

## BOUND RESIDUES OF $^{14}\text{C}$ – 2,4-D IN THE SOIL – THEIR SIGNIFICANCE FOR THE ENVIRONMENT

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**Abstract:** The results of studies showed that residues of 2,4-D bound in the soil are available for wheat plants. It was found that most of radioactive residues detected in the soil were accumulated in fulvic acids fraction soluble in the water. Therefore, this part of residues can be available not only for progressive plants, but also for soil and aquatic organisms. The binding of 2,4-D residues with the soil matter turned out to be a partly reversible process, which progressed under biotic as well as abiotic factors. The soil microorganisms play a beneficial role in the soil environment. Due to their activity the bound residues are released in small amounts into soil solution, and mineralized more than under abiotic factors.

**Key words:** 2,4-D, degradation, soil, bound residues, bioavailability

### INTRODUCTION

The agrochemicals introduced into the environment are dispersed in all its elements, independent on the method of application. Degradability of these compounds in the air, soil and water is the most important factor determining their fates in the environment, and possibility of occurrence of the adverse effects on human organism.

The pesticides that reached the soil are affected by many environmental factors, which caused their gradual disappearance. The reduction of active ingredient concentration in the top layer of the soil can be a result of evaporation to the atmosphere, uptake by plant, leaching into deeper layers of soil profile, chemical and biological decomposition or formation of complexes with the soil matter. The formation of nonextractable (bound) residues in the soil is the key factor that ought to be considered in the evaluation of the impact of pesticides and other toxic chemicals on the environment.

The tendency for binding of the compound is generally determined by its chemical structure, its stability and the presence of active groups. On the other hand, soil properties also are essential. The soil ability to retain of xenobiotics is attributed of

adsorption phenomena and chemical reactions on the active surfaces of mineral particles and humus. Due to binding the mobility of these compounds in the soil system is greatly restricted, therefore they are less likely to contaminate of ground-water. Moreover the bound residues often exhibit reduced toxicity and bioavailability than their free (extractable) forms.

For the environmental risk assessment it is essential to study whether the bound residues are permanently retained or gradually released to the soil solution and therefore become again available for plants and living organisms. The bound residues, as possible threat to the environment, are continuously the subject matter of many studies (Calderbank 1989; Liu et al. 2000; Madhun and Freed 1990).

In respect of chemical nature of bound residues it is not possible to determine their concentration in cultivated soil, using common methods of residue analysis. Any estimations and previsions of the impact to the environment are based on results of laboratory experiments, conducted under controlled conditions, and using compounds labeled with radioisotopes.

In this paper the results of investigations on the fates of 2,4-D residues bound in the soil are presented, in the aspect of their bioavailability for successive plants and living organisms. The studies were carried out in three steps. In the first – the plant uptake of bound residues was tested. In the next two steps the causes of biological uptake were searched. In the second one – the accumulation of residues in water-soluble fractions of soil organic matter was determined. In the last – the release of previously bound residues into soil solution was observed in presence of natural soil microflora or mineral enrichment.

## MATERIALS AND METHODS

In the experiments unlabeled 2,4-dichlorophenoxyacetic acid (“cool”) and  $^{14}\text{C}$  – labeled standard were used. The isotope label was located in the carboxyl group of acetic acid chain. The basic material for the investigations was the soil with high content of organic matter, which was treated with the mixture of “cool” and labeled 2,4-D dissolved in water-methanol solution, at a dose of 2,4-D equivalent to 20 ppm. The physical and chemical properties of the soil were summarized in table 1. The treated soil was adjusted to 40% of maximum water holding capacity (WHC), and stored in incubator for 3 months at 23°C, in the darkness. After incubation the extractable residues of 2,4-D were removed from the soil. The soil was purified using the exhaustive methanol-extraction procedure. The level of bound  $^{14}\text{C}$  – residues in extracted soil was determined by combustion of dry material to  $^{14}\text{CO}_2$ .

The possibility of biological uptake of bound residues from the soil was studied in greenhouse experiment. The test plants were cultivated in the extracted soil enriched either by soil with natural microflora or minerals.

The investigation was performed in plastic flowerpots (5.0–7.5 cm diameter, 7-cm height) filled with a 1:4 mixture of soil with  $^{14}\text{C}$  – residues and natural soil (without any treatment), or with pure silica sand. The 50 seeds of spring wheat (cultivar Helia) were sown into each pot and then transferred to greenhouse for 4 weeks. During the vegetation the plants were watered as needed. Moreover a part of them was amended two times with mineral fertilizer solution, equivalent to

21.6-mg of N; 7.2-mg of P; 10.8-mg of K for each pot. The experiments were carried out in triplicate for each variant.

In control experiments the wheat plants were grown in pots filled with the mixture of methanol extracted soil, not treated with 2,4-D, and clean silica sand or unextracted (natural) soil. During wheat growth, 14 h intervals of light and 10 h of dark were applied. The plants were harvested after 4 weeks of germination. The green parts of wheat were cut close to the soil surface. The roots and seed shells were carefully separated from the soil by shaking and washing in a stream of tap water. After 24-h of drying at 40°C, the plant materials were weighted and shredded using the mill. The total amount of <sup>14</sup>C – residues in dry plants was analyzed by combustion to <sup>14</sup>CO<sub>2</sub> and then counted on liquid scintillation counter (LSC).

The range of accumulation of <sup>14</sup>C – 2,4-D in the main fractions of soil organic matter was studied using the fractionation method according to Helling and Krivonak (1978). The principle of the method was the lixiviation of fulvic and humic acid fractions using 0.5-N NaOH solution and separation of humic acids from the solution by precipitation with 5-N HCl. The radioactivity remained in insoluble in alkali fraction of humin was determined in post-extracted solid, by combustion to <sup>14</sup>CO<sub>2</sub>.

The investigations of release and mineralization of bound 2,4-D residues were carried out in biometer flasks. The soil samples, with bound residues of 2,4-D mixed with clean silica sand or natural soil (control) were distributed in Erlenmeyer unit and adjusted to 40% of WHC. The closed biometer flasks with trap solutions for volatile compounds were placed in incubation chamber. The soil mixtures were incubated for 12 weeks, at 23°C in the darkness. Within this period the amounts of volatile radioactive compounds evolved from the soil were observed. The trap solutions were exchanged at 1-week intervals. After the experiment, the soil was analyzed on extractable and bound <sup>14</sup>C – residues.

The radioactivity of solutions was counted on Beckman Liquid Scintillation Counter model LS 5000TD. The results obtained in units of radioactivity [Dpm] were re-counted into residue units [mg/kg] or explained as percent [%] of total activity present in analyzed material.

## RESULTS AND DISCUSSION

The radioactivity of the soil after exhaustive methanol extraction was equivalent to residues of 2,4-D at the level of 2.67 mg/kg. It means that within 3-months of incubation, the 13.35% of initial amount were bound with soil matter. The label of <sup>14</sup>C placed at the carboxyl group allows consider the radioactive residues as unchanged parent 2,4-dichlorophenoxy acetic acid residues.

In the experiment on biological uptake, the soil with bound residues of 2,4-D was up to 20% of weight of whole substratum placed in one flowerpot. Therefore the concentration of bound residues was established at 0.53 mg/kg. During the plant vegetation no differences were observed between their growth and control plants. It allows to conclude that the presence of bound residues of 2,4-D at investigated level did not cause any adverse effects on the growth of wheat plants. It suggests also the inaccessibility of bound residues for plant roots. However, the results of radiochemical analyse of plant material shows that these residues were uptaken

from the soil by test plants. The  $^{14}\text{C}$ - residues were found in the leaves and plant roots. The most of them were found in roots. The levels of residues determined in dry plant material and final residues in substratum are summarized in table 2.

In plants grown on substratum with mineral admixture, watered only during vegetation (variant III), the radioactivity found in leaves was equivalent to residues at the level of 0.104 mg/kg and 1.279 mg/kg in roots. In particular parts of plants cultivated on the same substratum, but with mineral fertilization, the residues were 0.157 and 0.605 mg/kg, in leaves and roots respectively (variant I). Somewhat different results obtained in the case of uptake by plants grown on substratum contained the soil with natural microflora as the admixture (variants II and IV). In these cases generally lower residues were determined, than in previous variants with silica sand. In plants watered only during vegetation (variant IV) the residues of 0.112 mg/kg in leaves and 1.167 mg/kg in roots were found. The radioactivity in leaves of plant fed with mineral fertilizer (variant II) was similar to variant IV, but in roots was approximately a half lower, and corresponded to 0.54 mg/kg of residues. To take into consideration the whole mass of plants grown in each flower pot (data not shown), the plant uptake of bound residues of 2,4-D from the soil turned out to be small. The uptake by plants fed with mineral fertilizer ranged from 0.53% to 0.55% of bound  $^{14}\text{C}$  and 0.74%–0.77% by plants watered only. The results showed that fertilization and the natural soil microflora are two factors, which limited the uptake of bound residues from the soil by plants.

In the next step of studies a search focused on the question about the reason of availability of bound residues for plants. For this purpose, the soil contained only bound residues of 2,4-D was extracted with 0.5-N NaOH, which removed extractable humic material together with humus-bound radioactivity from humin, insoluble fraction in alkali. It has been stated that about of 5% of radioactivity remained unextracted in humin. After acidification with HCl from the solution was precipitated the amorphous sediment of humic acid, in which 3% of the bound radioactivity was found. The most of bound  $^{14}\text{C}$  – residues, approximately 92%, were lo-

Table 1. Physiochemical properties of the soil

Characteristic	
pH	6.70
organic matter (%)	3.74
sand (%)	63.00
silt (%)	21.00
clay (%)	16.00
WHC <sup>a</sup> (g/100g)	34.00
CEC <sup>b</sup> (meq/100g)	14.75

<sup>a</sup>water holding capacity, <sup>b</sup>cation exchange capacity

Table 2. Uptake of  $^{14}\text{C}$  – 2,4-D residues bound in the soil by wheat plants

Variant	Admixture	Fertilization	Residues in plant [mg/kg]*		Final residues in substratum [mg/kg]
			leaves	roots	
I	silica sand	+	0.157	0.605	0.060
II	natural soil	+	0.115	0.541	0.082
III	silica sand	-	0.104	1.279	0.036
IV	natural soil	-	0.112	1.167	0.040

\* residues in dry plant material

calized in unprecipitated fulvic acids. The presence of 2,4-D in fulvic acids fraction can explain their availability for plant roots, which was observed in plant uptake experiment. The high accumulation of residues in fulvic acids showed that these residues can contaminate of the successive plants. The solubility of fulvic acids in water permits to consider this part of residues as potentially accessed not only for plants, but also for soil and water fauna (Lewandowska and Weymann 2002).

The formation of bound residues phenomena was already observed in the investigations with other pesticides belonging to different chemical groups, and with different biological activity. The previous studies proved, as mentioned above, that mobility and toxicity of bound pesticide residues for living organisms and plants usually are lower than the free ones. In the estimation of significance of bound residues for the environment and probability of negative changes occurrence, in the long time after the pesticide treatment, where it is possible, very important is to recognise of the mechanism of their bioavailability. From this reason the essential is to investigate the stability of complexes formed with soil matrix. In practice these studies lead to tests of release of bound residues to soil solution as again “free” form and determination of the range of their mineralization.

The results of soil incubation experience showed that binding of 2,4-D residues with soil matter is partly reversible process (Tab. 3). During incubation in solutions absorbing the volatile products, evolved from the contaminated soil, the radioactive compounds were found. In variant with silica sand admixture and the soil with active microflora, the release of  $^{14}\text{C}$  – residues took place. In both cases, either with silica sand or natural soil, a part of radioactivity of trap-solutions originated from  $^{14}\text{CO}_2$ . It has been stated that in the presence of soil microflora the mineralization was about 30% greater, than under abiotic interactions, which dominated in the variant with silica sand. From the soil incubated with silica sand evolved together about 18.7% of previously bound radioactivity, and about of 12.0% were found as  $^{14}\text{CO}_2$ . In variant with natural soil, in trap-solutions determined of 22.5% of volatile products, but 18.5% of them were from  $^{14}\text{CO}_2$ .

Table 3. The results of incubation test of the soil contaminated with bound 2,4-D residues

Before incubation	After 12 weeks of incubation					Recovery [%]
	volatile compounds [%]			residues [%]		
	$\text{CO}_2$	other	total	extractable	bound	
100% of bound 2,4-D						
+ silica sand	12.07	6.66	18.73	24.53	30.05	73.31
+ natural soil	18.54	3.93	22.47	10.30	45.22	67.50

The analyse of the soil after 12 weeks of incubation indicated that also the percent of residues remained in bound form, in the presence of microflora was greater, than with silica sand admixture. Inversely presented the levels of  $^{14}\text{C}$  – extractable residues. In the variant with silica sand the higher concentration of extractable  $^{14}\text{C}$  – residues was found (at the range of 24.5%), while in the presence of microorganisms the amount of residues was a half lower (10.3%). It seems that the bound residues released into free form are immediately mineralized to  $\text{CO}_2$  and partly bound with soil particles again.

## CONCLUSIONS

The results of experiments showed that bound residues of 2,4-D could be available for plant roots. The presence of bound residues of 2,4-D in the soil, at investigated level did not cause any adverse effects on the growth of test plants. The reason of observed plant uptake may be their high accumulation in fulvic acids fraction of soil organic matter, which are water-soluble. Also the release of previously bound residues into soil solution, made them accessible for plant roots. Thereby these residues can appear as a source of progressive plants contamination, resulted from repeated herbicide treatments. The data presented above confirmed the important role of microorganisms in soil remediation process. The natural microflora is an essential factor removing of 2,4-D residues from the soil environment. Under its influence the mineralization process advanced with higher effectiveness, than under abiotic factors. Also the higher level of bound remainder showed its participation either in controlled release or secondary binding of released 2,4-D. Therefore soil microflora protected the environment against the impact of immobilized xenobiotics.

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## POLISH SUMMARY

### ZWIĄZANE POZOSTAŁOŚCI $^{14}\text{C}$ – 2,4-D W GLEBIE – ICH ZNACZENIE DLA ŚRODOWISKA

Wyniki badań wskazują, że związane pozostałości 2,4-D są dostępne dla roślin pszenicy. Większość radioaktywnych pozostałości wykrytych w glebie było zakumulowanych we frakcji kwasów fulwowych, które są rozpuszczalne w wodzie. Dlatego ta część pozostałości może być potencjalnie dostępna nie tylko dla roślin następczych, ale również dla organizmów glebowych i wodnych. Wiązanie pozostałości 2,4-D z materią gleby okazało się procesem częściowo odwracalnym, który postępuje pod wpływem czynników biotycznych i abiotycznych. Mikroorganizmy glebowe spełniają dobroczynną rolę w środowisku glebowym. W wyniku ich aktywności związane pozostałości uwalniane są do roztworu glebowego w niewielkich ilościach oraz mineralizowane w większym stopniu, niż pod wpływem czynników abiotycznych.