



Article

Biodegradation Rate of EDTA and IDS and Their Metal Complexes

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Abstract: Ethylenediaminetetraacetic acid (EDTA), when used as a main chelator for complex plant microfertilizers, causes many negative environmental effects; therefore, new compounds or new use of the known compounds to replace EDTA have been widely studied. In the present study, biodegradation rate, plant (*Raphanus sativus*) growth stimulation and ecotoxicity towards *Daphnia magna* and *Chlorella vulgaris* of iminodisuccinic acid (IDS), considered as an alternative for EDTA in agriculture, has been investigated. It was demonstrated that IDS' biodegradation rate over 28 days was 28.1%, which is 4.5 times higher than that of EDTA. Similar to EDTA, complexation with metals led to an increase in the degradation rate by 1.8-fold. The majority of compounds were degraded within first 7 days. The GI values for IDS implemented at concentrations of 100 mg/L (both in pure form and in combination with microelements) were 2.4–2.6 times higher than those of EDTA. The ecotoxicity index EC10 of IDS was estimated to be 2.0 g/L and 5.8 g/L towards *D. magna* and *Ch. vulgaris* which are 4.1- and 10-fold higher than those for EDTA, respectively. It can be concluded that IDS is a promising agent to chelate microelements used in plant nutrition.

Keywords: biodegradation; complex microelement fertilizer ethylenediaminetetraacetic acid; iminodisuccinic acid; non-target biota; toxicity



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1. Introduction

The world's population is increasing every year, which leads to an increase in the amount of food requirements. The use of fertilizers can increase yields of crops in agriculture. The most effective is the joint application of macro- (N, P, K, Ca, Mg) and microelement (Cu, Fe, Zn, B, Mo, Co, Mn) fertilizers into the soil. However, the use of fertilizers also leads to negative consequences for the environment, for example, pollution of ground and surface waters, increased eutrophication of water bodies, soil degradation, damage to the environment, loss of biodiversity and greenhouse gases emission [1]. Microelement fertilizers in the form of mineral salts are characterized by low bioavailability, high toxicity, and are quickly washed out from the soil [2]. A way to increase the bioavailability and to reduce the ecotoxicity of microelements when used as fertilizers is their use in the form of organomineral complexes—chelates. Chelating agents should meet several requirements such as efficient transport of the microelements into plant cells, high biodegradability and low toxicity of the initial compounds and their biodegradation metabolites for plants and the soil microbiome. It is desirable that the metabolites of chelating agents could be used by plants for the synthesis of peptides, for example, in transamination reactions during the biosynthesis of glutamic acid, which is necessary for the formation of chlorophylls [3], or by the soil microbiome as a source of carbon. A commonly used chelating agent is ethylenediaminetetraacetic acid (EDTA) [4], which meets the majority of requirements listed above. However it is reported that EDTA is slowly degradable, and, as a result, it

accumulates in ground and surface waters and in soil [5–8]. In the soil, EDTA being an acid, lowers the pH and promotes the desorption of heavy metals (copper, zinc, cadmium, chromium), converting them into a soluble form, and making them available for absorption by plants, which contributes to their further transfer along the trophic chains [5–11]. Furthermore, EDTA was demonstrated to be able to destroy the outer membranes of some soil bacteria [12]. In recent studies, it was revealed that in high concentrations that can be reached as a result of environmental accumulation, EDTA even possesses cytotoxic and slightly genotoxic properties [13,14]. From surface water, EDTA may be transferred to humans and animals causing adverse effects on the reproductive function and development of animals. Thus, according to the World Health Organization, the EDTA concentration in drinking water should not exceed 600 µg/L [14].

Microorganisms capable of EDTA degradation are quite rare [15]. In connection with the accumulation of EDTA in the environment and the negative consequences of such accumulation described above, there is an active search for new agents and new ways to remove EDTA. Thus, in 1990 Lauff with co-authors described *Agrobacterium* sp., which was able to degrade EDTA [16]. Later, Thomas with co-authors found 13 strains belonging to the *Methylobacterium*, *Variovorax*, *Enterobacter*, *Aureobacterium* and *Bacillus* genera that were able to biodegrade 0.1–0.2 mmol EDTA or EDTA complexes with metals per g biomass in an hour. The authors found that the primary degradation of EDTA is catalyzed by EDTA-monooxygenase [17]. A.D. Satroutdinov with co-authors demonstrated that *Agrobacterium* sp. is capable of destroying the Fe(III)–EDTA, while the Gram-negative BNC-1 strain is capable of destroying Mg–EDTA, Ca–EDTA, Mn–EDTA and Zn–EDTA complexes [18]. The mechanisms of microbial degradation of EDTA are described for the microbial community of activated sludge through ethylenediamine triacetate [19,20]. However, the main pathway of EDTA degradation in the environment is still abiotic decomposition under the action of UV rays. The intensity of this pathway depends on climatic conditions and the areas of EDTA accumulation (ground, underground, etc.) [8,15,21,22].

As an alternative to EDTA, it is proposed to use other chelating agents that are also related to aminopolycarboxylates, but of natural origin. The advantage of natural aminopolycarboxylates is that they form low-toxic chelate complexes with metals with comparable chelating ability [23]. The best-known representatives of aminopolycarboxylate complexes are nitrilotriacetic acid, methylglycine diacetic acid, glutamic-N,N-diacetic acid, ethylenediaminesuccinic acid, ethylenediaminedimalonic acid, 3-hydroxy-2,2-iminodisuccinic acid, 2-hydroxyethyliminodiacetic acid and pyridine-2,6-dicarboxylic acid [6,24]. Based on their chemical structure, it can be assumed that, in addition to low ecotoxicity, high biodegradability and the ability to provide plants with macro- and microelements, they will stimulate plant growth due to the metabolites formed during their biodegradation. The first described natural polyaminocarboxylic acid—ethylenediamine-N,N′-succinic acid—was isolated from the culture fluid of actinomycete *Amycolatopsis orientalis* [21]. According to McDougall [25], ethylenediamine-N,N′-succinic acid is an effective biodegradable alternative to EDTA. Interestingly, in areas other than agriculture, alternative EDTA complexonates have been used for quite some time [23,26]. In agriculture (as a chelating agent for trace elements), EDTA is still dominant, although this area has more impact on human health than others [27].

The most common alternative for EDTA (used in 13% of the world fertilizer market in 2020 [28]) in the field of fertilizers is diethylenetriamine pentaacetate (DTPA); however this chelating agent belongs to the same group of synthetic aminopolycarboxylates and has similar disadvantages. In addition, its use is suggested for an important, but rather narrow area—the elimination of iron deficiency in the soil and plants. Ligands such as ethylenediamine-N,N′-disuccinic acid or its salts, glutamic acid diacetic acid, methylglycine diacetic acid and iminodisuccinic acid (IDS), are suggested as alternatives in the scientific literature [29–31]. However, no industrial technology of inexpensive production of those compounds has been implemented yet. Additionally, data on their ecotoxicity as well as biodegradability are still poor.

In the present study, we analyzed the ecotoxicity and biodegradability of the IDS and IDS–metal complexes that potentially might be used as microfertilizers in agriculture. The water flea *Daphnia magna*, the green algae *Chlorella vulgaris* and the higher plant *Raphanus sativus* were used as test-objects in bioassays. Biodegradability was estimated using compost microbial community on the basis of carbon dioxide release. For comparison, the analogous set of data for EDTA and EDTA–metal complexes were obtained. It was hypothesized that (i) due to the presence of the NH group bound with two succinic fragments in their chemical structures, the IDS–metal complexes are more biodegradable as compared with EDTA complexes, because even the very first step of biodegradation leads to forming fumaric and aspartic acids (EDTA complexes at the same time undergo step-by-step biodegradation via tri-, two- and monoacetic acids as well as iminodiacetic acid, ending with ethylenediamine), and (ii) because of slight decrease in binding capacity, IDS–metal complexes are non-toxic for aquatic organisms but effective enough as chelated fertilizer.

2. Materials and Methods

2.1. Samples

Iminodisuccinic acid (IDS) was investigated as a biodegradable chelating agent, ethylenediaminetetraacetic acid (EDTA) was used as a comparison. For this, $(\text{NH}_4)_3\text{IDS}$ was synthesized following known protocols [32,33] and EDTA disodium salt was purchased from Sigma Aldrich (27285-1KG-R). The commercial preparation Akvarin Universal, produced by Buyskii Chemical Plant (Russia) on the base of EDTA salts, was chosen as a prototype of a complex fertilizer based on EDTA. Based on the ratio of trace elements in it, 2 complex fertilizers based on EDTA and IDS were prepared (Table 1).

Table 1. Samples.

Sample	Na ₂ EDTA	K6-EDTA	(NH ₄) ₃ IDS	K6-IDS
Chelating agent	EDTA	EDTA	IDS	IDS
Fe	-	0.054%	-	0.054%
Mn	-	0.042%	-	0.042%
Zn	-	0.014%	-	0.014%
B	-	0.02% (non chelated)	-	0.02% (non chelated)
Cu	-	0.01%	-	0.01%
Mo	-	0.004% (non chelated)	-	0.004% (non chelated)
Molecular Formula	$\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$		$(\text{NH}_4)_3\text{C}_8\text{H}_{20}\text{N}_4\text{O}_8$	

The first of them is a complete analogue of the commercial preparation Aquarin, and it was prepared using individual solutions of commercial salts of Zn (EDTA), Cu (EDTA), Mn (EDTA), Fe (DTPA) and B (KBO_2), all produced by the Buyskii Chemical Plant (Russia) as well as Mo (hexaammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$). The second was based on IDS and was prepared using individual mixing solutions of IDS salts (IDS*Fe, IDS*Zn, IDS*Cu, IDS*Mn) as well as H_3BO_3 and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ keeping the same ratio of micronutrients. Individual IDS salts were prepared by reaction of $(\text{NH}_4)_3\text{IDS}$ with, individually, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, in a 1:1 molar ratio with respect to the metals.

2.2. Biodegradation

The biodegradability of individual chelating agents and complex fertilizers was evaluated in a statistical water test system according to GOST R ISO 9439-2016 «Water quality. Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium. Carbon dioxide evaluation test» Standartinform: Moskau, Russia, 2016, Test No. 301 OECD [34]. For this, an aqueous mixture of 0.03 L was prepared, consisting of the test substance, the only source of carbon, micro- and macroelements (K, P, Na, N, Cl, Mg, Fe) and microbial inoculum. As a microbial inoculum, a microbial community isolated from compost, passaged 2 times, was used. A daily culture was added to the aqueous test system so that the concentration of microorganisms varied in the range of 1×10^4 – 1×10^5 CFU/mL

of the final mixture. Checking the activity of the microbial inoculum was carried out using a control compound—glucose at a concentration of 30 g/L of the final mixture—with a theoretical value of released carbon dioxide (ThCO_2) of 3.3 mg. The concentration of the studied substances in the aqueous mixture was chosen so that the final concentration of organic carbon was 30 g/L. The mixtures were stirred in test vessels with a volume of 0.03 L, saturated with air without CO_2 and kept at 25 °C in the dark for 28 days. On days 1, 4, 7 and 28, the CO_2 content in the gas–air mixture was determined using the gas chromatography method on a GC-2010 Plus device (Shimadzu, Japan). The biodegradability of the control compound (glucose) corresponds to 92% of the theoretically expected one, which enables to use the isolated microbial community of composts to assess biodegradation. The abiotic degradation of fertilizers was evaluated in parallel with the biotic one; for this, similar test vessels were prepared without the introduction of microbial inoculum.

2.3. Toxicity

Toxicity was determined by tests using the water flea *Daphnia magna* (ISO 6341, 2012) [35], the green algae *Chlorella vulgaris* [36] and the higher plant *Raphanus sativus* (ISO 22030, 2005) [37]. For the negative control water was used.

The tests with *D. magna* were performed in 50 mL beakers, filled with 20 mL of the test dilution. Five test organisms (aged 6–24 h) were subsequently added to each solution and were not fed during the experiment. Individuals were exposed to the test concentrations at 20 ± 2 °C in a constant light:dark photoperiod (16:8). After 48 h, the number of immobilized specimens (mortality) was determined visually.

The germination experiments with *R. sativus* were carried out on filter paper in Petri dishes. In total, 5 Petri dishes were used, each of which contained 5 seeds of radish. The test dilution (5 mL) was placed into the dishes, and demineralized water was used as a control. The dishes were placed in a germination chamber 22 ± 2 °C at 6:18 dark:light regime. After 3 days, the root lengths were measured.

For tests with *D. magna* the percentage inhibition (I, %) for each of the dilutions was determined by comparing the number of immobilized or dead test organisms with the number of organisms at the start. Subsequently, an inhibition curve was fitted to calculate the lowest value of the dilution factor of the water extract that exhibits 10% inhibition of a selected biological response (EC10). For this, the inhibition values (I) for each dilution were plotted against the corresponding dilution factor. The desired values of EC10 were inferred from the intersection of the straight lines with lines parallel to the abscissa at ordinate values of 10%. Eluate was considered as not toxic if EC10 was equal to 1. For tests with *R. sativus*, the germination index was then calculated according to Zucconi [38].

2.4. Statistics

All measurements were conducted in three replicates; the results obtained are expressed as the mean \pm standard deviation. The Mann–Whitney U Test and Kruskal–Wallis test were used to determine statistically significant differences ($p < 0.05$). Statistical analysis was performed in Statistica 10.0 software (StatSoft Inc., Tulsa, OK, USA).

3. Results

3.1. Degradation Ability of EDTA, IDS and Their Complexes with Metals

Degradation ability of EDTA, IDS and their complexes with metals was estimated from two variants—with and without microbial community in order to reveal biotic and abiotic degradation, respectively (Figure 1a). The lowest degradation ratio was registered for pure EDTA (6.3%) while the degradation rate of EDTA–metal complexes was 2.2-fold higher. The degradation rate of IDS was estimated to be 28.1% which is 4.5 times higher than that of EDTA.

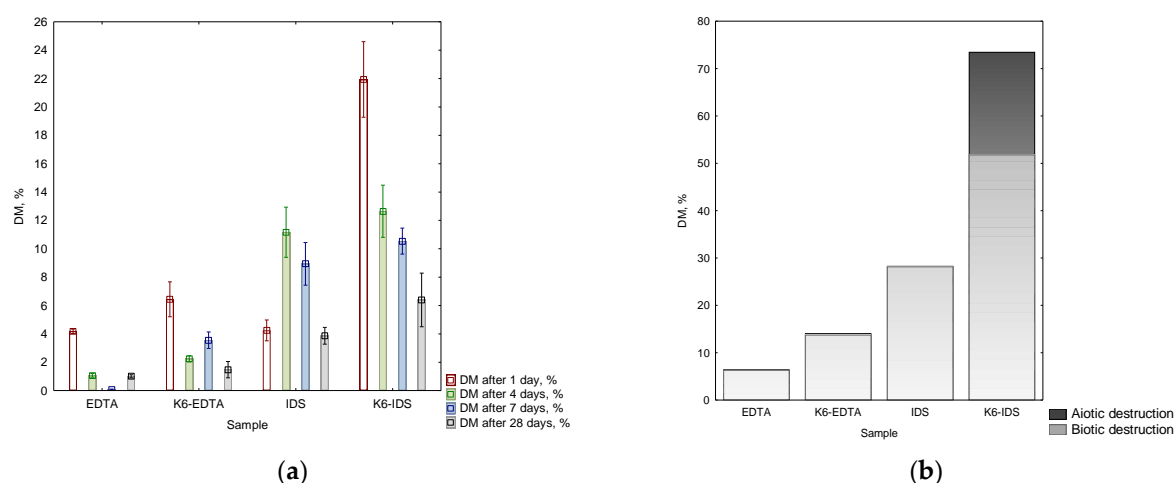


Figure 1. Biotic and abiotic destruction of individual chelating agents and complex fertilizers based on them ((a) dynamic, (b) cumulating).

The ratio of the abiotic degradation of IDS was negligible compared to the case of EDTA. Similar to EDTA, complexation with metals led to an increase in the degradation rate by 1.8-fold. The rate of the degradation was different over the 28 days of the experiment (Figure 1b). The majority of compounds were degraded within the first seven days, then the degradation rate slowed down.

3.2. Ecotoxicity of EDTA, IDS and Their Complexes with Metals

The IDS chelating agent, as well as a fertilizer based on it, were evaluated for toxic effects on radish plants, unicellular algae and water flea. The data obtained were compared with those for EDTA. Figure 2 shows the results of an assessment of the impact of chelating agents and chelate complexes on radish plants. The evaluation used 100 g/L solutions corresponding to the maximum solubility of EDTA in water [39], i.e., the highest concentration of substances that can be achieved in the soil solution as a result of repeated use and accumulation of chelates. As a test function, we used the seed germination index GI, which takes into account the number of germinated seeds and the length of the seedling root. It was found that EDTA at the concentration used inhibits the development of radish plants by 72%. The complex fertilizer based on EDTA was more toxic (GI = 18%), which is probably due to the negative impact of Fe, Zn, Cu, Mn, Mo and B in high concentrations on plants. In the case of IDS, higher toxicity was also observed in the case of a complex fertilizer rather than a pure chelating agent (GI = 47%). However, in general, the toxicity values for IDS (both in pure form and in combination with microelements) were 2.4–2.6 times lower. Complexes based on EDTA and IDS were evaluated at a concentration of 10 g/L, corresponding to the maximum recommended application rate of chelated fertilizer based on EDTA. A 10-fold decrease in concentration led to a decrease in the phytotoxicity of individual chelating agents EDTA and IDS by 1.5 times and 1.3 times, respectively. It should be noted that at the recommended concentration, chelate complex compounds had less phytotoxicity than individual compounds ($p < 0.05$). Similar data were obtained by Dufková et al. [40]. Two chelating agents—EDTA and IDS—at a concentration of 1 g/L (recommended concentration for root application for chelated microelement fertilizer) were non-phytotoxic since GI values were estimated to be 94–118% and 102–121%, respectively. The average GI values for EDTA and IDS did not differ from each other ($p > 0.05$). For complexes based on EDTA and IDS, GI values were 1.5 times higher than those for individual chelating agents.

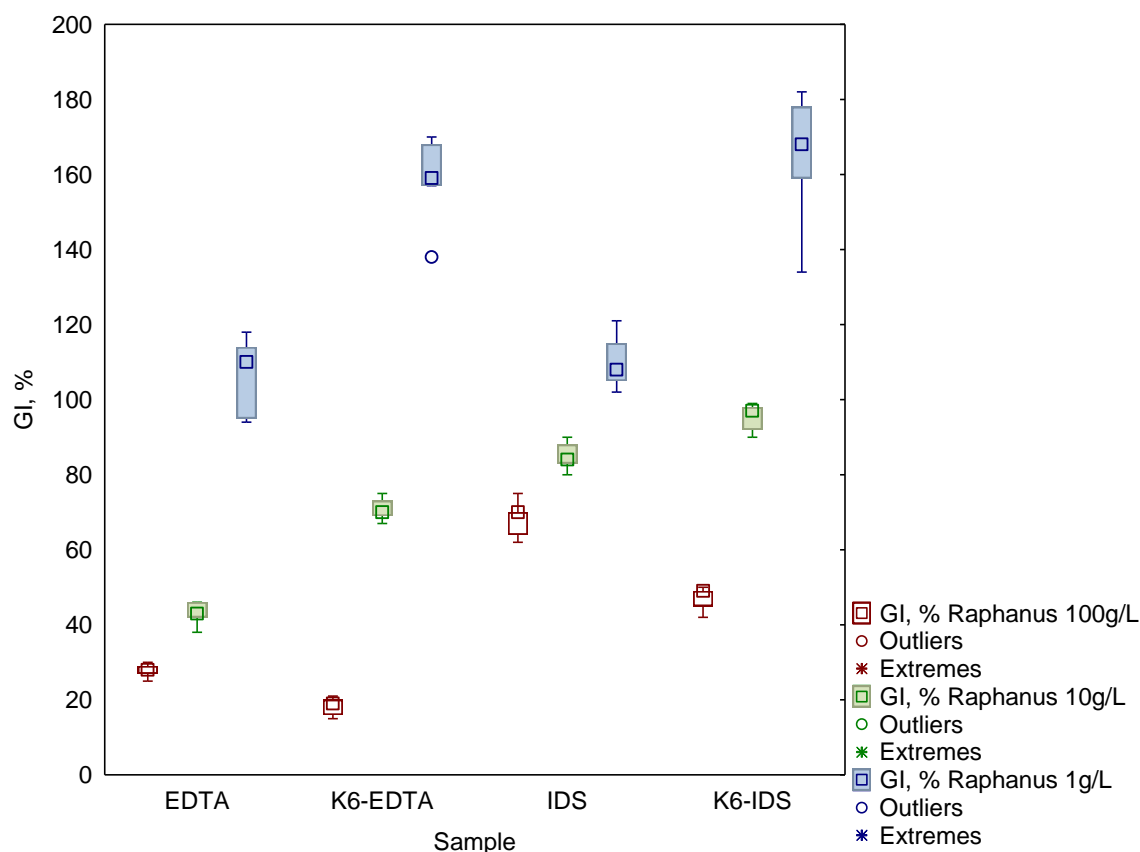


Figure 2. Phytotoxicity of individual chelating agents and complex fertilizers based on them in relation to *Raphanus sativus*.

It was found that for *Ch. Vulgaris*, EDTA was toxic at 1.4 g/L (EC10), i.e., 10 and 100 times lower than that estimated in experiments with radish (Figure 3a). As with radishes, IDS was less toxic than EDTA. Complexes with trace elements in both cases were more toxic than the corresponding acids; however, as in the case of higher plants, the complex with IDS had a less negative effect on plants.

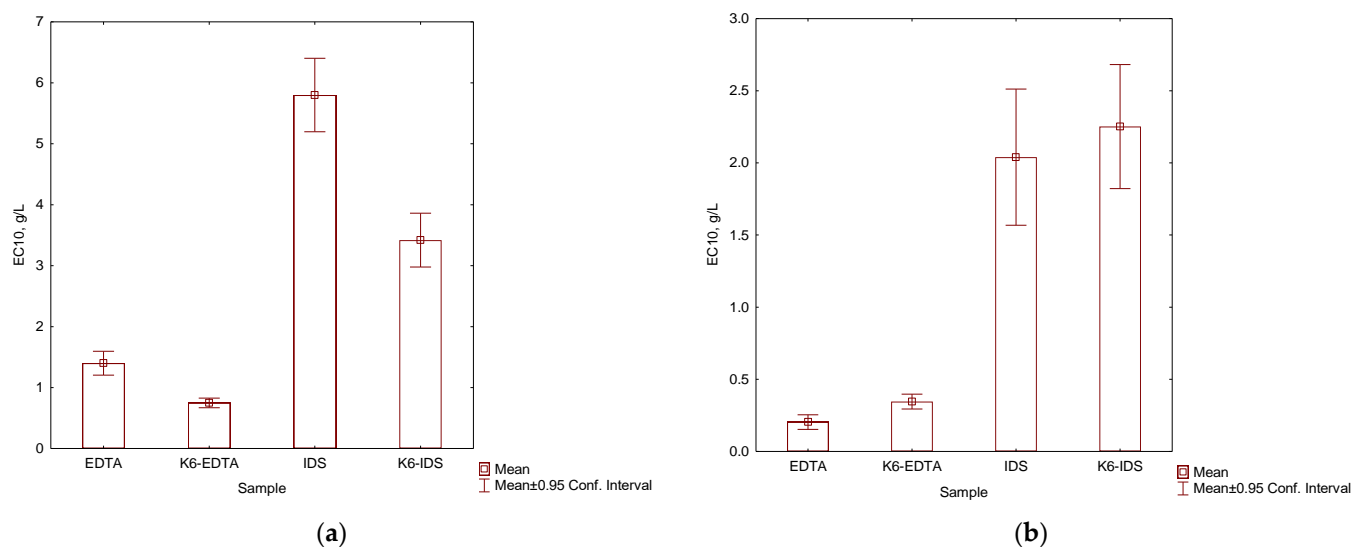


Figure 3. Toxicity to (a) *Ch. vulgaris* and (b) *D. magna* of individual chelating agents and complex fertilizers based on them.

When evaluating the effect of the studied compounds on *D. magna*, it was shown that, as well as for *Ch. Vulgaris*, the toxicities of both the individual chelating agent IDS and the complex fertilizer based on it are lower than those of EDTA (Figure 3b). Unlike microalgae, daphnia did not “distinguish” between chelating agents and complexes—in both cases, EC10 did not differ significantly from each other ($p < 0.05$). This is probably due to differences in the cell walls of plants and animals, and the peculiarities of the penetration of such large molecules as chelate complexes. The last assumption requires additional studies.

4. Discussion

In the present study, the biodegradability of IDS-based microelemental fertilizer containing several microelemental complexes was estimated and compared with that of EDTA-based fertilizer as well as with EDTA and IDS chelating agents. In the studies of the other authors published before, this biodegradability was assessed for only one microelemental complex [41,42]. The ability to be rapidly degraded in the environment is extremely important for microfertilizers since they are supposed to not influence soil and plant flora after completing their main goal of providing plants with microelements. Interestingly, that the ratio of abiotic degradation of EDTA and of IDS was minor and did not exceed 0.3% in both cases. This is due to the experimental conditions that excluded light and therefore prevented photolysis of EDTA that is reported to be the main cause of EDTA degradation in the environment. On the other hand, metal complexes are used as soil fertilizers where sunlight is absent or limited, and therefore our experimental conditions corresponded to the real ones. Thus, T. Smirnova with coauthors reported that light is main factor of abiotic degradation of IDS [43]. Biodegradation of EDTA, IDS and their complexes with metals were higher than abiotic degradation. Complexation of EDTA and IDS with metals led to an increase in the biodegradation rate by 1.8-fold. Intensification of biodegradation of chelated complexes as compared with pure chelating agents was described by other authors before and may be caused by better supply of degrading microorganisms with microelements [44,45]. Interestingly, for IDS–metal complexes the role of abiotic degradation was higher than that of EDTA–metal complexes by about 21.7%. It should be noted that the method used allows one to estimate the amounts of EDTA/IDS that were fully mineralized to carbon dioxide while a significant amount of the compounds can be degraded to intermediates. Thus, IDS can be metabolized to L-aspartic acid and D-aspartic and succinic acids, alanine and serine. Further degradation is possible with the formation of fumaric acid and ammonia [31,45–48]. Previously, IDS and IDS complexes were demonstrated to significantly exceed EDTA and its complexes in the speed of decomposition [7,31,41,46,47,49–52]. However, the data are available on individual (Fe- or B-) complexes but not mixtures of several metals' complexes.

In order to substitute EDTA for chelation of microelements in agriculture, several chelating agents have been recently suggested, and IDS is one of them. However, information about one of the important aspects of its implementation, in particular, its potential hazard for various environments was missing. Presumably, such fertilizers and their components can affect soil organisms through direct contact, as well as aquatic organisms when they enter water bodies as a result of surface or underground runoff. To our knowledge, this is the first report on ecotoxicity of IDS towards aquatic organisms such as *D. magna* and *Ch. vulgaris*, and one of the few reports on ecotoxicity of IDS towards higher plants [53].

Indeed, EDTA unchelated with trace elements inhibits cell division, chlorophyll synthesis, alters cell biochemical processes and leads to necrotic leaf lesions [40,53,54]. In the study of Zhang et al. [54], it was shown that with an increase in IDS concentration (from 5 to 100 mmol/L), corn seed germination was slowed down and the length of seedlings was reduced; in the presence of Pb IDS, conversely, the length of corn seedlings was 20–67% higher. The introduction of IDS as a fertilizer into the soil may lead to its entry not only into the soil but also into aquatic ecosystems. Therefore, at the next stage of our work, we

assessed the ecotoxicity of chelating agents and chelate complexes based on them using two aquatic test-objects, microalgae (*Ch. vulgaris*) and water flea (*D. magna*).

According to the US EPA (United States Environmental Protection Agency), the toxicity of EDTA to *D. magna* (EC50) is 100 mg/L [55,56], which is consistent with our data. According to Sorvari and Sillanpää [57], toxicity to *D. magna* after 24 h of exposure, expressed in EC50, is determined by the chelated metal. Thus, the minimal toxicity of the solo EDTA as expressed in EC50 was estimated to be 900 mg/L, while that of the complexes was found to be 1000, 60 and 11 mg/L, for Zn, Cu and Fe, respectively. As for IDS, it is not classified as a hazardous compound for aquatic organisms according to various regulating documents and research works (voluntary safety information following the Safety Data Sheet format according to Regulation (EC) No. 1907/2006 [24,57,58]), and the numerical information on its ecotoxicity is absent. The data on IDS toxicity are, however, available for mammals (skin application, ingestion in rats). According to the Chemical Index Database, IDS can be classified as a chemical suitable to be used in households [59].

5. Conclusions

It can be concluded that IDS is a promising agent for chelating microfertilizers for agriculture. Thus, IDS–microelement complexes stimulate plant growth more efficiently compared with those of EDTA, which is the most widespread chelating agent for microfertilizers worldwide. IDS is more biodegradable than EDTA both as a single compound and as a complex; this prevents its accumulation in environments and further negative consequences such as lowering pH and the mobilization of metal salts in soil and water. Furthermore, IDS complexes possess lower ecotoxicity towards aquatic test-objects such as *D. magna* and *Ch. vulgaris*. However, to recommend the IDS–microelement complexes as an agricultural fertilizer, further studies are required. Thus, pathways of IDS biodegradation should be investigated, the effect of this novel microfertilizer on various plants' yield and yield quality under hydroponics, greenhouse and field conditions should be estimated, and the influence of IDS complexes on soil and plant endosphere microbial communities, playing an important role for plant wellbeing, should be discovered.

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