Original Research

The Effect of Phenoxyacetic Herbicides on the Uptake of Copper, Zinc and Manganese by *Triticum Aestivum* L.

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Abstract

2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) are active substances in many weed-killer agents commonly used for the protection of crops. The literature data accompanied by our former studies showed that in weak-acid and neutral environments these compounds react with bivalent metal ions and form complexes of limited solubility in water. It was assumed that the formation of the complexes can also occur between metals present in soil and herbicides used for plant spraying, which can reduce the uptake of the metals by the above-ground parts of the plants. Our studies concerned copper, zinc and manganese determination in wheat cultured using the pot method under laboratory conditions. Cultures were treated after sprouting with either 2,4-D or MCPA. The content of copper, manganese and zinc was determined in the wheat straw. In comparison with the content in plants untreated with herbicides, the respective values were lower by ~50% for Mn, 20% for Cu and 13% for Zn.

Keywords: soil, crops, herbicides, heavy metals uptake

Introduction

Pesticides which are commonly used in agriculture are biologically active and often highly toxic synthetic organic compounds. These xenobiotics are deliberately introduced by man into the environment in order to increase crops and improve their quality. However, if used unskillfully or excessively, they can pose a threat to the environment. The pesticides and the products of their decomposition pollute the soil, ground and surface waters, agricultural and food products. This should be prevented and the effects of the use of pesticides on the environment should be continually examined.

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In this work, two main herbicides from the phenoxyacetic group were used: 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyactetic acid (MCPA). They exhibit selective weed-killer properties as broad-leaved plants are susceptible to their action, while the plants of the *Graminae* family, including crop plants, are resistant to that. Therefore, phenoxyacetic acids are used as active substances in many herbicides applied for the protection of wheat, rye, barley and oat. Since these crops are grown in big areas, the use and economic importance of these herbicides is very high. In Poland pesticides containing 2,4-D or MCPA account for ~20% of all crop protection products used in agriculture. Phenoxyacetic herbicides are usually applied in the form of a suspension at the time when crops are sprouting. Due to the low solubility of phenoxyacetic acids in water, the suspension contains their salts, amides or esters, which undergo hydrolysis when they find their way into the plants, which leads to the generation of proper active substances, i.e. the acids. After spraying, the herbicides also get into soil, where they undergo complex reactions leading to their complete biodegradation. The stability of phenoxyacetic acids in soil depend on the composition and physicochemical properties of the soil and the type and amount of microorganisms. The half-life period of 2,4-D and MCPA is considered to be 2-4 weeks [1].

In a weak-acid or neutral medium 2,4-dichlorophenoxyacetic and 4-chloro-2-methylphe-noxyactetic acids react with ions of many bivalent metals and form complexes which are slightly soluble in water and have a general formula of $ML_2 \cdot nH_2O$, where L = 2,4-D or MCPA anion and n = 0-6. Complexes were usually formed as a result of a reaction of water-soluble sodium of phenoxyacetic acid salts with nitrates of the following metals: Cu(II), Zn(II), Cd(II), Ni(II), Co(II), Mn(II) and Pb(II). They were characterized by chemical analysis, IR spectrophotometry, thermal analysis, X-ray methods and determination of solubility of the formed compounds [2-9]. The crystal structures of some of the complexes were also examined [10-16].

It was assumed that similar complex formations can also occur between phenoxyacetic herbicides and metals present in the soil. The formation of complexes slightly soluble in water should cause metals to stay in soil and underground parts of the plants and stop their transport to their above-ground parts. This assumption was further verified experimentally and the effect of spraying wheat with 2,4-D and MCPA on the uptake of Cu, Zn and Mn by the wheat stems was examined.

Although there is abundant literature concerning studies on the uptake of metals by plants and the conditions which affect bioavailability of metals, those studies were limited either to phytoremediation or searching for novel hyperaccumulator plants [17-22]. To the best of our knowledge the effect of herbicides on the uptake of metals from soil has not been described in detail so far. The importance of that subject has been raised by Y. X. Chen at al. [23]. They proved that 2,4-dichlorophenol (structurally similar compound to 2,4-D) applied to ryegrass influences the level of water-soluble copper and zinc.

Materials and Methods

Chemicals

Analytically pure 2,4-dichlorophenoxyacetic acid (98%, Aldrich, molecular weight 221.04) was purified by recrystallization from toluene. The melting temperature of the herbicide after purification was 141°C (lit. m.p. 140.5°C) [24]. Technical 4-chloro-2-methylphenoxyactetic acid (MCPA), (Z. Ch. "Organika-Sarzyna", Company, Poland) of purity 96.1% was purified by recrystallization from water - methanol (1+1 by volume). Purified MCPA melts at 118°C (lit. m.p 118-119°C) [24].

The maximum dose of phenoxyacetic herbicides recommended in agriculture is 2 kg ha^{-1}. In this study the following doses used for spraying of the sprouting wheat used 2 kg ha^{-1} or 4 kg ha^{-1}. Four solutions prepared for spraying contained, like those available on the market, sodium salts of 2,4-D and MCPA. They were made by dissolving 37.4 mg and 74.8 mg of each of the herbicides in water with the addition of 0.1 mol L^{-1} NaOH in 500 mL volumetric flasks. The pH of sodium salt solutions was ~7 (pH-meter Delta 355, Mettler-Toledo). The obtained concentrations of the herbicides were 0.0748 mg L^{-1} and 0.1496 mg L^{-1}, respectively. The pots of the surface of 0.11×0.17 = 0.0187 m^2, were used for the wheat cultures, so 50.00 mL of the above-mentioned solutions ensured the application of the planned herbicide doses.

Copper, zinc and manganese stock solutions (1,000 mg L^{-1} as nitrate salts in 0.5 mol L^{-1} nitric acid) were purchased from Merck. Working solutions were prepared by appropriate dilution of stock standards.

All other reagents used were of analytical grade. Hydrochloric, nitric, sulphuric and hydrofluoric acids, magnesium chloride and potassium chloride were obtained from POCh (Poland) and hydrogen peroxide from Chempur (Poland).

In all analyses deionized water (conductivity < 0.1 µS cm^{-1}) obtained using a Polwater K-35 system (Poland) was used.

Before use, all laboratory glassware was soaking in 10% HNO_3 acid for 24h, and carefully washed with deionized water only.

Soils and Wheat Grains

Two types of soil used for agricultural purposes, collected in central Poland (the area near Bełchatów and Skierniewice), was used for the experiment. The soils were brought to the air dry state, ground and sieved through a screen with 2 mm mesh diameter. The properties of the soils were determined by pH measurement of the suspension of the soil in distilled water and in the 1 mol L^{-1} KCl solution [25] (pH meter Delta 350, Mettler-Toledo). Evaluation of the content of organic matter was performed using the gravimetric method (determination of the mass loss after heating at 550°C). Additionally, the granulometric analysis was made [26] (Table 1). Total Cu, Zn and Mn content in the soil (decomposition with the use of hydrofluoric acid) [27], the content of bioavailable forms (extraction with 1 mol L^{-1} HCl solution) [28-30] and the content of exchangeable forms (extraction with 1 mol L^{-1} $MgCl_2$ solution) [31] were also determined. Metal determination was carried out using FAAS (spectrometer AAS 932, GBC) (Table 2).

Wheat of the Rywalka variety, quality grade A, introduced into the register of crop varieties in Poland in 2003, was used for the cultures.

Certified reference material IAEA-V-10 lucerne hay (*Medicago sativa*) was used for validation procedure used for the determination of metals.

Table 1. General characteristics of soil used for wheat cultivation.

Soil	Reaction		Content of the organic matter [%]	Granulometric composition [%]		
	pH – H_2O	pH – KCl		1.0–0.1 mm	0.1–0.02 mm	< 0.02 mm
1	6.26	5.16	3.19	92.25	6.60	0.45
2	7.37	6.89	6.15	65.70	33.95	0.80

Laboratory Wheat Cultures

Four series of cultures were grown, which differed by the type of soil (acid or neutral) or the herbicide used for plant spraying (2,4-D or MCPA). The pot method was used for the cultures. Each of the two series of cultures in acid soil and in neutral soil involved, respectively, 9 and 18 pots. Samples (400 g) of soil were weighed and placed in a pot, wheat grain (20 g) were planted and covered with another 100 g of soil. The pots were placed in the room, the temperature was ~20ºC, and the moisture of soils 5-10%. Cultures were watered with measured amounts of distilled water. In each series the pots were divided into three groups (of 3 or 6 pots, respectively). In one series the plants grew without any additional treatment, and in the other two cases, a week after the planting, sprays were made after sprouting, with a solution (50 mL) of one of the herbicides. The amount of the herbicide corresponded to the doses of 2 kg ha^{-1} or 4 kg ha^{-1}. After another 3 weeks (in stage 1-3 leaves, over a dozen centimetres high) the above-ground parts of wheat were cut.

Determination of Metals in Plant Samples

The cut wheat stems were washed, rinsed with distilled water and cut into smaller pieces. Next they were dried to constant weight at 55°C and ground. Microwave mineralization of the weighed samples (about 0.7 g) was performed in a closed system using an oxidizing mixture consisting of 6 mL of concentrated HNO_3 and 2 mL of 30% H_2O_2. Heating of the sample was carried out in three cycles at increasing time, pressure and power of microwave energy (5 min., 17-20 atm., 60%; 5 min., 32-35 atm., 80%; 10 min., 42-45 atm., 100%). After cooling, the sample was transferred and diluted into a 25 mL volumetric flask. Concentrations of copper, zinc and manganese in the solutions were determined by FAAS method. Absorbance was measured against a blank sample prepared by mineralization of oxidizing mixture in the same conditions as described above (Table 3).

Accuracy of the procedure of microwave mineralization and FAAS determination of metals in the dry mass of the above-ground wheat parts, was confirmed by analysis of certified reference material IAEA-V-10 (pulverized dried Medicago sativa hay). The results of the analysis of the latter material were compared with data from a reference sheet and calculated recovery are given in Table 4. Good precision of determination of all the metals was confirmed. The obtained results demonstrate very good accuracy of copper determination (100% recovery) and satisfactory accuracy of zinc determination (87% recovery) [32]. Mn recovery is only 73%, but its content in the standard is not a certified value.

Results and Discussion

Soil Characteristics

The soils used in the study (Table 1) belong to mineral soils, as they contained less than 10% of organic matter but they varied in pH. According to Polish Standard [25] soil (1) was acid while soil (2) was neutral. The soils also had different granulometric composition – soil (1) was a sandy one type while soil (2) is sandy loam [26]. In both cases the content of silty minerals (fraction < 0.02 mm) was very low. Organic matter and silty minerals lead to strong binding of many metals, including copper and zinc, in the surface layer of soil. The acid reaction of soil and low content of organic matter and silt in the soils are conducive to higher levels of bioavailable metals [33].

The results of determination of total content of Cu, Zn and Mn as well as the content of their forms extracted with1 mol L^{-1} HCl or 1 mol L^{-1} $MgCl_2$ solutions are presented in Table 2. Hydrochloric acid is recommended by Polish Standards [28-30] as the extraction solvent for the determination of the so-called bioavailable forms of the metals examined in the present study, whereas $MgCl_2$ solution was successfully used by Tessier [31] in the procedure of sequence extraction as the solvent which releases the so-called exchangeable forms. Total content of zinc and manganese in both type of soil and the content of Cu in soil (2) are at natural levels. The content of zinc and copper correspond with the average content in soils in Poland, while the content of manganese is considerably lower than the average [33]. In soil (1) the content of copper is increased and corresponds with 3rd degree contamination by this element [34]. For the purposes of the study, however, the content of bioavailable forms of the metals is more important. Despite a large difference in the total content of copper in both soils, the amount of its bioavailable and exchangeable forms is similar and it is worth noting that the amount of the exchangeable forms is very low. The total zinc content is higher in the neutral soil (2) than in the acid one, but the proportion of bioavailable zinc content is reversed. There is more bioavailable zinc in the acid soil.

Table 2. Results of determination of the studied metals content in soils expressed as $\bar{x} \pm ts_{\bar{x}}$, p = 0.95, n = 3.

Soil	Metal	Determined contents					
		Total [mg kg^{-1}]	RDS [%]	Bioavailable forms [mg kg^{-1}]	RDS [%]	Exchangeable forms mg kg^{-1}	RDS [%]
1	Cu	66.9 ± 1.4	0.8	6.4 ± 0.1	2.0	0.14 ± 0.16	44.6
	Zn	46.4 ± 1.0	0.2	29.2 ± 1.9	0.6	8.6 ± 1.0	9.6
	Mn	162.5 ± 24.1	6.0	146.3 ± 4.4	3.0	5.0 ± 0.3	1.2
2	Cu	11.5 ± 1.0	3.4	5.7 ± 0.4	3.0	0.16 ± 0.05	13.3
	Zn	53.7 ± 5.4	4.0	21.1 ± 2.0	3.8	0.67 ± 0.13	8.6
	Mn	149.0 ± 18.7	5.0	110.8 ± 6.0	2.2	10.1 ± 0.9	3.5

Table 3. Results of determination of the studied metals content in stalk of wheat expressed as $\bar{x} \pm ts_{\bar{x}}$, p = 0.95, soil (1) n = 3, soil (2) n = 6

Series	Dose of herbicides [kg ha^{-1}]	Determined contents					
		Cu [mg kg^{-1}]	RDS [%]	Zn [mg kg^{-1}]	RDS [%]	Mn [mg kg^{-1}]	RDS [%]
I	0	9.7 ± 0.8	3.6	52.5 ± 2.9	2.3	53.6 ± 3.7	2.7
soil (1)	2	8.8 ± 0.9	4.0	45.7 ± 1.5	1.3	26.9 ± 0.2	0.3
2,4-D	4	7.8 ± 1.1	5.8	45.6 ± 4.6	4.1	26.9 ± 1.4	2.0
II	0	9.6 ± 1.0	4.3	51.9 ± 2.6	2.3	54.3 ± 2.9	2.1
soil (1)	2	8.4 ± 1.0	4.7	46.5 ± 2.3	2.0	26.8 ± 2.5	3.7
MCPA	4	7.7 ± 0.4	2.2	45.9 ± 3.1	2.7	22.2 ± 2.4	5.4
III	0	11.8 ± 1.5	12.4	42.6 ± 3.5	7.9	21.5 ± 0.7	3.1
soil (2)	2	9.6 ± 0.6	5.8	41.8 ± 0.6	1.2	13.0 ± 0.6	4.5
2,4-D	4	9.3 ± 0.4	3.7	41.1 ± 0.7	1.4	12.0 ± 0.8	6.7
IV	0	10.4 ± 0.8	6.9	39.8 ± 1.2	2.9	22.3 ± 1.0	4.4
soil (2)	2	9.9 ± 0.5	3.7	40.3 ± 0.8	1.9	16.6 ± 1.2	6.9
MCPA	4	9.5 ± 0.6	5.8	42.5 ± 1.2	2.8	16.5 ± 1.1	6.1

There are also considerable differences in the content of exchangeable Zn in the soils – in the acid soil (1) it is twelve times higher than in soil (2) with the neutral reaction. A large proportion of the manganese present in both soils is in bioavailable forms, the amount of its exchangeable forms are also high and there is twice as much of them in soil (2) than in soil (1). The metals that occur in exchangeable forms are readily available to plants and thus can also react with the herbicides introduced into the soil.

The Effect of the Herbicides on the Content of Metals in Wheat Stems

The results of the determination of copper, zinc and manganese content in the above-ground parts of wheat grown in the four series of cultures are presented in Table 3. The metals discussed in the present work exhibit a different ability to be transported to above-ground parts of plants. Zinc and manganese belong to elements of medium mobility and the mobility of copper is low so that it is accumulated in the roots [33]. As a result, stalks of wheat accumulated higher amounts of Zn and Mn than Cu. In most of the studied cases the decrease of amount of metals after treatment with 2,4-D as well as MCPA was observed. The discussion of this phenomenon for the three mentioned metals in four series of cultures is given in detail below.

Copper

The amount of copper taken up under analogous conditions in two soils of different pH and different total content of Cu (Table 4) are similar. It results from the similar

Table 4. Results of determination of the studied metals content in certified reference material expressed as $\bar{x} \pm ts_{\bar{x}}$, p = 0.95, n = 3 compared to data given in certificate.

Metal	Determined		Recommended		Recovery [%]
	$\bar{x} \pm ts_{\bar{x}}$ [mg kg^{-1}]	RDS [%]	Recommended value, [mg kg^{-1}]	95% confidence interval, [mg kg^{-1}]	
Cu	9.4 ± 0.1	0.6	9.4	8.8–9.7	100
Zn	20.9 ± 1.7	3.3	24	23–25	87
Mn	34.6 ± 0.5	1.4	47*	44–51*	74

* Information values

Table 5. Solubility of the examined metals complexes with phenoxyacetic acids (mol L^{-1}).

	2,4-D	MCPA
Cu(II)	$1.1 \cdot 10^{-3}$	$1.6 \cdot 10^{-3}$
Zn(II)	$7.0 \cdot 10^{-3}$	$1.1 \cdot 10^{-2}$
Mn(II)	$8.0 \cdot 10^{-3}$	$9.9 \cdot 10^{-3}$

content of bioavailable and exchangeable forms of Cu in the soil samples, which facilitate the uptake of the metal by plants. In the acidic soil the plant which was not treated with the herbicides took up the same amounts of copper in series I and II. Spraying of wheat with either 2,4-D or MCPA led to a similar decrease of the amount of copper transferred to the above-ground parts of the plant. The decrease of Cu content in both cases is distinctly related to the herbicide dose – the use of the 2 kg ha^{-1} dose in the spray causes a drop in the uptake of Cu by ~10%, while the dose of 4 kg ha^{-1} decreases the content of Cu in the wheat stems by ~20%. In series III and IV, in which neutral soil was used, the content of Cu in the plants that were not sprayed with the herbicides do not show such a close similarity as in series I and II. A decrease of Cu uptake by wheat stems affected by 2,4-D or MCPA is also observed, but its dependence on the dose of the herbicide is less distinct. The decrease of Cu uptake in spite of the very low content of its exchangeable forms in the soils suggests that in this case the bioavailable forms can also react with phenoxyacetic acids.

Zinc

The amount of zinc taken up by the stems of wheat grown in acid soil (series I and II) is considerably higher than those found in the plants grown in neutral soil (series III and IV). This is obviously related to the content of exchangeable and bioavailable forms of zinc in the soil samples, which is higher in the acid soil (Table 2). In series I and II spraying of the plants with 2,4-D or MCPA causes a clear decrease in the content of Zn in the wheat stems (by about 9-13%), which however, does not depend on the dose of the herbicide applied. In series III and IV, in which the plants were grown in the soils with a very low content of exchangeable Zn, no effect of 2,4-D and MCPA on the uptake of zinc by the above-ground parts of wheat is observed. These findings imply that the herbicides react with exchangeable zinc forms present in the soils.

Manganese

The plants grown in series I and II took up twice as much manganese than those in series III and IV. Total content of manganese and the amount of its bioavailable forms are higher in soil (1), but soil (2) contains more exchangeable forms (Table 2). Spraying the plants with 2,4-D or MCPA causes considerable decrease in the uptake of Mn by wheat stems. In the case of cultures grown in acid soil (1), both 2,4-D and MCPA cause a decrease in Mn content in the plants by about 50%, while in neutral soil (2) the effects of the herbicides are weaker and different: 2,4-D decreases the content of Mn by about 40% and MCPA by about 25%. As soil (2) contains more exchangeable forms of Mn than soil (1), and the effects of the herbicides on the content of manganese in wheat stems cultivated in soil (2) are smaller, there can be other factors apart from the exchangeable forms of the metal, which cause the observed decrease of metal uptake by the plants.

In the beginning we assumed that the decrease of the uptake of Cu, Zn and Mn by above-ground parts of wheat after treating them with 2,4-D or MCPA can be caused by the formation of a slightly water soluble metal – herbicide complexes. These complexes precipitate from solution in reaction of metal nitrates(V) with 2,4-D or MCPA sodium salts at pH 6-7. The decrease of bioavailability was affirmed for copper and manganese, regardless of the pH of the soil, in which the culture was grown. In the case of Zn, the same effect occurred only in the plants grown in acid soil. Discussing the possibility of complex formations, such factors as pH of soil (Table 1), content of exchangeable forms of metals (Table 2) and solubility of metal – herbicide complexes [8, 9] (Table 5) were considered.

The examination of the uptake of Zn by the stems of wheat grown in two different types of soil suggests that only metals in the exchangeable generate form complexes with 2,4-D and MCPA. The soil of neutral pH contains very low amount of exchangeable Zn forms and the content of Zn in the wheat grown in this soil does not change as the result of the added herbicides. The results of determination of Mn confirms this assumption to a large extent – both soils contain exchangeable forms of Mn and spraying the plants with the herbicides causes a substantial decrease of the content of the metal in the plants grown in them. However, if we accept this assumption, the explanation of the decrease of Mn content, which is greater in soil (1) containing less exchangeable Mn than soil (2) can be more complex. We believe that the pH value of water suspensions of the soils is in the optimum range for complex formation reactions (pH 6-7) only in the case of soil (1), whereas pH of soil (2) is higher than 7.

Interpretation of the results of determination of Cu requires consideration of another factor which can affect the formation of complexes – the solubility of the resulting compounds. Although both soils contain very slight amounts of exchangeable forms of Cu, a decrease of Cu content was observed in the above-ground parts of the plants grown in them, being proportional to the dose of the herbicide. Cu complexes with 2,4-D and MCPA are less soluble than Zn or Mn complexes and more readily precipitate from solutions. Therefore, it is postulated that the herbicides applied in the spray react also with the forms of Cu bound in the soil more strongly than the exchangeable forms. 2,4-D complexes of the metals under study are less readily soluble than those with MCPA and this can explain the stronger effect of 2,4-D than MCPA on the content of Cu and Mn in the wheat grown in soil (2). The relatively high solubility of Zn and Mn complexes and alkaline reaction of water suspension of soil (2) may also explain the lack of effect of herbicides upon the content of Zn and their weaker effect on Mn content in the stems of wheat grown in this soil.

The results of our preliminary studies [35] indicate that the formation of the metal-herbicide complex in soil is possible. We have observed that the content of bioavailable Cu and Zn forms in soils extracted with deionized water decreased after the soils were treated with the examined pesticides.

Conclusions

Our studies have demonstrated for the first time that 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyactetic acid (MCPA) decrease the uptake of copper, zinc and manganese by wheat stems. In comparison with the relevant content in plants untreated with herbicides, the largest decrease (~50%) was found for manganese. In the case of copper the maximum decrease was ~20%, and for zinc ~13%. The dependence of the decrease in metal uptake related to the dose of the herbicides was observed only in the case of copper, and wheat cultivated in acid soil. The observed effect depends on soil reaction, and is more distinct in acid soil, and also related to the content of exchangeable forms of the metals in the soil. This effect can be caused by the formation of complexes slightly soluble in water occurring in soil and additionally depends on the solubility of complexes.

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