

Elke Bloem¹, Silvia Haneklaus¹, Robert Hänsch², Ewald Schnug¹

Evaluation of soil EDTA applications on crop performance and uptake of macro- and micronutrients by agricultural crops

EDTA Applikation zum Boden beeinflusst Pflanzenwachstum und Aufnahme von Haupt- und Spurenelementen

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Abstract

Chelates such as ethylenediaminetetraacetic acid (EDTA) enter the environment from various sources but its impact on crop growth and mineral uptake has been evaluated only sporadically. In a pot experiment with graded EDTA applications the impact of free EDTA on crop performance, macro- and microelement uptake was assessed. The sensitivity towards EDTA decreased from sunflower > oilseed rape > maize. Maize was the least sensitive crop showing no visual toxicity symptoms, however, a reduction in biomass development. In comparison, oilseed rape and sunflower displayed necrotic lesions on their leaves and biomass development was significantly reduced when higher rates of EDTA were applied. Soil EDTA application increased the uptake of Mn and Zn in shoots of all three crops and in roots of maize and sunflower. In maize EDTA increased not only the uptake of Mn and Zn, but also all other investigated micronutrients in shoots with the only exception of copper. In oilseed rape EDTA applications increased the uptake of Cu, Mn and Zn in shoots while the Fe, Mn and Mo content decreased in roots. Changes in the micronutrient content in shoots of sunflowers were similar to that in oilseed rape. In roots EDTA increased the Mn uptake. Next to micronutrients EDTA influenced the macronutrient uptake of the tested crop plants.

Key words: Chelator, EDTA, oilseed rape, sunflower, maize, macro- and micronutrients, microelement mobilization

Zusammenfassung

Chelatoren wie das Ethylenediamintetraessigsäure (EDTA) gelangen über unterschiedliche Kontaminationspfade in die Umwelt. Dennoch wurde bislang die Wirkung von EDTA auf das Pflanzenwachstum im Allgemeinen und die Wirkung auf die Mineralstoffversorgung im Besonderen nur unzureichend erforscht. In einem Gefäßversuch wurde daher der Einfluss steigender EDTA Konzentrationen auf das Wachstum sowie die Aufnahme von Haupt- und Spurenelementen bei unterschiedlichen Kulturpflanzen untersucht. Die Sensitivität der untersuchten Kulturpflanzen hinsichtlich EDTA sank in der Reihenfolge Sonnenblume > Raps > Mais. Mais reagierte also am wenigsten sensitiv auf eine Behandlung mit EDTA und zeigte keine visuellen Symptome, die Hinweis auf eine EDTA Toxizität geben würden. Der Biomassertrag war jedoch bei Mais in der höchsten EDTA Stufe deutlich reduziert. Im Vergleich dazu wurden an Winterraps und Sonnenblume nekrotische Läsionen in Verbindung mit EDTA festgestellt und die Biomasseentwicklung war signifikant reduziert, wenn EDTA dem Boden in höheren Mengen zugesetzt wurde.

Institute

Julius Kühn-Institut (JKI) – Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Braunschweig, Germany¹

Technical University Braunschweig, Institute of Plant Biology, Braunschweig, Germany²

Correspondence

Dr. Elke Bloem, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Bundesallee 50, 38116 Braunschweig, Germany, E-Mail: elke.bloem@jki.bund.de

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Die Aufnahme von Mangan (Mn) und Zink (Zn) in den Spross wurde bei allen drei untersuchten Kulturen durch die Applikation von EDTA zum Boden signifikant erhöht. Mn und Zn wurden auch stärker in die Wurzel aufgenommen, nur beim Raps wurde eine signifikant geringere Mn Aufnahme in die Wurzel in Verbindung mit EDTA festgestellt. Bei Mais wurden nicht nur Mn und Zn verstärkt in den Spross aufgenommen, wenn EDTA appliziert wurde, sondern auch alle anderen untersuchten Spurenelemente mit Ausnahme von Kupfer (Cu). Bei Raps zeigten sich unterschiedliche Trends: während die Aufnahme von Cu, Mn und Zn in den Spross durch EDTA Applikation erhöht wurde, sank gleichzeitig der Gehalt an Eisen (Fe), Mn und Molybdän (Mo) in den Wurzeln. Bei der Sonnenblume zeigten sich ähnliche Veränderungen in der Spurenelementaufnahme wie beim Raps, nur der Mn Gehalt in der Wurzel stieg im Gegensatz zu Raps mit EDTA Applikation an. EDTA hatte nicht nur einen starken Einfluss auf die Verfügbarkeit der Spurenelemente, sondern beeinflusste auch die Aufnahme an Hauptnährelementen signifikant.

Stichwörter: Chelatoren, EDTA, Raps, Sonnenblume, Mais, Haupt- und Spurenelemente, Mobilisierung von Spurenelementen

1 Introduction

Ethylenediaminetetraacetic acid (EDTA) is a commonly used complexing substance that is used besides DTPA (diethylenetriaminepentaacetic acid) or NTA (nitrilotriacetic acid) in several industries such as paper- and pulp-making, electroplating, photography, textile finishing, and leather manufacturing to sequester metal ions (OVIEDO and RODRIGUEZ, 2003).

Divalent and trivalent metals such as calcium (Ca), magnesium (Mg), copper (Cu), nickel (Ni), iron (Fe), lead (Pb), zinc (Zn) and cadmium (Cd) can form strong complexes with EDTA (EKLUND et al., 2002) which is the reason why EDTA and other complexing agents have been employed in many industries. A second important source of EDTA entering the environment is its usage in cleaning agents and cosmetic articles. In Germany, considerable quantities of EDTA have been used in cleaning agents since the phosphate limiting regulation came into force in 1980 with the consequence that triphosphates were substituted by complexing substances to increase their cleaning activity through reducing water hardness. In cosmetic products EDTA was used as a preserving agent or stabilizer; in foods and aliments EDTA is known as the food additive E385, which is promoting color retention in dried and canned foods (ANONYMUS, 2014; OVIEDO and RODRIGUEZ, 2003). The direct application of EDTA in the field occurs via the application of micronutrient fertilizers such as Fe(III)-, Cu- and Zn-EDTA.

EDTA is seen critically because of its high environmental persistence and low biodegradability (ALLARD et al., 1996; KARI and GIGER, 1996; NÖRTEMANN, 1999; 2005).

Consequently EDTA is a common water contaminant because of industrial sewage water disposal in natural water bodies such as rivers and lakes (BERGERS and DE GROOT, 1994; KARI and GIGER, 1995; OVIEDO and RODRIGUEZ, 2003). Since the 1990 s the industrial release of EDTA is regulated in many countries by law (ANONYMUS, 2012) and alternative compounds were investigated (EVANGELOU, 2007) which partly replaced EDTA in cosmetics and other consumer products (KATATA et al., 2006). But still EDTA is used in high quantities. For example in Germany, the annual consumption accounts for 3700 tons of EDTA and 1600 tons of DTPA (average value for the time period of 2005 to 2009) and environmental loads originate in comparable shares from industry (~60%) and municipal sewage plants (~40%) (ANONYMUS, 2012).

EDTA is considered to be harmless for humans and mammals in environmental concentrations. A concentration of 2.2 mg L⁻¹ was predicted by the European Union Risk Assessment as the no effect concentration for EDTA in water (EUROPEAN CHEMICALS BUREAU, 2004). Higher concentrations can be toxic for soil and water organisms as well as plants. Toxic environmental effects were attributed to the ability of EDTA to increase the bioavailability and phytotoxicity of heavy metals in sewage sludge or contaminated soils and to change the permeability of cell membranes (BERGERS and DE GROOT, 1994; GRČMAN et al., 2001; HUGENSCHMIDT et al., 1993; SILLANPÄÄ et al., 1995; VASSIL et al., 1998). The toxicity of EDTA in its free form is much higher than when it is chelated with micronutrients (HUGENSCHMIDT et al., 1993). EDTA in its free form has been shown to produce toxic effects in photosynthetic algae by inhibiting cellular division, chlorophyll synthesis and biomass production while chelated with microelements no such toxicity was observed (DUFKOVA, 1984). Free EDTA displays antibacterial activity upon gram negative bacteria by disrupting their outer membranes by removing the divalent cations Ca²⁺ and Mg²⁺, causing a loss of lipopolysaccharides which makes the cells susceptible to many substances (HANCOCK, 1984; BERGAN et al., 2001).

GRČMAN et al. (2001) found necrotic lesions on leaves of Chinese cabbage in response to soil EDTA application accompanied by an increased uptake of Pb, Zn and Cd in the aboveground biomass. Strong phytotoxic effects were observed in red clover (*Trifolium pratense*) where EDTA inhibited the development of arbuscular mycorrhiza when applied as a single dose of 5 to 10 mmol kg⁻¹ soil (GRČMAN et al., 2001). The plant toxicity of EDTA in high concentrations is mainly caused by a disturbance in the mineral nutrition (ANONYMUS, 2014) and toxicity symptoms show similarities to symptoms caused by the severe deficiency of essential metals (OVIEDO and RODRIGUEZ, 2003).

Thus the toxicity of EDTA at higher rates is caused by different factors: its ability to increase the mobility of heavy metals causing an increased uptake by plants, enhanced leaching into water bodies, a disturbance of important membrane structures which again result in a disturbance in mineral uptake, and a negative impact on soil microorganisms.

It was the aim of the presented study to determine the effect of free EDTA on crop growth and mineral uptake of maize, oilseed rape and sunflower under controlled greenhouse conditions.

2 Material and methods

2.1 Greenhouse experiments

In a greenhouse experiment maize (*Zea mays* var. Prinz), oilseed rape (*Brassica napus* var. Akela), and sunflower (*Helianthus annuus* var. Sonja) were grown in sand culture. Eight plants were sown in "Mitscherlich" pots containing 7–8 kg of washed sand. All pots were fertilized uniformly at sowing and start of main vegetative growth each time with nitrogen (N) in form of NH_4NO_3 (750 mg N per pot) and Ca and phosphor (P) in form of $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (100 mg Ca and 155 mg P per pot). Sulfur (S) was fertilized at a dose of 250 mg per pot to oilseed rape and 50 mg S per pot to maize and sunflowers in form of K_2SO_4 at sowing. All pots received a uniform application of a magnesium-microelement solution at sowing supplying 50 mg Mg, 10 mg Fe, 1 mg Mn, Zn, 0.5 mg molybdenum (Mo), 0.2 mg Cu and 0.1 mg boron (B). The same amount of micronutrients was applied again when main growth started. EDTA was applied 4 weeks after sowing. The EDTA treatment was split into 4 rates which were dissolved in water before being applied together with distilled water for routine irrigation. EDTA was applied at rates of 0, 0.5, 1.7, and 3.3 g per pot equaling 0, 150, 550 and 1050 kg ha^{-1} EDTA which were applied in a field experiment (BLOEM et al., 2016). Oilseed rape plants were thinned to 4 plants per pot when main vegetative growth started and maize to 3 plants per pot. In case of sunflowers plants were much smaller and several plants died off after EDTA treatment so that no thinning was required. Vegetative aboveground plant parts and roots of maize were harvested when the third leaf collar was visible (GS 12/13), in case of oilseed rape at early stem elongation (GS 30) and leaves and stems from sunflower when internodes were visibly elongated (GS 30). In case of sunflower results for shoots refer to leaves and stems unless depicted separately. Growth stages were determined on basis of the phenotypical codes of STAUSS et al. (1994). In shoots, stems and roots the total concentration of macro- and microelements was determined.

2.2 Determination of macro- and micronutrients in plant material

Plant samples, shoots and roots of all plant species and stems of sunflower were dried in a ventilated oven at 60°C until constancy of weight. Afterwards samples were fine-ground to a particle size of ≤ 0.1 mm using the ultracentrifugal mill ZM 1000 (Retsch GmbH, Haan, Germany) before microwave digestion. For microwave digestion 0.5 g dry plant material was digested with 6 mL HNO_3 (65%) + 1.5 mL H_2O_2 (30%) in a microwave oven (CEM/Mars xpress, Kamp-Lintfort, Germany) at 600 Watt and 120°C for 2 minutes followed by an extraction step

at 200°C for 15 minutes. After cooling down the samples were filled up to 50 mL with bi-distilled water and filtrated. Ca, K, Mg, P, S, Cu, B, Mn, Zn and B were determined by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES icap 6000, Thermo), Mo and other microelements at lower concentrations by high resolution sector field ICP-MS (Element XR, Thermo).

2.3 Statistical analysis

Statistical data analysis was conducted using the COSTAT software package employing analysis of variance (ANOVA) and by Tukey's test. Significant differences were determined at $p < 0.05$.

3 Results

In a sand culture experiment the effect of EDTA on growth and mineral uptake of three different plant species (*Zea mays*, *Brassica napus*, *Helianthus annuus*) was studied. The plants were sufficiently fertilized with all essential plant nutrients and received four different doses of EDTA (0, 0.5, 1.7, and 3.3 g pot^{-1} EDTA). All three species were harvested at main vegetative growth: maize when the third leaf collar was visible, oilseed rape at early stem elongation and sunflower when internodes were visibly elongated. Shoots and roots were harvested and the macro- and micronutrient content determined. The impact of graded doses of EDTA on plant biomass is shown in Table 1.

The different plant species showed strong differences in their sensitivity towards EDTA: sunflower was the most sensitive crop and reacted already to a dose of 0.5 g pot^{-1} with a biomass reduction of more than 50% and necrotic lesions on the leaves (Table 1, Fig. 1). At 1.7 g pot^{-1} EDTA an elevated number of necrotic lesions on leaves was counted and at the highest dose of 3.3 g pot^{-1} EDTA plants almost died off (Fig. 2). At the highest EDTA dose the biomass of sunflower was reduced by 90% (Fig. 1).

Necrotic lesions could be detected also on older leaves of oilseed rape when 1.7 and 3.3 g pot^{-1} EDTA were applied. Shoot biomass of oilseed rape was significantly reduced by 43% at 1.7 g pot^{-1} EDTA (Table 1) in comparison to the control (Fig. 1). Maize was least sensitive against EDTA and no visible symptoms were observed (Fig. 2), but shoot biomass was still reduced at the highest EDTA dose by up to 32% and root biomass by 57% compared to the control (Fig. 1).

Visible necrotic lesions were only observed in the dicotyledonous crops oilseed rape and sunflower. Lesions first appeared on older leaves but also stems showed toxicity symptoms after EDTA application (Fig. 3). Oilseed rape plants regularly lose older leaves during main growth. Plants that were affected by EDTA lost more leaves when compared to the control plants.

Root growth of all crops was stronger reduced by EDTA than that of shoots and the effect was dose dependent (Fig. 1). Root biomass was reduced by 57%, 76%, and

Table 1. Influence of graded doses of EDTA on shoot and root biomass of maize, oilseed rape, and sunflower [g fresh weight]

Biomass at harvest [g fresh weight] Crop	EDTA application [g pot ⁻¹]				LSD _{5%}
	0	0.5	1.7	3.3	
Maize (<i>Zea mays</i>)					
Shoots (3 plants)	163	147	139	111	21.4
Roots (3 plants)	62.3	39.7	39.2	26.9	8.4
Oilseed rape (<i>Brassica napus</i>)					
Shoots (4 plants)	113	99.8	64.9	62.1	27.6
Roots (4 plants)	43.7	19.6	11.1	11.0	13.0
Sunflower (<i>Helianthus annuus</i>)					
Shoots (1–8 plants*)	43.1	20.5	7.1	4.3	8.8
Roots (1–8 plants*)	9.3	4.3	2.3	1.6	2.7

* originally 8 plants were sown per pot but with increasing EDTA rates plants died off resulting in a varying number of harvested plants per pot.

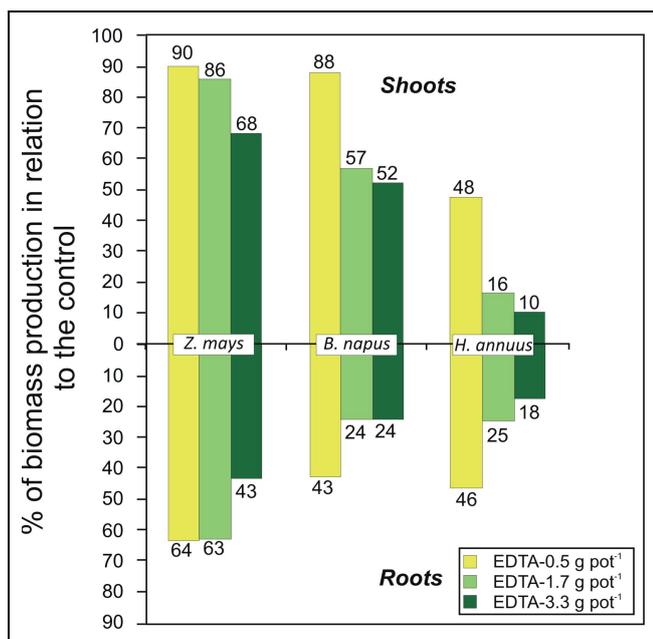


Fig. 1. Relative biomass development (control plants = 100%) of shoots and roots of maize, oilseed rape and sunflowers in relation to EDTA rates.

82% in maize, oilseed rape and sunflower when compared to the control by the highest EDTA rate (Fig. 1).

EDTA negatively affected shoot and root growth of oilseed rape, maize and sunflower. Microelements such as Fe and Mn form EDTA complexes and by this their availability and plant uptake is significantly increased (VASSIL et al., 1998). In the presented experiment the supply with essential plant nutrients was sufficiently high for maxi-

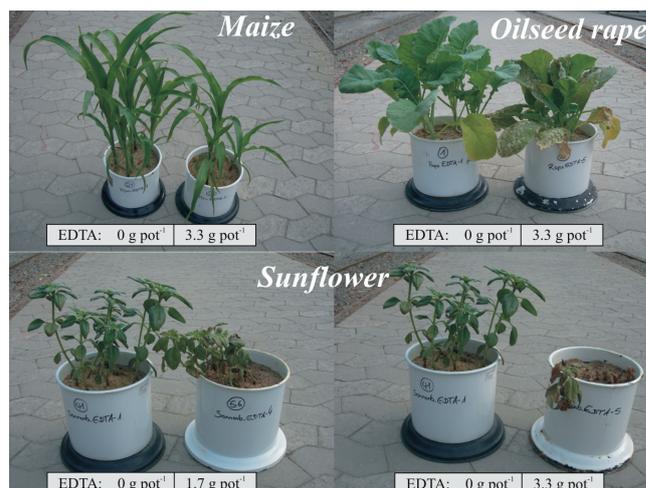


Fig. 2. Influence of soil EDTA application on growth of maize, oilseed rape and sunflower.

imum growth. Expectedly EDTA influenced the uptake of minerals. The effect of graded doses of EDTA on the uptake of macro- and micronutrients is summarized in Table 2 for maize and oilseed rape and in Table 3 for sunflower. The data in Table 2 and 3 reveal that all crops were sufficiently supplied with macro- and micronutrients (REUTER and ROBINSON, 1997).

The plant species were affected differently by EDTA application. EDTA application increased the uptake of all macronutrients in oilseed rape. In case of maize EDTA yielded a higher K, P and S uptake, but reduced that of Ca. With a view to sunflowers EDTA increase the K uptake in



Fig. 3. Symptoms of EDTA toxicity on leaves of oilseed rape (A) and sunflower (B), and stems of sunflower (C).

leaves, while that of Ca and Mg decreased. In stems a higher K, P and S content was found and in roots a higher Ca, P and S concentration (Table 2). All of these effects proved to be significant.

Not only macro-, but also patterns of micronutrient concentrations varied between the tested crop plants. EDTA increased the micronutrient concentration in shoots and roots of maize. A similar effect was observed in shoots of oilseed rape, but in roots EDTA caused a significant decrease of the Fe, Mn and Mo content (Table 2). With reference to sunflower the Mn content increased significantly in roots, stems and leaves, the Cu and Zn in leaves and the Fe content in stems (Table 3). EDTA significantly enhanced the Mn and Zn uptake of all three plant species.

EDTA application increased the uptake of several macro- and micronutrients (Table 2, 3), but it negatively influenced biomass production (Table 1). The off-take of macro- and micronutrients with harvest products reflects changes in nutrient uptake and crop growth in relation to EDTA rates. In Table 4 values for the mean nutrient off-take per pot by shoots are shown for all three crop plants in relation to EDTA rates.

The nutrient off-take of most macro- and microelements decreased with EDTA application because of its negative effect on growth. Only in case of maize where EDTA had only minor effects on plant growth the Fe, Mn and B off-take increased together with the EDTA dose. The concentration of these nutrients increased over-proportionally and strongest in relation to the EDTA rate compared to Cu, Zn and Mo (Table 2). Striking is that the B and Mn off-take of all crops exceeded consistently the supply with the nutrient solution irrespective of the EDTA treatment. For both elements the nutrient concentration is in the range of the upper limit for an optimum supply. This suggests that plants mobilized B and Mn from impurities of the sand that were not washed off by water and EDTA apparently reinforces this effect.

4 Discussions

4.1 High rates of soil-applied EDTA negatively affected plant biomass

Pot experiments in sand culture deliver the possibility to investigate the direct effects of EDTA on plant performance

under controlled conditions as there are for instance no interactions with clay minerals or humic substances. The highest EDTA rate of 3.3 g pot^{-1} caused considerable yield reductions in all three crop plants. The response of the plant species to graded rates of EDTA proved to be markedly different. Toxicity symptoms were strongest in sunflower. Some plants died off at the highest EDTA level. Distinctive toxicity symptoms expressed as necrotic lesions on leaves and stems occurred if $\geq 1.7 \text{ g pot}^{-1}$ EDTA were applied. At lower rate (0.5 g pot^{-1} EDTA) symptoms were still visible and occurred together with a biomass reduction of more than 50%. A sensitivity of sunflower against EDTA was described by CHEN and CUTRIGHT (2001). The same authors found a dose of $0.5 \text{ g EDTA kg}^{-1}$ substrate to be most efficient for plant metal accumulation in the context of phytoremediation. This dose corresponds with the highest EDTA level in the presented experiment and caused a significant reduction of biomass. It can be expected that on natural soils where interactions between EDTA and the soil matrix take place the toxicity of EDTA is less pronounced.

Oilseed rape was less sensitive than sunflower but considerably more susceptible than maize. Oilseed rape showed necrotic lesions on older leaves when $\geq 1.7 \text{ g pot}^{-1}$ EDTA was applied to the soil. In case of maize the highest dose of 3.3 g pot^{-1} EDTA reduced shoot and root biomass significantly but no visual toxicity symptoms appeared. SHEN et al. (2002) determined a different response of plant species (cabbage, mung bean, and wheat) to EDTA application in a pot trial on a soil contaminated with Pb. Cabbage reacted most sensitive with a biomass reduction of 38% in comparison to 11% of mung beans and 27% of wheat. CHEN et al. (2004) determined differences in the sensitivity of dicotyledonous and monocotyledonous species to a treatment with $5 \text{ mmol EDTA kg}^{-1}$ soil. On average the shoot dry weight of the monocotyledonous species was less affected by the EDTA application. RENGEL (1999) found adverse effects on the growth of wheat at an H-EDTA concentration of $50 \mu\text{M}$ in hydroponics while no toxicity symptoms were observed at concentrations $\leq 25 \mu\text{M}$. He assumed that toxic effects are induced by direct toxicity of the chelators caused by interferences with molecular structures and functions at higher H-EDTA concentrations.

In the presented experiment it was most likely this direct toxicity of EDTA that caused the strong negative

Table 2. Total macro- and micronutrient concentration in shoots and roots of maize and oilseed rape

EDTA [g pot ⁻¹]	0	0.5	1.7	3.3	LSD _{5%}	0	0.5	1.7	3.3	LSD _{5%}
Maize (GS 12/13)										
Element	Macronutrient concentration [g kg ⁻¹] in shoots					Macronutrient concentration [g kg ⁻¹] in roots				
	Ca	5.8*	5.9	4.7	4.5	0.9	8.1	7.3	5.9	4.7
K	5.6	5.0	6.7	7.5	1.1	2.8	3.0	3.3	4.0	1.0
Mg	3.5	3.3	3.1	3.0	0.6	1.9	2.3	2.1	1.9	0.5
P	3.1	2.9	3.6	3.8	0.4	1.4	1.6	2.0	2.3	0.4
S	1.0	0.9	1.2	1.4	0.2	0.6	0.8	1.0	1.3	0.2
Element	Micronutrient concentration [mg kg ⁻¹] in shoots					Micronutrient concentration [mg kg ⁻¹] in roots				
	B	34	36	21	58	33	15	14	10	18
Cu	8.3	8.5	9.0	8.2	1.4	8.9	11.6	13.6	11.6	2.4
Fe	74	141	132	148	25	3399	2783	3141	2654	1374
Mn	231	440	445	593	117	357	943	1422	1270	664
Mo	0.59	0.79	0.72	0.84	0.22	0.53	0.63	0.76	0.94	0.18
Zn	24	38	36	36	9	25	46	47	58	12
Oilseed rape (GS 30)										
Element	Macronutrient concentration [g kg ⁻¹] in shoots					Macronutrient concentration [g kg ⁻¹] in roots				
	Ca	11.7	12.9	14.5	16.0	3.1	3.2	3.3	4.3	5.2
K	12.5	16.5	18.4	21.0	4.9	7.2	10.2	14.3	15.1	4.4
Mg	2.6	3.2	3.3	3.3	0.5	1.4	1.2	1.5	1.7	0.3
P	6.0	6.7	7.9	8.2	1.2	2.8	4.0	5.3	6.0	1.1
S	5.2	7.5	8.2	8.9	2.1	3.0	4.8	5.5	6.0	1.5
Element	Micronutrient concentration [mg kg ⁻¹] in shoots					Micronutrient concentration [mg kg ⁻¹] in roots				
	B	55	39 a	57	34	46	16	15	16	15
Cu	9.6	12.7	12.1	9.6	2.0	0.16	0.28	0.29	0.30	0.17
Fe	703	1198	949	539	393	6425	3380	2627	2482	1696
Mn	803	1338	1780	1863	395	1029	1525	992	891	530
Mo	2.3	1.7	1.8	2.0	0.9	1.3	1.1	0.9	0.9	0.3
Zn	73	114	104	96	27	46	65	79	96	11

* Bold letters indicate a significant increase or decrease (shaded) in elemental concentrations in relation to EDTA application.

impact on plant performance as no critical heavy metal concentrations existed in the growth medium. EDTA was applied in its free acidic form and it was shown by VASSIL et al. (1998) that free H-EDTA is more phytotoxic than metal-bound EDTA, and that it caused stronger growth reductions. The plant available heavy metal fraction is often low in agricultural soils due to the strong association with organic matter, Fe-Mn-oxides, clay minerals or precipitations as carbonates, hydroxides and phosphates (MCBRIDE, 1994). EDTA can enhance the mobility of such heavy metals and thus cause its accumulation in shoots. Toxicity symptoms like the formation of necrotic lesions is accompanied by a significant loss of water from shoot tissue (VASSIL et al., 1998) and may be attributed to the presence of free protonated EDTA which can bind to var-

ious essential divalent cations, disrupting the biochemistry of the cells and ultimately causing cell death. It was speculated by VASSIL et al. (1998) that EDTA-metal complexes were taken up by plants when a threshold concentration of EDTA is reached above which the physiological barrier(s) in roots are destroyed that usually function to control uptake and translocation of solutes. The sensitivity of different plant species against EDTA can be explained probably by differences in their physiological barriers: in case of the most sensitive crop of this research, sunflower, biomass development of roots and vegetative parts were similarly affected by EDTA while in case of more resistant crops the root biomass was stronger affected than that of the vegetative parts (Fig. 1) indicating more efficient barriers.

Table 3. Total macro- and micronutrient content in leaves, stems and roots of sunflower at GS 30

EDTA [g pot ⁻¹]	0	0.5	1.7	LSD _{5%}	0	0.5	1.7	LSD _{5%}	0	0.5	1.7	LSD _{5%}
Element	Macronutrient concentration [g kg ⁻¹] in leaves				Macronutrient concentration [g kg ⁻¹] in stems				Macronutrient concentration [g kg ⁻¹] in roots			
Ca	39*	33	26	6	12	13	14	3	3	9	19	9
K	17	26	27	3	27	37	41	7	10	17	10	11
Mg	7.8	5.7	4.6	0.9	9.2	8.9	8.3	2.1	1.7	2.3	2.6	1.1
P	8.9	8.7	9.3	0.7	4.9	6.8	8.4	0.5	3.3	7.0	8.8	3.7
S	4.1	4.5	3.9	0.6	2.7	3.6	4.3	1.1	1.0	1.9	2.2	0.9
	Micronutrient concentration [mg kg ⁻¹] in leaves				Micronutrient concentration [mg kg ⁻¹] in stems				Micronutrient concentration [mg kg ⁻¹] in roots			
B	207	280	190	239	30	44	35	38	18	40	30	39
Cu	25	32	30	4	15	15	18	7	16	28	27	13
Fe	613	867	1012	413	126	211	721	484	3425	2946	3978	1750
Mn	913	1601	1464	396	131	327	592	271	201	743	1796	889
Mo	0.7	0.7	0.9	0.3	0.4	3.3	0.9	5.4	3.0	3.2	6.1	4.1
Zn	87	121	127	16	46	50	67	22	50	80	84	36

* Bold letters indicate a significant increase or decrease (shaded) of elemental concentrations in relation to EDTA application in different plant parts; the highest dose of 3.3 g pot⁻¹ EDTA was not included in the statistics as too many plants in this treatment died off.

4.2 High rates of soil-applied EDTA influenced the mineral uptake of crop plants

Free EDTA can disrupt membrane functions by removing divalent cations and lipopolysaccharides, thus making cells susceptible against various substances (HANCOCK, 1984; PELLETIER et al., 1994; BERGAN et al., 2001). This happens as EDTA is able to chelate divalent cations such as Mg²⁺ which are essential for the stabilization of outer membranes. This way the membrane permeability is increased and under extreme conditions even cell lysis may occur in response to EDTA (PELLETIER et al., 1994). SHAHID et al. (2012) concluded from their studies that a disruption of the Casparian strip is most likely responsible for a higher Pb accumulation in aerial plant parts when plants were exposed to EDTA.

It was shown in different studies that application of chelates to the soil did not only increase the total dissolved metal concentration but did also change the primary route of metal uptake from the symplastic to the apoplastic pathway as damage of physiological barriers in plant roots can cause a rapid uptake from soil solution via the xylem into the shoots (CHEN et al., 2007; LUO et al., 2006; NOWACK et al., 2006; WENGER et al., 2005). WENGER et al. (2005) supposed that a high-affinity transport system exists together with a low-affinity system for the uptake of metals and the proportions of these transport pathways can be changed by the availability of ligands. As chelation and sequestration in vacuoles are part of the natural plant defense mechanisms and metals are not bioavailable in this form, it is possible that plants

do not perceive metals bound in this form and will not activate feed-back inhibition to reduce further uptake. COLLINS et al. (2002) concluded from their studies that for Zn-EDTA species specific uptake routes exist: while *Hordeum vulgare* and *Solanum tuberosum* took up Zn-EDTA via the apoplastic pathway, *Brassica juncea* was only able to take up Zn-EDTA after physiological damage. In another study with two different genotypes of sunflower no effect of EDTA in the nutrient solution (0.1 mM EDTA) on metal uptake (Pb) or even plant growth was detected (DONCHEVA et al., 2013).

All previously mentioned studies demonstrate that besides metal and EDTA concentration there are species-dependent differences in metal uptake in relation to EDTA. A comparison of the existing studies suggests that experimental conditions seem to influence kind and strength of observed effects.

In the present pot experiment a significant increase of the Mn uptake took place in all three plant species which resulted in tissue concentrations that can be regarded as high (HUMPHRIES et al., 2007). Obviously Mn was mobilized from the substrate by EDTA application and accumulated in the plant tissue. This effect was most pronounced in case of sunflower when 1.7 g pot⁻¹ EDTA was applied. Then the Mn concentration increased in roots by factor 9 and in vegetative parts by factor 1.6–4.5 in comparison to the control. For the same treatment the Mn content increased by factor 4 in roots and by factor 1.9 in shoots of maize. Only in oilseed rape an opposite effect was determined. Here, the highest Mn content was deter-

Table 4. Nutrient off-take by shoots of maize, oilseed rape and sunflower in relation to EDTA rates

EDTA [g pot ⁻¹]	0	0.5	1.7	3.3	0	0.5	1.7	3.3	0	0.5	1.7	3.3
Element	Nutrient off-take [mg pot ⁻¹] of maize				Nutrient off-take [mg pot ⁻¹] of oilseed rape				Nutrient off-take [mg pot ⁻¹] of sunflower			
Ca	128	123	90	72	193	161	125	110	159	74	25	18
K	125	106	129	121	205	209	161	147	109	81	35	32
Mg	77	70	60	48	44	41	28	23	44	20	6.5	5.8
P	69	62	68	62	98	84	69	57	40	22	8.9	9.4
S	21	20	23	22	86	95	71	61	19	12	4.0	3.6
B	0.73	0.75	0.42	0.98	1.0	0.49	0.50	0.24	0.78	0.41	0.11	0.13
Cu	0.18	0.18	0.18	0.13	0.16	0.16	0.10	0.07	0.12	0.07	0.02	0.03
Fe	1.6	3.0	2.6	2.4	11	15	8.1	4.0	2.4	1.6	0.74	1.2
Mn	5.1	9.3	8.6	9.7	13	17	15	13	3.5	3.2	0.97	1.2
Mo	0.013	0.017	0.014	0.014	0.036	0.022	0.015	0.014	0.003	0.005	0.001	0.001
Zn	0.52	0.80	0.71	0.57	1.2	1.4	0.90	0.69	0.39	0.26	0.09	0.10

mined in the roots of the control plants. In the shoots of oilseed rape the Mn concentration increased twofold.

Based on the analyzed data a translocation concentration factor (TCF) can be calculated which equals the shoot to root concentration of an element (DE LA ROSA et al., 2007). The TCF delivers information about the translocation of minerals from roots to shoots. Values < 1 indicate a higher accumulation of an element in the roots, while values > 1 reflect an accumulation in shoots. In case of oilseed rape the TCF for Mn increased from 0.8 (control) to 2.1 (highest EDTA dose). In contrast, in maize and sunflower the TCF proved to be consistently < 1 irrespective of the EDTA dose for Mn. These data show that the response of plants to graded doses of EDTA is species-specific.

Mn and Zn were the only metals that showed a significant response to EDTA in all crop plants. The Cu concentration boosted with graded EDTA rates in leaves and stems of sunflowers while this effect was not observed for maize and oilseed rape. A similar effect was found for the Mo concentration in the shoots of maize. A heterogeneous effect was also established for Fe: the concentration was elevated in shoots of maize and leaves and stems of sunflower, but reduced in roots of oilseed rape.

The presented experiment revealed that graded doses of soil-applied EDTA increased the micronutrient concentration in maize, sunflower and oilseed rape. At the same time the off-take of micronutrients declined because of the negative impact on plant growth (Table 4). This strong growth effect is restricted to pot experiments, in particular those in hydroponics and sand culture because of an unnaturally high root density and lack of buffer capacity of natural soils. Accordingly, it was shown in a field experiment that EDTA application rates similar to that in

the presented pot trial had only a slight negative impact on plant growth because of the interaction of EDTA with the soil matrix and the translocation of EDTA within the soil profile (BLOEM et al., 2016). The most distinctive effect found in the current study is that EDTA significantly increased Mn and Zn concentrations of shoots in all three crops. Hereby mobilization of Mn and Zn seems to be fortified strongly by EDTA (see above).

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