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ORIGINAL ARTICLE

Responses of microorganisms and enzymes to soil contamination with metazachlor

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Abstract A greenhouse pot experiment was conducted at the University of Warmia and Mazury in Olsztyn, with the aim of describing the influence of metazachlor on counts and biodiversity of soil microorganisms, soil enzymatic activity, physicochemical properties of soil and yield of spring oilseed rape. The first experimental factor was soil contamination with increasing rates of metazachlor: 0 (soil without the herbicide), 0.333 (recommended by the manufacturer), 6.666, 13.332, 26.665, 53.328, 106.656 and 213.312 mg kg^{-1} dm of soil, while the second factor comprised two dates of determination: 30 and 60 days after starting the experiment. The tested herbicide had an adverse effect on reproduction of all analyzed microorganisms: oligotrophic bacteria and their endospore forms, *Azotobacter* spp. bacteria, organotrophic bacteria, actinomycetes and fungi. The values of the colony development and eco-physiological diversity indices decreased under the influence of excessive doses of the herbicide. Also, significant fluctuation in the enzymatic activity of soil was observed in response to the higher doses of metazachlor and depending on the date of determinations. The herbicide had an invariably negative influence on the activity of soil enzymes, causing the inhibition of dehydrogenases, catalase, urease, acid phosphatase, alkaline phosphatase, arylsulfatase and β-glucosidase. The physicochemical status of soil depended significantly on the degree of soil contamination with the herbicide, same as the yield of spring oilseed rape.

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Introduction

Herbicides are broadly used in agriculture for the sake of improving yields of crop plantations and quality of crops (Arias-Estéves et al. 2008). However, the fate of herbicides in the environment is a serious problem because only a very small percentage of these substances reach target organisms, while the remaining amounts can affect humans, animals and plants. Although herbicides are very useful in farming, under certain circumstances they may turn into pollutants, deteriorating the quality of nature, including soils (Anwar et al. 2009; Baćmaga et al. 2014). Soil is one of the main regulators of the mobility of herbicides. The chemical and biochemical processes which occur in soil largely determine the transfer of these chemicals. Persistence of plant protection preparations differs relative to their chemical structure. Herbicides are composed of one up to a few biologically active substances, which makes their rate of decomposition dependent on several physical, chemical and biological factors (Arias-Estéves et al. 2008). Among the factors that distort the homeostasis of soil are herbicides, which may disrupt the proper functioning of a soil ecosystem and become hazardous to soil microorganisms. The toxic influence of herbicides on microorganisms can be manifested through some changes in the biochemical and physiological characteristics. Most herbicides are a source of carbon and nutrients for microorganisms, which is why they can be completely decomposed by single species or whole consortia of microbes down to non-toxic compounds transposons (Mukherjee et al. 2006).

Metazachlor (2′,6′-dimethyl-N-(pyrazol-1-ylomethyl)-chloroacetanilide) is an active substance added in an amount of 500 g dm^{-3} to the herbicide Fuego 500 SC, made by Feinchemie Schwebda GmbH. Metazachlor is a compound of the chloroacetanilide chemical family, which is most often used to control weeds in fields of potato, oilseed rape, soybean and tobacco. It acts as an inhibitor of plant enzymes, entering into plants through roots and the hypocotyl. This preparation affects the cell division process in plant seedlings, inhibiting the biosynthesis of proteins, fatty acids and lignin. In soil, metazachlor typically undergoes quick degeneration, and its half-life is from 19 to 82 days. The expected concentration of metazachlor in soil ($PEC_{soila20d}$) is 0.069 mg kg^{-1}. This substance was first produced by BASF in 1976, in the form of aqueous suspension. It is an active substance in many herbicides, e.g., Alpha Metazachlor 50 SC, Rapsan 500 SC, and its widespread use means that metazachlor can readily penetrate into various components of the natural environment (Griesser et al. 2004). Mohr et al. (2008) state that metazachlor can easily reach aquatic habitats, posing a threat to water-dwelling organisms. Due to the use of metazachlor in the winter season, it may undergo slow biodegradation in the environment. In the studies conducted by Mohr et al. (2007) and (2008) observed a negative impact of metazachlor on biomass of macrophytes and algae. The authors claim that when metazachlor of more than 5 μg dm^{-3} seeps into groundwater, it may have significant and long-lasting effects on the aquatic organisms and proper functioning of this ecosystem. The fish are also exposed to the toxic effect of the metazachlor. Lazartigues et al. (2013) observed accumulation of this substance in the muscle tissue of carp and perch. Besides, it disturbs the synthesis of long chains of fatty acids in plants, which makes cellular membranes lose their rigidity and permeability. This in turn results in leaks and disruptions of the cell division, resulting in the retarded growth of a plant (Eckermanna et al. 2003). Accumulation of these substances in living organisms may contribute to their rapid movement in the trophic chain. This represents a danger to the organisms at the end of the chain, especially humans. Metazachlor frequently enters the human body through the consumption of food and drinking water contaminated with this material, as well as through direct contact, inter alia, by the application (Oosterhuisa et al. 2008).

According to Laue et al. (1996) metazachlor in the soil environment converts to ethanesulfonate, which is a substance whose degradation and activity in soil are not well understood yet. According to Beulke and Malkomes (2001), metazachlor deposited in the soil clearly influences the activity of soil microorganisms. Side effects of metazachlor on microorganisms may depend on environmental conditions such as soil type, its temperature and humidity. This may be related to the impact of these factors on the stability and availability of metazachlor and its toxicity to soil microorganisms. The authors claim that soil with a higher organic matter content and higher temperature facilitates faster degradation of metazachlor, so that microorganisms are less susceptible to the damaging effects.

In view of the above considerations, the present study has been undertaken in order to assess the influence of metazachlor, an active substance in the herbicide Fuego 500 SC, on counts and biodiversity of soil microorganisms, enzymatic activity of soil, its physicochemical properties and yields of spring oilseed rape.

Materials and methods

Conditions of the experiment

The plant growing experiment was conducted in a greenhouse at the University of Warmia and Mazury in Olsztyn. Plants were grown in four replications, in polyethylene pots each filled with 3 kg of air-dry soil. Before being placed in the pots, soil was mixed with metazachlor and mineral fertilizers. Rates of mineral fertilizers were adjusted to the nutritional demands of spring oilseed rape, and their quantities, expressed in amounts of the pure component, were as follows (in mg kg^{-1} of soil): N—100 $[CO(NH_2)]_2$, P—44 $[KH_2PO_4]$, K—100 $[KH_2PO_4 + KCl]$, Mg—25 $[MgSO_4\ 7H_2O]$, Cu—5 $[CuSO_4\ 5H_2O]$, Zn—5 $[ZnCl_2]$, Mn—5 $[MnCl_2\ 4H_2O]$, Mo—2.5 $[Na_2MoO_4\ 2H_2O]$ and B—0.33 $[H_3BO_4]$. During the whole experiment (60 days), the soil moisture content was maintained at a level of 50 % of the capillary water capacity, and any loss of water was replenished with demineralized water. Soil samples for microbiological, biochemical and physicochemical analyses were taken on days 30 and 60 of the experiment.

Soil

The soil used for the trials originated from the Experimental Station in Tomaszkowo, which belongs to the University of Warmia and Mazury in Olsztyn. It was collected from the arable and humus layer, at a depth of 0–20 cm; afterwards, its grain-size distribution was determined by the areometric method. According to the World Reference Base of Soil Resources (2006), the soil belonged to Eutric Cambisols. Its texture corresponded to sandy loam. Some of the characteristics of the soil are given in Table 1.

Herbicide

The pot experiment was set up to test metazachlor, which is applied to control weeds in spring and winter oilseed rape

Table 1 Physicochemical properties of the soil

Grain-size distribution			C_{org} (g kg^{-1})	N_{total} (g kg^{-1})	pH_{KCl}	HAC (mmol(+) kg^{-1})	TEB (mmol(+) kg^{-1})	CEC (mmol(+) kg^{-1})	BS (%)
Sand (%)	Silt (%)	Clay (%)							
72.00	21.00	7.00	7.05	0.86	7.00	8.00	111.00	119.00	93.28

HAC hydrolytic acidity, *TEB* total exchangeable base cations, *CEC* cation exchange capacity, *BS* base saturation, C_{org} organic carbon, N_{total} total nitrogen

fields. In our experiment, metazachlor was applied to soil as water emulsion in the following doses (in mg kg^{-1}) as recalculated into active substance: 0 (soil without the herbicide), 0.333 (an optimum dose), 6.666, 13.332, 26.664, 53.328, 106.656 and 213.312.

Counts of soil microorganisms

On the set dates (30 and 60 days of the experiment) microbiological assays were performed with three replications, using the culture dilution method. Counts of oligotrophic bacteria and their spores were determined on a 100-fold diluted medium according to Onta and Hattori (1983) after 21 days of incubation; organotrophic bacteria were counted on a medium designed by Bunt and Rovira (Alexander 1973) with added soil extract after 7 days of incubation; bacteria from the genus *Azotobacter* were determined on a medium according to Fenglerowa (1965) after 3 days; actinomycetes were grown on the Küster and Williams' medium using the antibiotics nystatine and actidion (Parkinson et al. 1971) after 7 days of incubation, and counts of fungi were determined on 5-day cultures grown on the Martin's medium (1950) with added Rose Bengal and aureomycin. Petri dishes were incubated at a constant temperature of 28 °C.

Biodiversity of soil microorganisms

On the set dates (days 30 and 60 of the experiment), microbiological assays of soil samples were performed with three replications. The results were then used to calculate the colony development (CD) and eco-physiological biodiversity (EP) indices for organotrophic bacteria, actinomycetes and fungi in soil contaminated with the metazachlor. Our identification of the structure of microbial communities relied on the rate of development of microbial colonies on plates with agar medium. For that purpose, specific dilutions of the soil suspension were cultured on Petri dishes, i.e., r. 10^{-5} for organotrophic bacteria and actinomycetes and r 10^{-3} for fungi. Counts of grown cultures of microorganisms were checked daily for 10 days.

The colony development (CD) index for microorganisms was derived from the equation by Sarathchandr et al. (1997):

$$CD = [N_1/1 + N_2/2 + N_3/3. \ldots . N_{10}/10] \cdot 100$$

where:

CD	Colony development index,
N_1, N_2, N_3,.... N_{10}	Count of colonies grown after 1, 2, 3, …, 10 days

The eco-physiological (EP) index of the diversity of microorganisms was calculated from the formula given by De Leij et al. (1993):

$$EP = -\sum(pi \cdot \log 10pi)$$

where:

EP	Index of the diversity of microorganisms,
Pi	Count of colonies of microorganisms achieved on a given day divided by the number of all colonies.

The enzymatic activity of soil

In the reported plant growing experiment, on days 30 and 60, the following determinations were made with three replications: the activity of dehydrogenases (EC 1.1) by the Lenhard's method modified by Öhlinger (1996), catalase (EC1.11.1.6), urease (EC 3.5.1.5), arylsulfatase (EC 3.1.6.1) and β-glucosidase (EC 3.2.1.21) according to the procedure by Alef and Nanipieri (1998), acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) in line with the protocol described by Alef et al. (1998). The activity of dehydrogenases was expressed in μmol TPF kg^{-1} dm h^{-1}, catalase—in mol O_2 kg^{-1} dm h^{-1}, urease—in mmol N-NH_4 kg^{-1} dm h^{-1}, acid phosphatase, alkaline phosphatase, arylsulfatase and β-glucosidase—in mmol PNP kg^{-1} dm h^{-1}. Except for catalase, the activity of all the other soil enzymes was measured on a Perkin-Lambda 25 spectrophotometer. The activity of dehydrogenases was measured at the wavelength of $\lambda = 485$ nm, arylsulfatase at $\lambda = 420$ nm, β-glucosidase at $\lambda = 400$ nm, while the activities of urease, acid phosphatase, alkaline phosphatase were assayed at the wavelength of $\lambda = 410$ nm.

Physicochemical properties of soil

The basic physicochemical characteristics were determined, with three replications, in soil samples collected on

day 60 of the experiment: soil pH by potentiometry in aqueous solution of KCl of the concentration of 1 mol dm^{-3}, hydrolytic acidity (HAC) and total exchangeable bases (TEB) by the Kappen's method (Carter 1993), as well as the content of organic carbon (C_{org}) with the method designed by Tiurin (Nelson and Sommers 1996).

Yield of spring oilseed rape

On the day of setting up the experiment, spring oilseed rape cv. Hybryda was sown, 25 seeds per pot. Next, after the seeds had germinated, the seedlings were thinned, leaving 8 plants per pot. When the experiment was terminated (day 60), all the plants were collected and weighed. Afterwards, they were dried to determine the yield of dry matter in g per pot.

Statistical analyses

The results were processed statistically using the following applications from a Statistica software suite (Statsoft, Inc, Statistica 2010): Duncan's multiple range test as well as one- and two-factorial analysis of variance at $p = 0.05$. In addition, correlation coefficients were calculated between the dose of metazachlor and counts of microorganisms or the activity of soil.

Results and discussion

Counts of soil microorganisms

In our experiment, soil contamination with metazachlor disturbed its microbiological balance, mainly due to changes in counts of oligotrophic bacteria and their spores, *Azotobacter* spp., organotrophic bacteria, actinomycetes and fungi. In general, the optimal dose (0.333 mg kg^{-1}) did not cause any substantial modifications in the aforementioned groups of microorganisms, but higher doses (from 6.666 to 213.312 mg kg^{-1}) retarded the multiplication of the discussed groups of microorganisms. In our experiment, metazachlor applied to soil in the form of water emulsion inhibited the development of all the analyzed soil microorganisms (Table 2). The count of oligotrophic bacteria was positively correlated with the rate of metazachlor on day 30 of the experiment ($r = 0.381$). On the first date of determinations, the dose of 26.664 mg kg^{-1} caused a decrease in the count of these bacteria by 29.89 %; on the second date (day 60), the decline was 37.73 % against the control. Regardless of the dose of metazachlor, counts of oligotrophic bacteria on day 60 of the experiment decreased by 10.70 % against day 30. The count of sporulating oligotrophic bacteria depended on the dose of metazachlor and date of determinations. The technological dose of the herbicide increased the count of these bacteria on both days 30 and 60 of the trial, by 17.40 and 29.97 %, respectively, against the soil not contaminated with the herbicide. On day 30, all the doses of metazachlor stimulated the reproduction of sporulating oligotrophic bacteria except the highest dose (213.312 mg kg^{-1} dm of soil), which depressed their count by 8.67 %. The application of the tested chemical in doses from 6.666 to 26.664 mg kg^{-1} dm of soil significantly decreased counts of sporulating oligotrophes in the range of 6.08–51.62 %. The *Azotobacter* spp. bacteria responded significantly negatively to soil contamination with metazachlor, both on days 30 and 60 of the trial, which was reflected by negative coefficients of the correlation between the dose of the active substance and counts of the above bacteria ($r = -0.765$ on day 30 and $r = -0.369$ on day 60). The dose of 213.312 mg kg^{-1} dm of soil most strongly inhibited the multiplication of bacteria of the genus *Azotobacter* on both determination dates, depressing their count by 82.79 % on day 30 and 97.61 % on day 60. On the second date of determinations, the manufacturer recommended dose lowered the count of these bacteria by 5.1-fold versus the control soil. Counts of organotrophic bacteria depended on the rate of metazachlor as well as the duration of exposure. All the tested doses of the herbicide, irrespective of the date of determinations, caused a significant depression in the count of organotrophic cells, including the dose recommended by the producer. The rate of 213.312 mg kg^{-1} dm of soil was responsible for the most destructive changes in the reproduction of these bacteria. This rate decreased the count of organotrophic bacteria by 44.86 % on day 30 and by 37.66 % on day 60. The adverse influence of herbicides on multiplication of bacteria has also been reported by Sebiomo et al. (2011), who tested atrazine, primeextra, paraquat and glyphosate, or Araùjo et al. (2003) who ran an experiment with glyphosate. Milošević and Govedarica (2002) claim that bacteria of the genus *Azotobacter* are the ones which most readily respond to the soil contamination with herbicides and therefore can be regarded as a reliable indicator of soil health status. In our experiment, *Azotobacter* cells were most highly sensitive to metazachlor introduced to soil. An over 90.00 % decrease was noted on day 60 of the experiment when the highest dose of the herbicide had been applied. Similar conclusions were drawn by Kucharski and Wyszkowska (2008), who noticed a significant decline in the count of these bacteria after an application of the herbicide called Apyros 75 WG.

Like all other groups of microorganisms, actinomycetes responded to changes induced by metazachlor. Excessive doses of the herbicide, irrespective of the date of

Table 2 Effect of metazachlor on counts of soil microorganisms, 10^n cfu kg^{-1} dm of soil

Dose of metazachlor mg kg^{-1}	Date of analysis (days)											
	[a]Olig × 10^9		[b]Oligp × 10^8		[c]*Az* × 10^3		[d]Org × 10^9		[e]Act × 10^9		[f]Fun × 10^6	
	30	60	30	60	30	60	30	60	30	60	30	60
0.000	13.764	13.899	5.719	3.140	9.637	60.394	12.067	13.899	11.124	11.851	18.016	41.986
0.333	13.721	14.096	6.714	4.081	13.137	11.953	10.468	12.530	10.342	10.717	16.056	29.471
6.666	12.475	11.488	7.348	2.949	10.378	3.094	10.171	10.870	9.527	6.930	16.190	26.401
13.332	11.283	10.759	7.481	1.519	6.649	3.746	9.413	10.510	6.151	7.658	16.208	26.430
26.664	9.650	9.493	6.503	2.831	4.970	4.372	8.201	8.535	4.639	5.288	14.289	21.026
53.328	12.177	8.655	6.432	4.255	3.330	2.685	8.201	8.820	3.372	4.276	10.616	15.079
106.656	13.329	10.079	6.425	4.719	3.119	1.863	7.569	8.879	4.949	2.670	7.901	12.418
213.312	14.137	11.304	5.223	5.693	1.658	1.444	6.654	8.664	6.135	2.496	7.048	8.458
Average	12.567	11.222	6.481	3.648	6.610	11.194	9.093	10.338	7.030	6.486	13.291	22.659
[A]*r*	0.381	−0.287	−0.656	0.782	−0.765	−0.369	−0.808	−0.608	−0.441	−0.766	−0.901	−0.801
[B]$LSD_{0.05}$												
a	5.299		4.333		2.290		7.951		1.120		2.236	
b	2.650		2.166		1.145		3.525		n.s.		1.118	
ab	7.495		6.128		3.234		9.971		1.584		3.162	

[a] *Olig* oligotrophic bacteria, [b] $Olig_p$ oligotrophic spores, [c] *Az* bacteria of the genus *Azotobacter*, [d] *Org* organotrophic bacteria, [e] *Act* actinomycetes, [f] *Fun* fungi

[A] *r* correlation coefficient

[B] $LSD_{0.05}$ for: *a* dose of herbicide, *b* date of determinations, *ab* interaction between the factors, *n.s.* non-significant

determinations, depressed the count of actinomycetes against the unpolluted soil sample. Metazachlor most strongly depressed the count of these microorganisms on the first date when it had been applied to soil in the amount of 53.328 mg kg^{-1} dm of soil (by 69.69 %). On the second date of analyses, the biggest decrease was due to the dose of 213.312 mg kg^{-1} dm of soil (by 78.94 %). Regardless of the applied dose of the herbicide's active substance, the highest count of actinomycetes was found on day 30 (on average $7.030 \cdot 10^9$ cfu kg^{-1} dm of soil). The negative effect of metazachlor on counts of actinomycetes is verified by the study completed by Kucharski and Wyszkowska (2008) on the preparation Apyros 75 WG, or another one reported by Sebiomo et al. (2011), who tested atrazine, primeextra, paraquat and glyphosate. Contrary to that, Martinez et al. (2008) recorded a significant increase in the count of actinomycetes in soil with sulfentrazone, similarly to Araùjo et al. (2003), who tested glyphosate.

The literature data show that herbicides can also have an adverse effect on the growth and development of fungi, which was confirmed by the present study, where fungi found a more favorable environment for development on day 60 of the experiment. Counts of fungi in soil, both unpolluted and metazachlor-contaminated, were higher on day 60. The tested herbicide depressed the count of fungi by 10.88 % on day 30 and by 28.58 % on day 60. However, the most drastic modifications in the reproduction of these microorganisms, both on the first and the second date of determinations, appeared in response to the highest dose of metazachlor (213.312 mg kg^{-1} dm of soil). This rate resulted in a decrease in fungi by 60.88 % on day 30 and 79.86 % on day 60. The negative influence of herbicides on fungi was also reported by Kucharski and Wyszkowska (2008), who tested Apyros 75 WG. In turn, Crouzet et al. (2010) and Zabaloy et al. (2010) observed an increase in the count of fungi after an application of weed killers. Crouzet et al. (2010) tested the herbicide mezotrion in rates from 0.45 to 45 mg kg^{-1}, while Zabaloy et al. (2010) examined the preparation 2,4-dichlorophenoxyacetate in doses from 1 to 10 mg kg^{-1}. Finally, Martinez et al. (2008) did not demonstrate any significant changes in the multiplication of fungi following an application of sulfentrazone.

The decrease in the count of the tested microorganisms under the influence of excessive doses of metazachlor may be due to the high toxicity of the metabolite formed during the transformation, which is ethanesulfonate. Cell death of a count of soil microorganisms can indirectly influence the reduction of biomass and the count of organisms. However, some species are able to use the herbicide as a source of carbon and nutrients. The ability of microbes to degrade pesticides partly contributes to the reduction of the toxicity of these substances, which may not only yield a positive effect on soil organisms, but also improve the biological properties of the soil. For instance, bacteria of the genus

Arthrobacter, exhibit a large capacity for degradation of atrazine. Ma et al. (2011) investigated the ability of HB-5 strain of bacteria *Arthrobacter* spp. to degrade atrazine brown and cinnamon soil. The authors, after introducing of 10 mg kg^{-1} of atrazine to the soils, reported only 10 % of the residues of the substance in both soil types.

Biodiversity of soil microorganisms

References (De Leij et al. 1993; Ratcliff et al. 2006) indicate that determination of the structure of soil microorganisms could be a suitable indicator diagnosing changes which take place in the soil environment due to imported chemical substances. Unfortunately, there is little information about the effect of weed controlling substances on structures of microbial communities and their biodiversity in soil. Soil pollution with heavy metals and polycyclic aromatic hydrocarbons has been a serious problem, and therefore there is more information about their effect on soil microorganisms. In their studies, Jiang et al. (2010) observed that cadmium and phenanthrene contributed to significant changes in the biodiversity of microorganisms. The results showed that the biodiversity of microorganisms was significantly lower in soil contaminated with cadmium and phenanthrene than that in the control. Joint activity of these substances contributed to the occurrence of a synergistic effect, which led to changes in the development and diversity of soil microorganisms.

In this experiment, metazachlor had a significant effect on the colony development index (CD) and eco-physiological diversity index (EP) (Table 3). The CD index provides a wealth of information about changes in the proportions between fast and slowly growing microorganisms. In soil microbiological research, r-strategists are microorganisms which grow rapidly on the agar substrate during 24–48 h, represented by live and active species. K-strategists, on the other hand, are microorganisms which grow more slowly or remain dormant. Generally, r-strategists dominate in less stable environments, in contrast to K-strategists. Our results confirm the claim that soil conditions can be a decisive factor ensuring, for example, the dominance of r-strategists or K-strategists (Cycoń and Piotrowska-Seget 2009). The value of the CD index for actinomycetes and fungi was higher on day 60 than day 30. A reverse tendency was observed for organotrophic bacteria. Nevertheless, the highest value of the CD index, irrespective of the date of determinations or the dose of metazachlor, was found for fungi (37.00 on average) and, subsequently, for organotrophic bacteria (32.345 on average) and actinomycetes (24.552 on average). The CD index calculated for the analyzed groups of microorganisms also changed under the influence of the highest doses of metazachlor. Organotrophic bacteria most negatively responded to the dose of 106.656 mg kg^{-1} dm of soil, which depressed the CD index by 29.06 % on day 30 and by 29.64 % on day 60. An identical dependence was noticed for actinomycetes. The same dose of the herbicide depressed the value of the CD index by 1.37-fold on day 30 and by 1.55-fold on day 60 against soil not contaminated with metazachlor. The value of the CD index calculated for fungi changed in response to metazachlor differently on the two dates of determinations. The largest decline in the CD index on day 30 was caused by the dose of metazachlor equal 213.312 mg kg^{-1} dm of soil (the value of the index fell by 43.58 %), whereas on day 60 it was the dose of 26.664 mg kg^{-1} dm of soil that was responsible for the biggest decrease (by 11.17 %). An increase in the CD index implies a higher share of r-strategists in soil while its low value means that K-strategists prevail (Sarathchandra et al. 1997). Such relationships were also noticed in our experiment, in which K-strategists gained the highest share.

Disturbances in the structure of microorganisms are often associated with changes in their biodiversity (Cycoń and Piotrowska-Seget 2009). According to Cycoń et al. (2010), a decline in the EP index implies that microorganisms sensitive to the soil contamination with herbicides are supplanted by more tolerant species. The EP index determined in our research demonstrated a significant effect of metazachlor on the biodiversity of the analyzed soil microorganisms. The highest EP index for organotrophic bacteria on day 30, equal 0.926, was recorded in soil with the herbicide introduced in the dose of 53.328 mg kg^{-1} dm of soil; on day 60, the EP for these bacteria was the highest (0.919) following an application of 213.312 mg kg^{-1} dm of soil. Regarding actinomycetes, the lowest EP index was achieved on the first date of determinations after the dose of 0.333 mg kg^{-1} dm of soil had been applied; on day 60, the EP was the lowest in the treatment with the dose of 26.664 mg kg^{-1} dm of soil. On both dates, the EP value was 0.877. For fungi, the values of the EP index ranged from 0.333 to 0.735. It was observed that metazachlor applied to soil in the dose of 6.666 mg kg^{-1} dm of soil reduced the value of the EP index by 11.86 % on day 30; on day 60, a decrease by 31.06 % in the value of this index was noticed in response to the dose of the herbicide equal 53.328 mg kg^{-1} dm of soil. Regardless the dose of metazachlor or date of determination, the highest value of the EP index was reached by bacteria (EP = 0.869), and the lowest one by fungi (EP = 0.435). Cycoń et al. (2010) report that lower values of the CD and EP indices are suggestive of certain disorders in the structure of indigenous communities of microorganisms inhabiting soil, which find confirmation in our experiment. Metazachlor applied to soil in excessive doses depressed both indices. Similar results were obtained by

Table 3 Effect of metazachlor on the colony development (CD) index and eco-physiological diversity (EP) index of soil microorganisms

Dose of metazachlor (mg kg^{-1})	Date of analysis (days)											
	Colony development index (CD)						Eco-physiological diversity index for microorganisms (EP)					
	Org		Act		Fun		Org		Act		Fun	
	30	60	30	60	30	60	30	60	30	60	30	60
0.000	36.599	33.476	24.615	29.578	39.372	43.993	0.837	0.829	0.855	0.782	0.607	0.483
0.333	37.616	31.121	24.727	30.848	35.884	42.545	0.830	0.897	0.877	0.767	0.583	0.489
6.666	38.118	31.628	24.352	30.312	36.182	41.237	0.837	0.860	0.846	0.775	0.535	0.501
13.332	41.549	35.140	24.278	32.705	32.494	39.276	0.800	0.861	0.856	0.782	0.630	0.505
26.664	37.966	36.945	23.181	23.329	33.550	39.080	0.832	0.879	0.849	0.877	0.623	0.504
53.328	28.582	26.433	23.560	21.784	31.179	43.920	0.926	0.909	0.865	0.873	0.649	0.333
106.656	25.964	23.553	18.009	19.093	31.395	43.163	0.899	0.897	0.826	0.780	0.735	0.345
213.312	26.013	26.808	20.439	22.011	22.213	46.116	0.867	0.919	0.773	0.749	0.658	0.368
average	34.051	30.638	22.895	26.208	32.784	42.416	0.854	0.881	0.843	0.798	0.428	0.441
[a]r	−0.821	−0.645	−0.776	−0.687	−0.925	0.641	0.475	0.687	−0.923	−0.265	0.606	−0.712

Org organotrophic bacteria, *Act* actinomycetes, *Fun* fungi

[a] r correlation coefficient

Cycoń and Piotrowska-Seget (2009), who tested the preparation diuron, or by Ros et al. (2006), who applied atrazine to soil.

The enzymatic activity of soil

Soil enzyme activity is to a large extent the parameter determining the quality of soil. Enzymes produced mainly by soil microorganisms and plants play a huge role not only in the synthesis and decomposition of organic compounds, but are also involved in the degradation of various types of pollutants that most often include heavy metals and pesticides. Gao et al. (2010) in their studies on cadmium and lead have noted a reduction in the activity of dehydrogenases, urease and phosphatases. They found that these enzymes can be used to evaluate the changes, not only under the influence of heavy metals, but also other chemicals. This study shows that soil contamination with excessive quantities of metazachlor had a significant effect on the biochemical activity of soil, the finding verified by negative correlation coefficients (Table 4). Dehydrogenases invariably responded negatively to the soil contamination with the tested herbicide. As the dose of metazachlor went up, the activity of dehydrogenases diminished. The strongest inhibitory effect on dehydrogenases on day 30 of the experiment was produced by the dose of 213.312 mg kg^{-1} dm of soil (a decline by 81.38 %), and on day 60 by the dose of 106.656 mg kg^{-1} dm of soil (a decline by 85.17 %). Irrespective of the dose of metazachlor, dehydrogenases were the most active on the second date of determinations, when their activity was on average twice as high as on the first date. High activity of dehydrogenases proves that active microorganisms are present in soil (Kucharski et al. 2009). In our study, dehydrogenases proved to be very sensitive to soil contamination with metazachlor. Similar results were attained by Kucharski et al. (2009), who investigated the influence of four herbicides (Harpun 500 SC, Faworyt 300 SL, Akord 180 OF and Mocarz 75 WG) and by Kucharski and Wyszkowska (2008), when assessing the impact of the herbicide Apyros 75 WG. In contrast, Baćmaga et al. (2012) noted that the preparation Aurora 40 WG left the activity of dehydrogenases nearly unaffected.

Next to dehydrogenases, catalase is another enzyme whose activity is commonly used as an indicator of the biological activity of soil (Shiyin et al. 2004). In this study, metazachlor added to soil in higher than recommended doses affected catalase in different ways. On day 30, the herbicide applied in the dose from 6.666 to 106.656 mg kg^{-1} dm of soil stimulated the activity of catalase in the range of 7.46–92.54 %. On day 60, all the doses of the herbicide inhibited the activity of this enzyme. The strongest inhibition was caused by the dose of 6.666 mg kg^{-1} dm of soil, which reduced its activity by as much as 99.72 %. However, the highest activity of catalase was observed on day 60 of determinations. In contrast, Yao et al. (2006) report that excessive amounts of acetamipirid do not affect the activity of catalase.

Urease, which belongs to hydrolases, is an enzyme quickly responding to changes in the soil environment. It participates in the transformations of organic nitrogen in soil which make it more readily available to plants

Table 4 Effect of metazachlor on soil enzymatic properties

Dose of metazachlor (mg kg^{-1})	Date of analysis (days)													
	Dehydrogenases (μmol TPF kg^{-1} dm h^{-1})		Catalase (mol O_2 kg^{-1} dm h^{-1})		Urease (mmol N-NH_4 kg^{-1} dm h^{-1})		Acid phosphatase (mmol PNP kg^{-1} dm h^{-1})		Alkaline phosphatase (mmol PNP kg^{-1} dm h^{-1})		Arylsulfatase (mmol PNP kg^{-1} dm h^{-1})		β-glucosidase (mmol PNP kg^{-1} dm h^{-1})	
	30	60	30	60	30	60	30	60	30	60	30	60		
0.000	4.559	12.301	0.067	0.281	0.305	0.573	3.460	4.566	4.791	3.926	0.291	0.396	0.281	0.345
0.333	4.193	9.928	0.057	0.162	0.672	0.684	3.961	5.431	4.726	3.763	0.296	0.350	0.270	0.274
6.666	4.115	6.598	0.072	0.072	0.537	0.454	3.455	4.517	4.461	3.622	0.293	0.329	0.274	0.248
13.332	4.028	6.526	0.190	0.084	0.362	0.427	3.422	4.416	4.665	3.353	0.312	0.269	0.275	0.244
26.664	3.846	3.907	0.195	0.074	0.273	0.217	3.157	4.191	4.757	3.333	0.325	0.311	0.271	0.235
53.328	1.371	2.584	0.116	0.170	0.169	0.272	3.069	3.319	4.792	3.063	0.335	0.254	0.256	0.220
106.656	1.071	1.824	0.129	0.186	0.137	0.241	3.106	2.978	4.379	3.304	0.334	0.271	0.248	0.221
213.312	0.849	4.812	0.059	0.134	0.035	0.239	2.755	2.693	4.792	3.385	0.308	0.244	0.250	0.222
Average	3.004	6.060	0.111	0.145	0.311	0.388	3.298	4.014	4.670	3.469	0.312	0.303	0.266	0.251
[a]*r*	−0.854	−0.508	−0.194	0.000	−0.764	−0.630	−0.738	−0.859	0.025	−0.419	0.326	−0.678	−0.833	−0.532
[b]$LSD_{0.05}$														
a	1.823		0.010		0.897		0.216		0.256		0.012		0.012	
b	0.911		0.005		0.449		0.108		0.128		0.006		0.006	
ab	2.578		0.014		1.269		0.306		0.362		0.017		0.016	

[a] *r* correlation coefficient

[b] $LSD_{0.05}$ for: *a* dose of herbicide, *b* date of determinations, *ab* interaction between the factors

(Rahmansyah et al. 2009). Overall, soil contamination with metazachlor had a destructive influence on the activity of urease. In samples analyzed on day 30, the doses of the herbicide between 0.333 and 13.332 mg kg^{-1} dm of soil raised the activity of urease. The strongest inhibitory effect on urease, both on days 30 and 60, was produced by the rate of 213.312 mg kg^{-1} dm of soil, which depressed its activity by 99.70 and 58.29 %, respectively, versus the unpolluted soil. Analogously to our results, inhibitory influence of herbicides on the activity of urease was reported by Kucharski and Wyszkowska (2008) testing Apyros 75 WG, or by Sukul (2006) investigating metalaxyl. In turn, Baćmaga et al. (2012), who tested the herbicide Aurora 40 WG, did not detect any distinct changes in the activity of urease.

Other enzymes used in soil quality evaluation assays are phosphatases, which are engaged in the mineralization of organic phosphate compounds (Rahmansyah et al. 2009). The herbicide metazachlor on day 30 as well as day 60 of the experiment affected significantly the activity of acid phosphatase and alkaline phosphatase. The activity of acid phosphatase was negatively correlated with the dose of metazachlor, both on day 30 ($r = -0.738$) and day 60 ($r = -0.859$) of the experiment. On both determination dates, the manufacturer recommended dose increased the activity of acid phosphatase by 14.48 and 18.94 %, respectively. The other doses retarded the activity of acid phosphatase, most strongly the rate of 213,312 m g kg^{-1} dm of soil, which depressed the activity of the said enzyme by 20.38 % on day 30 and by 41.02 % on day 60. Regardless of the dose of the active substance supplied to soil with the herbicide, higher activity of acid phosphatase was recorded on day 60 (on average 4.014 mmol PNP kg^{-1} dm h^{-1}).

Soil contamination with excessive quantities of metazachlor only slightly modified the activity of alkaline phosphatase. On day 30, its activity ranged from 4.379 to 4.792 mmol PNP kg^{-1} dm h^{-1}. On day 60, the rate of 53.328 mg kg^{-1} dm of soil depressed the activity of alkaline phosphatase by 21.98 %. On the earlier day of determinations, the activity of alkaline phosphatase was 1.35-fold higher than on the later date. Positive influence of metazachlor on the activity of phosphatases was noticed by Baćmaga et al. (2012) in a study on the preparation Aurora 40 WG. Wyszkowska and Kucharski (2004) also confirm that acid phosphatase and alkaline phosphatase were most resistant enzymes to the effect produced by Apyros 75 WG. With respect to the activity of phoshatases, weed control preparation can produce other than stimulating effects. Some can actually inhibit the activity of these enzymes. Such were the results demonstrated by Wyszkowska (2002) in an experiment which involved the herbicide Treflen 480 EC, or by Wyszkowska and Kucharski (2004) in another trial testing Triflurotox 250 EC.

An excellent indicator showing modifications in soil induced by chemical substances consists of the enzymes arylsulfatase and β-glucosidase, both belonging to the class

of hydrolases (Acosta-Martı′nez et al. 2008; Li and Sarah 2003). In our experiment, like other enzymes, arylsulfatase responded to soil contamination with metazachlor. On day 30, the herbicide was found to have stimulated the activity of that enzyme. Even the highest rate of metazachlor (213.312 mg kg^{-1} dm of soil) caused an increase in the activity of arylsulfatase by 5.84 % on day 30. On day 60, however, metazachlor inhibited the enzyme. The dose recommended by the producer depressed the activity of arylsulfatase by 11.62 % while the highest rate lowered it by 38.38 %. Metazachlor introduced to soil caused changes in the activity of β-glucosidase. Its activity was observed to have decreased under the influence of all tested doses of the herbicide, both on the first and the second date of determinations. The highest dose (213.312 mg kg^{-1} dm of soil) depressed the activity of β-glucosidase by 11.03 % on day 30 and 35.65 % on day 60. On both dates, its activity remained on a similar level. The response of arylsulfatase and β-glucosidase to plant protection chemicals permeating into soil is diverse. Some herbicides can stimulate, inhibit or be neutral to these enzymes (Uziak and Steinbrich 2005). In our investigations, metazachlor was shown to inhibit the activity of arylsulfatase and β-glucosidase. Also, Sukul (2006) reported lower activity of these enzymes following applications of metazachlor. The same findings were reported by Sofo et al. (2012), who tested five herbicides (cinosulfuron, prosulfuron, tifensulfuron-methyl, metylu, triasulfuron). In contrast, a stimulating effect of herbicides from the group of chloroacetanilides (alachlor, butachlor and pretilachlor) on the activity of β-glucosidase was detected by Saha et al. (2012).

Physicochemical properties of soil

The physicochemical properties of soil contaminated with metazachlor were demonstrated using the Principal Component Analysis (PCA) performed on standardized values (Fig. 1). The diagram shows that the vectors illustrating the soil pH, hydrolytic activity (HAC), total exchangeable base cations (TEB), cation exchange capacity (CEC) and base saturation (BS) are strongly inclined toward the main horizontal axis (X) and the vector showing the content of organic carbon (C_{org}) is inclined toward the main vertical axis (Y). The data obtained during our experiment prove that higher rates of metazachlor increased the soil's hydrolytic acidity (HAC), because this variable lies within

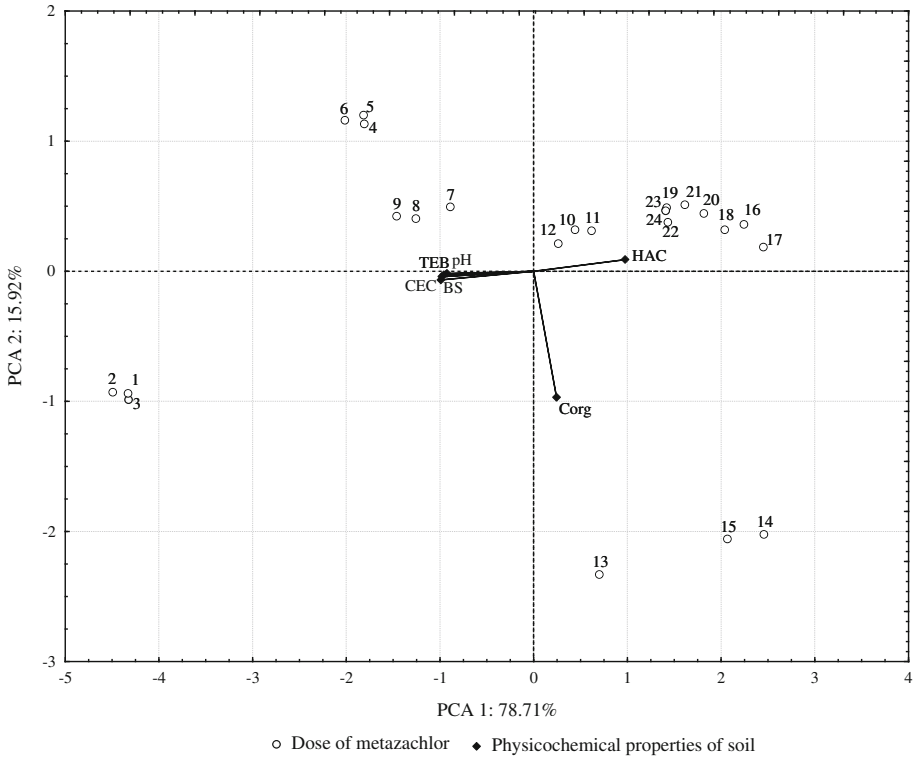

Fig. 1 Physicochemical properties of soil contaminated with metazachlor (M) represented with the PCA method. Vectors correspond to analyzed variables (pH, HAC, TEB, CEC, BS, C_{org}), while points correspond to soil samples with doses of metazachlor Key: *pH* soil reaction; *HAC* hydrolytic acidity; *TEB* total exchangeable bases; *CEC* cation exchange capacity; *BS* base saturation; C_{org} content of organic carbon; *1, 2, 3* control (M unpolluted soil); *4, 5, 6* 0.333 mg M kg^{-1} - dm of soil; *7, 8, 9* 6.666 mg M kg^{-1} dm of soil; *10, 11, 12* 13.332 mg M kg^{-1} dm of soil; *13, 14, 15* 26.664 mg M kg^{-1} dm of soil; *16, 17, 18* 53.328 mg M kg^{-1} dm of soil; *19, 20, 21* 106.656 mg M kg^{-1} dm of soil; *22, 23, 24* 213.312 mg M kg^{-1} dm of soil

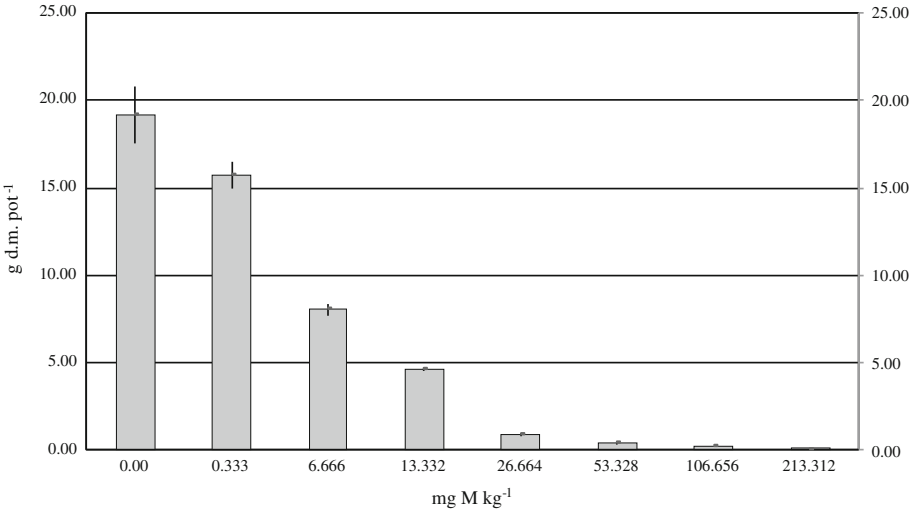

Fig. 2 Effect of metazachlor (M) on yields of spring oilseed rape, g of dm per pot^{-1}

positive values in the diagram. In respect of the pH of soil, total exchangeable base cations (TEB), cation exchange capacity (CEC) and base saturation (BS), reverse trends were detected (vectors representing values of these variables are inclined toward the main axis but within negative values). A study completed by Wyszkowską and Kucharski (2004) in which Triflurotox 250 EC was tested demonstrated that the said herbicide caused an increase in the degree of soil saturation with base cations, but the values of hydrolytic acidity and cation exchange capacity of soil were negatively correlated with the dose of this preparation.

Yield of spring oilseed rape

Metazachlor retarded the growth and development of spring oilseed rape, resulting in lower yields (Fig. 2). The yield of spring oilseed rape decreased by 18.12 % against the unpolluted soil under the influence of the rate recommended by the producer. However, the highest yield loss was noticed after an application of the dose of 213.312 mg kg^{-1} dm of soil (a 99.79 % yield loss). In a study with Triflurotox 250 EC applied in doses from 1.5 to 12 mm^3 kg^{-1} dm of soil, Wyszkowska and Kucharski (2004) determined a positive correlation between the dose of the herbicide and the yields of spring oilseed rape and white mustard. They also detected a significant decline in yields of the crops due to the application of doses 10- and 100-fold higher than recommended by the producer.

Conclusions

All the analyzed parameters, i.e., counts and biodiversity of soil microorganisms, enzymatic activity of soil, physicochemical properties of soil and spring oilseed rape yields, proved to be highly sensitive to the effect of metazachlor. Considering the average test results, metazachlor applied to the soil in contamination doses caused a reduction in the count of all tested microorganisms. This compound also caused changes to the structure of microorganisms units, which is indicated by values of CD and EP. Metazachlor added to the soil in excessive amounts decreased value of the CD index in all the microorganisms studied. EP index of organotrophic bacteria increased its value, while the EP of actinomycetes and fungi reduced under the influence of metazachlor. It was also found that this preparation had inhibitory effect on the activity of all the soil enzymes tested, the yield of spring rape and physicochemical properties of the soil. These parameters could be recommended for assessment of the toxicity of the tested herbicide. Metazachlor applied to soil in elevated doses acted destructively on some soil properties, which means it should be used for weed control with due caution. Uncontrolled introduction of metazachlor to the soil environment may lead to the destruction of soil habitats and spread the chemical substance to other components of the natural environment.

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References

Acosta-Martı′nez V, Acosta-Mercado D, Sotomayor-Ramı′rez D, Cruz-Rodrı′guez L (2008) Microbial communities and enzymatic activities under different management in semiarid soils. Appl Soil Ecol 38:249–260

Alef K, Nannipieri P (1998) Methods in Applied Soil Microbiology and Biochemistry. In: Alef K, Nannipieri P (eds) Academic Press, Harcourt Brace and Company, London, pp 316–365

Alef K, Nannipieri P, Trazar-Capeda C (1998) Phosphatase activity methods in applied soil microbiology and biochemistry. In: Alef K, Nannipieri P (eds) Academic Press, Harcourt Brace and Company, London, pp 335–344

Alexander M (1973) Microorganisms and chemical pollution. Bioscience 23:509–515

Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S (2009) Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by bacillus pumilus strain C2A1. J Hazard Mater 168:400–405

Araújo ASF, Monteiro RTR, Abarkeli RB (2003) Effect of glyphosate on the microbial activity of two Brazilian soil. Chemosphere 52:799–804

Arias-Estéves M, López-Periago E, Martinez-Carbaloo E, Simal-Gándara J, Garciario L (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric Ecosyst Environ 123:247–260

Baćmaga M, Boros E, Kucharski J, Wyszkowska J (2012) Enzymatic activity in soil contaminated with the Aurora 40 WG herbicide. Environ Prot Eng 38(1):91–102

Baćmaga M, Wyszkowska J, Borowik A, Tomkiel M, Kucharski J (2014) Response of fungi, β-glucosidase and arylsulfatase to soil contamination by Alister Grande 190 OD, Fuego 500 SC and Lumax 357.5 SE herbicides. Pol J Environ Stud 23(1):19–25

Beukle S, Malkomes HP (2001) Effects of the herbicides metazachlor and dinoterb on the soil microflora and the degradation and sorption of metazachlor under different environmental conditions. Biol Fertil Soils 33:467–471

Carter MR (1993) Soil sampling and methods of analysis. Canadian Society of soil science. Lewis Publishers, London

Crouzet O, Batisson I, Besse-Hoggan P, Bonnemoy F, Bardot C, Poly F, Bohatier J, Mallet C (2010) Response of soil microbial communities to the herbicide mesotrione: a dose-effect microcosm approach. Soil Biol Biochem 42:193–202

Cycoń M, Piotrowska-Seget Z (2009) Changes in bacterial diversity and community structure following pesticides addition to soil estimated by cultivation technique. Ecotoxicol 18(5):632–642

Cycoń M, Piotrowska-Seget Z, Kozdrój J (2010) Linuron effects on microbiological characteristics of sandy soils as determined in a pot study. Ann Microbiol 60(3):439–449

De Leij FAAM, Whipps JM, Lynch JM (1993) The use of colony development for the characterization of bacterial communities in soil and on roots. Microbiol Ecol 27:81–97

Eckermanna C, Matthesg B, Nimtzc M, Reiserb, Ledererb B, Bogerb P, Schrodera J (2003) Covalent binding of chloroacetamide herbicides to the active site cysteine of plant III polyketide synthases. Phytochemistry 64:1045–1054

Fenglerowa W (1965) Simple method for counting *Azotobacter* in soil samples. Acta Microbiol Pol 14:203–206

Gao Y, Zhou P, Mao L, Zhi Y, Shi W (2010) Assessment of effects of heavy metals combined pollution on soil enzyme activities and microbial community structure: modified ecological dose-response model and PCR-RAPD. Environ Earth Sci 60:603–612

Griesser UJ, Weigand D, Rollinger JM, Haddow M, Gstrein E (2004) The crystal polymorphs metazachlor. Identification and thermodynamic stability. J Therm Anal Cal 77:511–522

Jiang W, Wang J, Tang J, Hou F, Lu Y (2010) Soil bacterial functional diversity as influenced by cadmium, phenanthrene and degrade bacteria application. Environ Earth Sci 59:1717–1722

Kucharski J, Wyszkowska J (2008) Biological properties of soil contaminated with the herbicide Apyros 75 WG. J Elem 13(3):357–371

Kucharski J, Baćmaga M, Wyszkowska J (2009) Dehydrogenase activity as an indicator of soil contamination with herbicides. Ecol Chem Eng A 16(3):253–261

Laue H, Field JA, Cook AM (1996) Bacterial desulfonation of the ethanesulfonate metabolite of the chloroacetanilide herbicide metazachlor. Environ Sci Technol 30(4):1129–1132

Lazartigues A, Thomas M, Banas D, Brun-Bellut J, Cren-Olive C (2013) Accumulation and half-lives 13 pesticides in muscle tissue of freshwater fishes through food exposure. Chemosphere 91:530–535

Li X, Sarah P (2003) Arylsulfatase activity of soil microbial biomass along a mediterranean-arid transect. Soil Biol Biochem 35:925–934

Ma T, Zhu L, Wang J, Wang J, Xie H, Su J, Zhang Q, Shao B (2011) Enhancement of atrazine degradation by crude and immobilized enzymes in two agricultural soils. Environ Earth Sci 64:861–867

Martin J (1950) Use of acid rose Bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci 69:215–233

Martinez CO, Silva CMMS, Fay EF, Maia AHN, Abakerli RB, Durrant LR (2008) Degradation of the herbicide sulfentrazone in a Brazilian typic hapludox soil. Soil Biol Biochem 40:879–886

Milošević NA, Govedarica MM (2002) Effect of herbicides on microbiological properties of soil. Proc Nat Sci 102:5–21

Mohr S, Berghahn R, Feibicke M, Meineckie S, Ottenstroer T, Schmiedling R, Schmiediche R, Schmidt R (2007) Effects of herbicide metazachlor on macrophytes and ecosystem function in freshwater pond stream mesocosms. Aquat Toxicol 82:73–84

Mohr S, Feibicke M, Berghahn R, Schmiediche R, Schmidt R (2008) Response of plankton communities in freshwater pond and stream mesocosms to the herbicide metazachlor. Environ Poll 152:530–542

Mukherjee P, Alam S, Sardar D, Pahari A, Roy S, Chowdhury A (2006) Persistence and dissipation of linuron (Afalon-50 WP) in pea cropped soil and its effect on soil microorganisms. Bull Environ Contam Toxicol 76(3):407–414

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks DL (ed) Method of soil analysis: chemical methods. American Society of Agronomy, Madison, pp 1201–1229

Öhlinger R (1996) Dehydrogenase activity with the substrate TTC Methods in Soil Biology. In: Schinner F, Öhlinger R, Kandler E, Margesin R (eds) Springer, Berlin, pp 241–243

Onta H, Hattori T (1983) Oligotrophic bacteria on organic debris and plant roots in paddy field. Soil Biol Biochem 1:1–8

Oosterhuisa B, Vukmana K, Vagia E, Glavinasa H, Jablonkaib I, Krajcis P (2008) Specific interactions of chloroacetanilide herbicides with human ABC transporter proteins. Toxicology 248:45–51

Parkinson D, Gray FRG, Williams ST (1971) Methods for studying the ecology of soil micro-organism. Blackwell Scientific Publication, Oxford and Edinburgh IBP Handbook 19

Rahmansyah M, Antonius S, Sulistinah N (2009) Phosphatase and urease instability caused by pesticides present in soil improved by grounded rice straw. J Agri Biol Sci 4(2):56–62

Ratcliff AW, Busse MD, Shestak CJ (2006) Changes in microbial community structure following herbicide (glyphosate) addition to forest soils. Appl Soil Ecol 34:114–124

Ros M, Goberna M, Moreno JL, Hernandez T, Garcı′a C, Insam H, Pascual JA (2006) Molecular and physiological bacterial diversity of a semi-arid soil contaminated with different levels of formulated atrazine. Appl Soil Ecol 34:93–102

Saha S, Dutta D, Karmakar R, Ray DP (2012) Structure–toxicity relationship of chloroacetanilide herbicides: relative impact on soil microorganisms. Environ Toxicol Pharmacol 34:307–314

Sarathchandra SU, Burch G, Cox NR (1997) Growth patterns of bacterial communities in the rhizoplane and rhizosphere of with clover (*Trifolium repens* L.) and perennial ryegrass (*Lolium perenne* L.) in long-term pasture. Appl Soil Ecol 6:293–299

Sebiomo A, Ogundero WV, Bankloe SA (2011) Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. Afr J Biotech 10:770–778

Shiyin L, Lixiao N, Panying P, Cheng S, Liansheng W (2004) Effects of pesticides and their hydrolysates on catalase activity in soil. Bull Environ Contam Toxicol 72:600–606

Sofo A, Scopa A, Dumontet S, Mazzatura A, Pasquale V (2012) Toxic effects of four sulphonylureas herbicides on soil microbial biomass. J Environ Sci Health Part B 47:653–659

Statsoft, Inc, Statistica (2011) Data Analysis Software System, version 10. http://www.statsoft.com

Sukul P (2006) Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. Soil Biol Biochem 38:320–326

World Reference Base of Soil Resources (2006) A framework for international classification, correlation and communication. World Soils Resources Report. 103, FAO, Rome

Wyszkowska J (2002) Effect of soil contamination with Treflan 480 EC on biochemical properties of soil. Pol J Environ Stud 11(1):71–77

Wyszkowska J, Kucharski J (2004) Biochemical and Physicochemical Properties of Soil Contaminated with Herbicide Triflurotox 250 EC. Pol J Environ Stud 13:223–231

Yao X, Min H, Lii Z, Yuan H (2006) Influence of acetamiprid on soil enzymatic activities and respiration. Eur J Soil Biol 42:120–126

Zabaloy MC, Garland JL, Gomez MA (2010) Assessment of the impact of 2,4-dichlorophenoxyacetic acid (2,4-D) on indigenous herbicide-degrading bacteria and microbial community function in an agricultural soil. Appl Soil Ecol 46:240–246