049	nd	0,0041	nd	nd	0,01
Kamion 1	Sandy clay	nd	0,017	nd	0,0041	nd	nd	0,023
Kamion 2	Sandy clay	nd	0,028	nd	0,0082	nd	0,025	0,065
Kolonia Hołowienki	Loam	nd	0,0064	nd	nd	nd	0,0054	0,013
Rosocha	Clay loam	nd	0,016	0,0022	0,018	nd	0,061	0,10
Skierniewice 1	Sandy loam	nd	0,025	nd	0,0083	nd	0,020	0,057
Skierniewice 2	Sandy loam	nd	0,0053	nd	nd	nd	0,0069	0,013
Skierniewice 3	Sandy loam	nd	0,028	nd	0,014	nd	0,011	0,058
Stepniczka	Sandy clay loam	nd	0,072	0,0306	0,14	nd	nd	0,27
Suliszew	Sandy loam	nd	0,0033	nd	nd	nd	nd	0,004
carrot, root		nd	0,0089	nd	nd	nd	nd	0,010
Tchórznica	Clay loam	nd	0,015	nd	0,0095	nd	0,022	0,049
Wysokienice	Silt loam	nd	0,0016	nd	nd	nd	0,0022	0,004
Żabówko	Sandy clay loam	nd	0,029	0,0061	0,023	nd	nd	0,064

nd - not detected / nie wykryto

Source: own work / Źródło: opracowanie własne

Furthermore, DDT residues were not found (data not shown) in any edible part of the different plant species, which are following specified, that were sampled from the 11 contaminated sites: apple, blackberry, carrot, corn, dill, fennel, cucumber, green beans, lentil, mixture of cereals, parsley, parsnip, potato, pumpkin, red and white onion, red beet, rhubarb, sorrel, sour cherries, sweet cherries, tomato, zucchini. Even though early studies showed the possibility of uptake of DDT by plants [3], significant translocation from roots to shoots has been demonstrated only for *C. pepo* [10].

### 3. Results and discussion

The soil inoculation with consortia of microorganisms induced a reduction of soil DDT residues in comparison to the initial level; however, a similar reduction was observed also in the control at the end of the experiment (Table 3). Microorganisms' consortia containing mycorrhizal fungi have been mainly exploited for bioremediation of heavy metals pollution [2], thus the lack of their bio-degradation activity in absence of plants shown by our experiment con-

firms the inadequateness of their use in case of organic pollutants. However, when considering the amount of DDT measured in inoculated plants, all the inocula used favoured the uptake of DDT, even more than doubling the amount present in the plant in case of Micosat F, in comparison to plants without inocula (Table 3). The capacity of the plants to accumulate DDT was particularly related to root tissues; translocation to above-ground plant tissues was more effective in case of both Micosat consortia (Table 4). Plant inoculation with mycorrhizal fungi, also in consortia with rhizosphere bacteria, is used to increase the uptake of soil nutrients due to the increased soil volume explored by the fungal hyphae and to active metabolic pathways [1]. The same mechanisms could thus be involved to explain the higher uptake of plants colonized by these fungi, as observed in our experiment, also considering the synergy with *C. pepo* metabolism [9, 10]. The discrepancy between the sum of DDT recovered from the soil and the plant at the end of the experiment and the total amount present at the beginning can be accounted to volatilization or to effects of soil absorption [7].

Table 3. Effect of inoculation with different consortia of microorganisms on DDT content in the soil and *Cucurbita pepo* – zucchini plants

Tab. 3. Wpływ stosowania różnych konsorcjów mikroorganizmów na zawartość DDT w glebie i roślinach cukinii

Treatment	Content of DDT and its metabolites						Total DDT content [mg/kg]
	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT	
	LOD (limit of determination) 0.005 mg/kg						
Initial soil	nd	0,074	nd	0,12	nd	0,61	0,826
Soil							
Control (no inoculum)	nd	0,058	nd	0,057	nd	0,223	0,351
Micosat F	nd	0,049	nd	0,068	nd	0,216	0,345
Micosat Fito	nd	0,049	nd	0,057	nd	0,182	0,299
EmFarma	0,023	0,060	0,033	0,10	nd	0,081	0,322
EmFarma Plus	nd	0,049	0	0,044	nd	0,310	0,414

Cont. of Table 3

Treatment	Content of DDT and its metabolites						Total DDT content [mg/kg]
	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT	
	LOD (limit of determination) 0.005 mg/kg						
Initial soil	nd	0,074	nd	0,12	nd	0,61	0,826
Plant							
Control (no inoculum)	nd	0,072	nd	0,040	nd	0,061	0,186
Micosat F	nd	0,12	nd	0,080	nd	0,170	0,395
Micosat Fito	nd	0,11	nd	0,050	nd	0,078	0,253
EmFarma	nd	0,070	nd	0,046	nd	0,079	0,207
EmFarma Plus	nd	0,084	nd	0,055	nd	0,073	0,227

nd - not detected / nie wykryto

Source: own work / Źródło: opracowanie własne

Table 4. Effect of *Cucurbita pepo* – zucchini plants inoculation with microorganisms' consortia on accumulation and translocation of DDT

Tab. 4. Wpływ stosowania konsorcjum mikroorganizmów na gromadzenie się i przemieszczanie DDT roślinach cukinii

Treatment and plant organ analysed	Content of DDT and its metabolites						Total DDT content [mg/kg]
	o,p-DDE	p,p-DDE	o,p-DDD	p,p-DDD	o,p-DDT	p,p-DDT	
	LOD (limit of determination) 0.005 mg/kg						
Control (no inoculum)							
Shoots and leaves	nd	0.010	nd	0.006	nd	0	0.018
Roots	nd	0.062	nd	0.034	nd	0,061	0.168
Micosat F							
Shoots and leaves	nd	0.007	nd	0.008	nd	0.026	0.043
Roots	nd	0.115	nd	0.072	nd	0.145	0.352
Micosat Fito							
Shoots and leaves	nd	0.009	nd	0.009	nd	0.031	0.051
Roots	nd	0.098	nd	0.041	nd	0.047	0.202
EmFarma							
Shoots and leaves	nd	0.005	nd	0.005	nd	0.012	0.024
Roots	nd	0.064	nd	0.041	nd	0.067	0.183
EmFarma Plus							
Shoots and leaves	nd	0.009	nd	0.012	nd	nd	0.023
Roots	nd	0.075	nd	0.043	nd	0.073	0.204

nd - not detected / nie wykryto

Source: own work / Źródło: opracowanie własne

#### 4. Conclusions

DDT residues were detected in approximately 80% of soil samples taken from different sites located in 8 Voivodeships across the country. However, only 25% of the plants grown on polluted soils contained DDT residues, and only in root tissues. Inoculation with microbial consortia favored the uptake of DDT by *Cucurbita pepo* plants, which showed also the capacity to translocate the substances to above-ground organs, thus indicating a possible soil remediation strategy.

#### 5. References

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