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The influence of MCPA on soil ecotoxicity and the presence of genes involved in its biodegradation

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Abstract: The aim of this study was to evaluate the changes in ecotoxicity of agricultural soil under the influence of Agritox 500SL, an herbicide from the phenoxyacid group, containing MCPA (2-methyl-4-chlorophenoxyacetic acid), applied every 3 weeks for 3 months. Biodegradation potential in control and weathered soil was confirmed by the analysis of the functional gene *tfdA* using PCR technique. Properties of the soil were assessed by the analysis of granulometric composition, pH, the content of macroelements and heavy metals. The soil ecotoxicity was measured using biotests *Phytotoxkit*® and *Microtox*®.

The content of Ni (70 mg/kg) and Cr (21 mg/kg) was especially high in the soil with Agritox 500SL. The highest toxic effect for test organisms was observed in freshly spiked soil: 99% *L. sativum*, 97% *S. alba*, 66% *S. saccharatum* (% root growth inhibition) and 76% *V. fischeri* (% luminescence inhibition). Weathering processes significantly decreased the soil ecotoxicity being 36%, 34% and 3% for *V. fischerii*, *S. alba* and *L. sativum*, respectively. *S. saccharatum* showed 12% stimulation of the root length. Molecular analysis confirmed the potential of indigenous soil bacteria to biodegrade MCPA by the presence of *tfdAα* and *tfdA Class III* genes in the studied soil. The obtained results proved that either MCPA and residues of its decomposition or additional supporting substances in Agritox 500 SL, can influence enhanced soil ecototoxicity. The presence of functional *tfdA* genes in both: control and weathered soil, confirmed the high potential of indigenous soil bacteria to degrade MCPA.

Introduction

Phenoxy herbicides MCPA (2-methyl-4-chlorophenoxyacetic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid), according to Herbicide Resistance Action Committee classification belonging to growth regulators-synthetic auxins (Herbicide Resistance Action Commitee 2018), have been widely used in agriculture to control dicotyledonous weeds (Smith et al. 1994). MCPA is a selective agent usually used to protect wheat, which is one of the most extensively cultivated crops in Europe (Eurostat 2016) and Poland (GUS 2016), before weed infestation.

MCPA as a growth inhibitor, is characterized by structural similarity to phytohormones – natural auxins, which regulate elongation growth of plants. Its mode of action mimics indole-3-acetic acid (Skiba et al. 2017). Due to relatively short persistence of MCPA in soil ($t_{1/2}$=1–4 months) it was not considered as a serious environmental pollutant (Mcghee and Burns 1995). However, its persistence in soil is 3–4 times higher under acidic pH and low temperature (Mcghee and Burns 1995, Thompson et al. 1984) – frequently prevailing conditions in Poland (Siebielec et al. 2017). This has led to a long-term threat to soil ecosystem, reflected in increased soil toxicity, inhibition of plant development and damage of structure and functions of soil microbiota. Moreover, MCPA salts (e.g. MCPA-DMA, MCPA-Na) are characterized by high water solubility (866 mg/l) and low retention potential in soils (K_d= >0.21–2.7 l/kg) (López-Piñeiro et al. 2013, Nufarm 2012) which can lead to surface- and groundwater contamination, thus creating a risk to aquatic organisms.

The way of coping with soil MCPA contamination is the use of physical and chemical, as well as photochemical degradation processes. Such processes depend on several factors, such as soil organic matter content, which influences the distribution of sorbed and dissolved remains of herbicide degradation in a sorptive complex (Greert and Shelton 1992,

Poll et al. 2010), soil pH and moisture, temperature, light intensity as well as initial MCPA concentration.

Indigenous soil microbiotas are also capable of MCPA remains degradation. Consequently their activity accelerates the transformation of MCPA remains into inorganic metabolites. The first step in the MCPA biodegradation pathway is initiated by α-ketoglutarate-dependent dioxygenase encoded by *tfdA* or *tfdA-like* genes (Batogliu-Pazarbasi et al. 2013, Mcgowan et al. 1998), such as *tfdAα* detected in α-*Proteobacteria* (Batogliu--Pazarbasi et al. 2013, Itoh et al. 2004) and *tfdA Class I, II* and *III* belonging to *γ*- and *β*-*Proteobacteria* (Mcgowan et al. 1998, Poll et al. 2010). The oxidative degradation of MCPA mediated by bacteria harbouring *tfdA*-like genes reduces the remains of herbicide content in soil and mitigates its toxic effects.

The main aim of this study was to perform preliminary experiments evaluating the potential of indigenous soil bacteria to degrade MCPA under laboratory conditions. This was achieved by the investigation whether *tfdA*-like genes (*tfdA, tfdAα* and *tfdA Class I, II* and *III*) were present in agricultural soil previously spiked with MCPA. Furthermore, the effect of Agritox 500SL application on soil ecotoxicity and its further mitigation through degradation processes were measured using two commercial biotests (*Phytotoxkit*® and *Microtox*®) and four test organisms (*Lepidium sativum, Sinapis alba, Sorghum saccharatum* and *Vibrio fischeri*). The physicochemical analyses were conducted to provide further background information on the status of the used soil.

Materials and methods

Experimental conditions

The soil was collected from a farm field in Wólka Wojsławska, Zduńska Wola district, Łódź voivodeship, Central Poland (51°38'45.169"N, 18°56'14.582"E). The studied soil was under shifting cultivation, temporarily abandoned and allowed to revert to its natural vegetation. After collection, the soil was transported to the laboratory and divided into two parts: one used as control soil with no amendment, and the second spiked with commercial herbicide Agritox 500SL (Nufarm 2012) in the dose of 1.5 l/ha, containing MCPA salts (MCPA-DMA 26% and MCPA-Na 10.1%) (500 g/l) as active ingredients.

The control soil was sampled immediately after the collection of soil from a field, before any application of herbicide.

Agritox 500SL was applied every 3 weeks for 3 months in order to establish the bacteria community capable to use MCPA as the source of carbon. The soil was incubated in the dark in stable temperature of 22°C +/– 1°C (Celis et al. 2008) and watered periodically with deionized water.

The soil amended with Agritox 500SL (whether the herbicide was applied or active substance) was sampled twice during the incubation: after 3 months, in the day of last herbicide application and 3 weeks after, in order to assess the impact of fresh and weathered herbicide on physical and chemical properties of soil, the presence of genes involved in MCPA degradation and changes in soil ecotoxicity.

Soil physical and chemical properties

In air-dry sample of the soil, granulometric composition with use of the Casagrande method in Prószyński modification was determined (Siebielec et al. 2017). The content of organic carbon, nitrogen and sulphur were determined using the Vario Max Cube analyser, while the content of the remaining elements was determined using an inductively coupled plasma optical emission spectrophotometer (ICP-OES) Optima 7300 DV model by Perkin-Elmer. The total concentration of the macroelements Na, Mg, Ca, K, P, Na and heavy metals Fe, Mn, Ni, Cr, Zn, Pb, Cd in the soil was assessed after hot digestion in a mixture of HNO_3 and $HClO_3$ (3:2 v/v) acids (suprapure, MERCK). Bioavailable forms of phosphorus and potassium were analysed due to Egner-Reihm's method (Urbaniak et al. 2017). The soil reaction was determined potentiometrically in a suspension of water and 1 mol/dm^3 KCl. However, 1 mol/dm^3 KCl simulates the soil solution better than water (Goulding 2016). During the chemical analyses, each sample of the soil was analysed in three repetitions. Statistical calculations included average and standard deviation. The differences between averages were evaluated by Mann-Whitney U test at significance level of 0.05. The statistical analysis was performed using the *Statistica 12* software.

Soil ecotoxicity

The *Phytotoxkit*® (ISO 18763:2016, Phytotoxkit 2004) and *Microtox*® (Microbics Corporation 1992) tests were used to assess ecotoxicity for two variants: soil with weathered herbicide (3 weeks after last herbicide application) and soil freshly amended with herbicide agent. As reference, ecotoxicity of control soil was measured.

In the *Phytotoxkit*® three test plant species were the subject of the research: monocotyledonous *Sorghum saccharatum* and dicotyledonous plants *Lepidium sativum,* as well as *Sinapis alba*. The test was conducted in accordance with the procedure recommended by the manufacturer (MicroBioTests Inc. n.d.). Soil samples under study (90 cm^3) were placed on test plates (21/15.5/0.8 cm). 14 seeds of each test plant were placed on the top of analyzed soil covered with filter paper. The plates were incubated vertically in the dark at 25°C for 72 h. A photo of the test plate was then taken with a digital camera and subsequently root lengths were measured using *ImageJ* software. The measured parameter was the root length inhibition (RI). The percentage effect (PE %) of root growth inhibition of the studied plants was calculated according to the following formula:

$$PE = \frac{A - B}{A} \times 100$$

Equation 1. PE – % Root growth inhibition, A – plant root length in control soil; B – plant root length in studied soil

In the *Microtox*® test toxicity of soil samples was tested on *Vibrio fischeri* bacteria using an M 500 Analyzer. Elutriates from the soils were prepared by mixing one volume of the soil with four volumes of redistilled water and shaking mechanically for 24 hours (Baran and Tarnawski 2013). After that time, the samples were centrifuged for 10 minutes at a speed of 3000 rpm and filtered. The 81.9% Screening Test was carried out. Luminescence was measured before and after incubation of bacterial suspension with the analyzed

sample. The 15 min luminescence inhibition with *V. fischeri* was performed according to Microbics Corporation (1992) (Microbics Corporation 1992). Ecotoxicity results were expressed as Percent Effect (PE %) according to the formula:

$$PE = \frac{A - B}{A} \times 100$$

Equation 2. PE – % luminescence inhibition, A – luminescence in control soil; B – luminescence in studied soil

Molecular analysis

Soil bacteria DNA extraction was performed using a *GeneMATRIX Soil DNA Purification Kit EurX*®, following the manufacturer's instructions. PCR (Polymerase Chain Reaction) conditions were applied according to Bælum et al. (2006) and Bælum et al. (2010) with minor modifications of annealing temperatures. The 20 µl reaction mixture contained: sterile H_2O, 1× buffer, 3.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM primers, 0.1 mg/ml BSA, 1–2.5 U/µl Taq polymerase and 15–30 ng of template DNA.

Five sets of *tfdA* genes primers (Table 1) were used for amplification of given bacterial DNA fragments. The conditions for the PCR were as follows: 10 min at 95°C for enzyme activation; 30 cycles of 10s at 94°C for denaturation, 30 s at optimal annealing temperature (Table 1), and 1min at 72°C for elongation. A final extension step was preformed for 10 min at 72°C. PCR products were checked by introducing 1.5% agarose gel electrophoresis and stained with ethidium bromide. The following molecular ladders (DNA-Gdańsk) were used: M300-1000 bp for a band of length 856 bp (*tfdA* – class III) and M100-500 bp for a band of length 350 bp (*tfdAα*).

Results and discussion

Herbicides are important tools in agriculture that help to mitigate the economic losses caused by weeds. Although their application does improve crop yields and its value, herbicides are also recognized as a source of potential adverse effects observed at environmental (plants, soil and surface- and groundwater) and human health level.

In the view of the above, there is a need for a holistic approach to the problem of environmental contamination with remains of herbicide degradation, including those containing MCPA, and their effect on physical and chemical properties of the soil, as well as soil ecotoxicity, but also the influence of this herbicide on the potential of its degradation using indigenous soil bacteria.

Impact of MCPA on soil physicochemical properties

Comparing the percentage content of individual fractions, the dominance of sand (80%) fraction in the soil was determined. Silt and clay fractions accounted for 11% and 10% respectively. The soil had acid reaction. Our results (Table 2) indicate that the application of herbicide led to a slight increase in soil pH; pH of Agritox 500SL amended soil ranged between 4.56 (measured in KCl) and 5.92 (measured in H_2O), while pH of control soil amounted from 4.31 and 5.58 (in KCl and H_2O, respectively). The obtained values are within the range of mean soil pH values across Europe (between 4.5 and 5.5). Acidic pH can potentially increase MCPA sorption processes and therefore enhance its persistence in soil. According to Spadotto and Hornsby (2003), the sorption processes of herbicides such as MCPA in soil at low pH are stronger than in soil at higher pH.

The content of organic carbon and total content of macronutrients in soils are shown in Table 2. In the analysed soil samples, relatively low organic carbon contents were determined. Moreover, the study showed no significant differences between the above mentioned elements content in the soils depending on MCPA amended. High concentrations of bioavailable phosphorus and low concentration of bioavailable potassium were registered in the tested soils (Table 2). Compared to control soil, there were statistically significant differences in Ni and Cr concentration in samples spiked with MCPA (69.65±1.64 mg/kg vs. 4.07±0.13 mg/kg, 21.27±1.96 mg/kg vs. 10.18±2.95 mg/kg for amended and control soil, respectively) (Table 2). In the studies also an increased Cd content was found in the soil after the application of MCPA. Currently, producers are not obliged to specify chemical composition of additives for herbicide commercial products. High concentration of the mentioned heavy metals could have been derived from the content of MCPA agent, used for the experiment.

Table 1. Primer sequences used for amplification of the target *tfdA* and *tfdA-like* genes

Target gene	Primer (5'–3')	Fragment size (bp)	Optimal annealing temperature [°C]	Literature
tfdA	F:GAGCACTACGCRCTGAAYTCCCG R:CTTCGGCCACCGGAAGGCCT	210	64	Bælum et al. (2006)
tfdAα	F:CSGAGTTCKSCGACATGCG R:GCGGTTGTCCCACATCAC	350	66	Bælum et al. (2006)
tfdA Class I	F:GTGAGCGTCGTCGCAAAT R:GCATCGTCCAGGGTGGTC	856	56	Bælum et al. (2010)
tfdA Class II	F:TGAGCATCAATTCCGAATACC R:AAGACTGACCCCGTGGACT	882	53	Bælum et al. (2010)
tfdA Class III	F:TGAGCATCACTTCCGAATACC R:ACAGCGTCGTCCAACGTC	856	56	Bælum et al. (2010)

Impact of MCPA on soil ecotoxicity

One of the most apparent effects of the application of phenoxy acid herbicides, such as MCPA, is the increase in ecotoxicity to living organisms of amended soil, ground- and freshwater. Pereira et al. (2000) demonstrated negative effects of MCPA application on freshwater organisms such as the crustaceans *Daphnia magna*, *Thamnocephalus platyurus* and *Artemia franciscana*, and algae *Selenastrum capricornutum.* Podolska (2014), in turn, proved that MCPA caused the increase in soil ecotoxicity to buckwheat (*Fagopyrum esculentum* var. *Kora*), which resulted in stem deformation and discoloration of leaves. Also in our experiments, increased ecotoxicity as an effect of fresh amendment of soil with Agritox 500SL was observed. However, the results demonstrated that the used test plants (dicotyledonous *S. alba* and *L. sativum* and monocotyledonous *S. saccharatum*) showed diverse reactions to herbicide. Fresh application of Agritox 500SL significantly increased the ecotoxicity level (measured as % inhibition of roots growth, PE %), reaching 99% for *L. sativum* and 97% for *S. alba*; while *S. saccharatum* revealed the greatest tolerance to soil spiked with herbicide showing 66% inhibition of roots length. A similar reaction was observed for the *V. fischeri* – the luminescence inhibition of bacteria was 76% (Fig. 1). Three weeks of incubation process were found to have a positive effect on soil ecotoxicity reduction (Fig. 1): PE % dropped to 3%, 34% for *L. sativum* and *S. alba,* respectively; whereas *S. saccharatum* demonstrated 12% increase in root growth in comparison to non-spiked control soil. We observed also a decreased inhibition of luminescence of *V. fischeri* to 36% (Fig. 1).

The obtained differences in the test organisms' response against freshly spiked and aged soil could be related to their sensitivity to soil contamination. High sensitivity of dicotyledonous species esp. *S. alba*, was confirmed in other studies (Urbaniak et al. 2016). Since MCPA is a selective herbicide, used especially against dicotyledonous plant species, high tolerance exhibited by *S. saccharatum* belonging to monocotyledonous can be explained. On the other hand, lower tolerance of *S. alba* might result from higher metal content in samples amended with MCPA. In previous experiments it was shown that *S. alba* is not sensitive to organic contaminants but mostly to heavy metals (Baran and Tarnawski 2013, Steliga et al. 2012, Urbaniak et al. 2016). Therefore, high content of sand, low content of organic carbon and high concentrations of Ni and Cr present in the soil spiked with Agritox 500SL (Table 2) can explain the negative impact of these metals on the *S. alba* root growth and bacteria luminescence. Furthermore, phenoxy herbicides can form complexes with such heavy metals as Zn and Cu, increasing metals bioavailability and detectability in soil (Kobyłecka et al. 2009). Thus, the application of MCPA enhances heavy metal uptake by organisms, which could possibly result in a negative effect on their physiology (Skiba and Wolf 2017). Despite negative impact of the herbicide

Table 2. Mean and standard deviation for physical and chemical properties of the untreated soil (control soil) and soil amended with Agritox 500 SL at the dose of 1.5 l/ha (MCPA 500g/l).

Properties	Unit	Control soil	Soil amended with MCPA at the dose of 1,5 l/ha
pH (H_2O)		5.58	5.92
pH (KCl)		4.31	4.56
Macroelements			
N	%	0.10±0,00	0.09±0.01
C	%	0.98±0,02	1.00±0.07
S	%	0.02±0.00	0.015±0.00
P	g/kg	0.62±0.02	0.58±0.00
P_2O_5 (assimilable) phosphorus)	mg/kg	1515±5.00	166.00±4.95
K	g/kg	1.01±0.17	1.05±0.21
K_2O (assimilable) potassium)	mg/kg	345±1.60	38.40±1.98
Na	g/kg	0.11±0.00	0.12±0.02
Mg	g/kg	0.69±0.02	0.64±0.00
Ca	g/kg	0.77±0.06	0.77±0.02
Heavy metals			
Fe	g/kg	4.13±0.1	3.93±0.19
Cr	mg/kg	*10.18±2.95	*21.27±1.96
Mn	mg/kg	208.51±9.92	200.04±7.27
Ni	mg/kg	*4.07±0.13	*69.65±1.64
Cu	mg/kg	12.78±0.37	14.43±1.64
Zn	mg/kg	41.00±3.20	44.00±3.20
Cd	mg/kg	1.23±0.11	2.41±0.97
Pb	mg/kg	13.53±1.99	12.75±0.82

* **significant differences** at $\alpha \leq 0.05$ according to the Mann-Whitney U-test

compound itself, a range of organic solvents and surfactant additives present in a commercial MCPA product is able to generate additional negative effects. This was confirmed by Pereira et al. (2000), who compared MCPA toxicity in the form of the active compound to a commercial product using microbiotests: *Daphtoxkit magna* F^{TM}, *Thamnotoxkit* F^{TM}, *Artoxkit* M^{TM}, and *Algaltoxkit* F^{TM}. The obtained results showed that ecotoxicity is highly variable among different species; however, in general, the commercial product caused higher toxic effects.

Potential use of indigenous soil bacteria for MCPA removal

Indigenous soil bacteria are responsible for many ecosystem services such as nutrient transformation, litter degradation, promotion of plant growth and degradation of contaminants including herbicides such as MCPA.

Previous research on MCPA degradation pathways has identified set gene sequences involved in complete mineralization of this compound. The first step towards MCPA mineralization is decomposition of the ether bond resulting in a phenolic compound and acetic or propanoic acid (Xia et al. 2017). This step is catalyzed by an α-ketoglutarate-dependent dioxygenase encoded by *tfdA* and *tfdA*-like genes that are recognized as biomarkers for the growth of MCPA degraders in environmental soil samples.

In our case, molecular analysis showed that among 5 different targets *tfdA* genes (*tfdA, tfdAα, tfdA Class I, Class II* and *Class III*) only *tfdAα* and *tfdA Class III* were detected in both types of samples: control samples and in soil amended with Agritox 500SL (Table 3; Fig. 2). The *tfdA Class I* and *Class II* gene amplification products have not been obtained, even though modified PCR conditions were tested. Detected genes indicated the presence of microorganisms capable to degrade MCPA in the agricultural soil under study. These findings confirm observation made by Bælum et al. (2006), who showed that bacteria harboring *tfdA Class III* genes were able to proliferate during degradation of MCPA.

Interestingly, higher PCR product yields for both genes *tfdA Class III* and *tfdAα* were detected in control soil in comparison to the MCPA spiked soil samples. Similar situation was observed by Poll et al. (2010), who compared *tfdA* and *tfdAα* abundance in control and MCPA amended samples. This indicates that the soil was probably enriched with natural substrate for the growth of bacteria harboring *tfdAα* gene, not necessarily phenoxy acids (Itoh et al. 2004). On the other hand, the presence of *tfdAα* gene in control soil, which was not treated with MCPA in laboratory conditions, could indicate that this soil had been exposed to phenoxy herbicides for a longer time prior to collection. Consequently, it had already possessed desired biodegradation potential reflected in the existence of *tfdAα* gene; whereas the soil amended with MCPA under laboratory conditions could

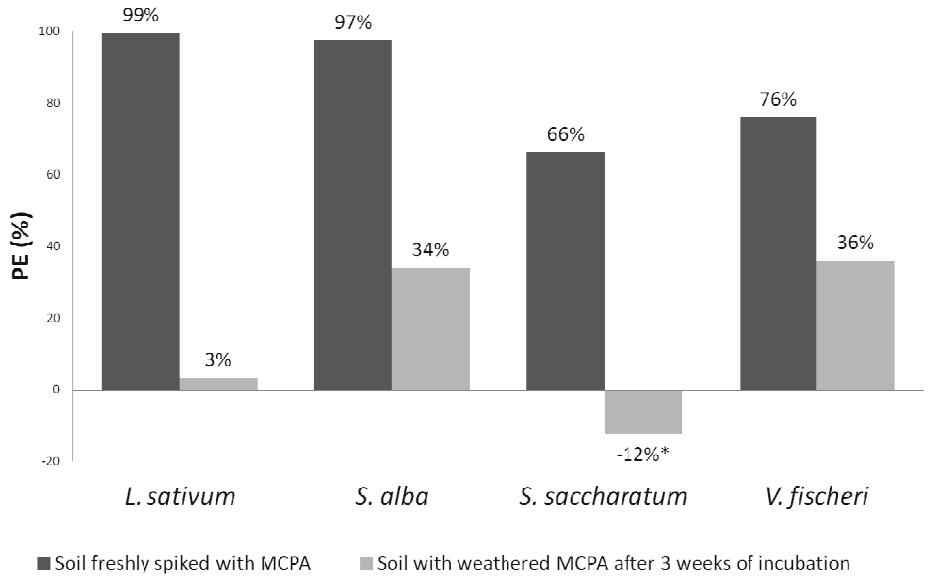

Fig. 1. Mean effect of commercial product Agritox 500SL containing MCPA on PE % (% root growth inhibition and % luminescence inhibition) in freshly amended soil and soil after 3 weeks of incubation measured using: dicotyledonous: *Lepidium sativum, Sinapsis alba*, monocotyledonous *Sorghum saccharatum* and bacteria *Vibrio fischeri*

Table 3. PCR results for target genes

Target gene	Control soil	Soil with MCPA
tfdA	No PCR product	No PCR product
tfdAα	One band on agarose gel (approx. 350 bp)	One band on agarose gel (approx. 350 bp)
tfdA Class I	No PCR product	No PCR product
tfdA Class II	No PCR product	No PCR product
tfdA Class III	One band on agarose gel (approx. 856) bp)	One band on agarose gel (approx. 856) bp)

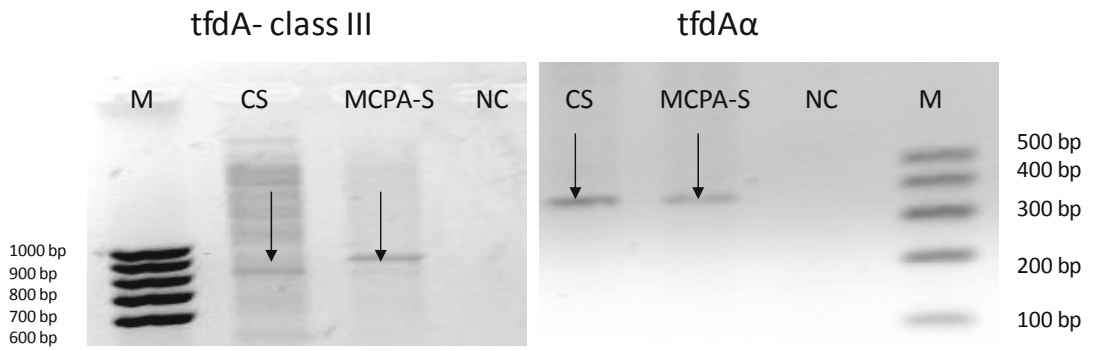

Fig. 2. The electrophoretic separation of the products of amplification of *tfdA-Class III* and *tfdA*α gene fragment in DNA samples (M: DNA marker *600–1000 bp* and *tfdA*α- 100-500 bp; CS: control soil not spiked with MCPA; MCPA-S: soil spiked with MCPA; NC: negative control with no DNA)

have had a toxic effect on microbial communities due to higher concentrations of heavy metals.

Conclusions

The results of this study showed that Agritox 500SL application led to the increase in soil ecotoxicity. The highest root growth inhibition was observed in soil freshly spiked with herbicide for *L. sativum* and *S. alba*. MCPA controls the growth of dicotyledonous weeds, thus the phytotoxicity was lower for monocotyledonous *S. saccharatum*. The values of root growth inhibition declined within the experiment for *S. alba* and *L. sativum*, while *S. saccharatum* demonstrated promotion of root length. In the present study both *Phytotoxkit*® (*S. alba, L. sativum*) and *Microtox*® (*V. fischeri*) test showed similar pattern of sensitivity to the used herbicide product. The highest luminescence inhibition of *V. fischeri* was observed in soil freshly spiked with MCPA agent. The decrease of toxic effect for bacteria was observed after 3 weeks. The increased concentration of heavy metals in the soil spiked with herbicide, especially Ni and Cr, could also enhance the high soil ecotoxicity. Thus, the increased ecotoxicity of the studied samples might have been caused either by remains of MCPA and its metabolites in soil or additional substances in Agritox 500SL.

In terms of molecular analysis, the obtained results demonstrated high potential of indigenous bacteria to degrade MCPA confirmed by the presence of the functional *tfdAα* and *tfdA Class III* genes. The research results showed that the biodegradation potential of soil is determined by the application of the herbicides agent in soil. Degradation processes of herbicide remains in soil influence the reduction in soil ecotoxicity. Further studies on MCPA biodegradation *in situ* can deepen the understanding of the processes accompanying microbial activity in the environment.

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Ocena wpływu MCPA na fitotoksyczność gleby oraz obecność genów biorących udział w procesach jego biodegradacji

Streszczenie: Celem przeprowadzonych badań była ocena zmian ekotoksyczności gleby rolniczej zanieczyszczonej środkiem ochrony roślin Agritox 500SL, zawierającym herbicyd z grupy fenoksykwasów MCPA (kwas 2-metylo--4-chlorofenoksyoctowy). Herbicyd był dodawano do gleby co 3 tygodnie przez okres 3 miesięcy. Potencjał biodegradacyjny gleby niezanieczyszczonej oraz gleby z dodatkiem MCPA, sprawdzono badając obecność genów funkcyjnych *tfdA* z wykorzystaniem techniki PCR. Dodatkowo zbadano właściwości gleby takie jak: skład granulometryczny, pH oraz zawartość makroelementów i metali ciężkich. Ekotoksyczność gleby oznaczono przy użyciu testów toksyczności *Phytotoxkit*® i *Microtox*®.

Stężenie Ni (70 mg/kg) i Cr (21 mg/kg) było szczególnie wysokie w glebie z dodatkiem MCPA. Najwyższą ekotoksyczność zaobserwowano w glebie świeżo zanieczyszczonej herbicydem, gdzie wynosiła ona: 99% dla *L. sativum*, 97% dla *S. alba*, 66% dla *S. saccharatum* (% inhibicja wzrostu korzeni) i 76% dla *V. fischeri* (% inhibicja luminescencji). Zachodzące w czasie inkubacji przemiany herbicydu przyczyniły się do zmniejszenia ekotoksyczności gleby do 36%, 34% i 3% odpowiednio dla *V. fischerii*, *S. alba* i *L. sativum;* natomiast w przypadku *S. saccharatum* zaobserwowano 12% stymulację wzrostu korzeni. Analizy molekularne tj. detekcja fragmentów genów *tfdAα* i *tfdA klasy III*, potwierdziły potencjał bakterii obecnych w glebie rolniczej do degradacji MCPA. Uzyskane wyniki potwierdzają że MCPA oraz produkty jego dekompozycji jak również substancje dodawane do komercyjnych środków ochrony roślin mogą przyczynić się do wzrostu ekotoksyczności gleby. Obecność genów funkcyjnych *tfdA* zarówno w glebie niezanieczyszczonej jak i zanieczyszczonej herbicydem, wskazuje na potencjał degradacyjny bakterii glebowych pod kątem usuwania z gleby herbicydów z grupy fenoksykwasów takich jak MCPA.