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*Original Research*

# Chelant-Assisted Accumulation of Cd, Cu, and Zn in Rapeseed (*Brassica napus* L.) Biomass as a Renewable Energy Feedstock

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## Abstract

This article discusses the environmental concerns regarding soil contamination by heavy metals and the possibility of growing a high biomass-yielding crop (i.e., rapeseed) as a tool of phytoremediation. The aim of our research was to investigate the growth parameters and the capacity of rapeseed to accumulate Cd, Cu, and Zn from the contaminated soil and to investigate the effects of the chelants (EDTA, EDDS) as potential heavy metal mobility-enhancing agents. A pot experiment was performed under greenhouse conditions where rapeseed was grown on heavy metal-contaminated soil taken from former septic drain fields. Chelants were applied twice using doses of 3 mmol $kg^{-1}$ of wet soil weight. Plants from contaminated soil produced more biomass and heavier seeds. The highest Cd concentrations were detected in rapeseed stems and leaves: Cu in roots and Zn in seeds and stems with leaves. Rapeseed in some cases exhibited translocation factor values for single plant parts greater than unity, whereas the bioconcentration factor was always below unity. Detected concentrations of Cd, Cu, and Zn in the biomass indicate that rapeseed may be considered an excluder rather than accumulator. Chelant application did not provide the expected enhancing effect on heavy metal uptake by rapeseed.

**Keywords**: heavy metal, contaminated soil, phytoextraction, chelate enhancement, bioconcentration factor

## Introduction

Heavy metals (HM) are essential components of the Earth's crust. However, due to natural processes like volcanism, erosion, and bioaccumulation, and anthropogenic activities such as mining, smelting, fossil fuel combustion, incineration, etc., HM are translocated to areas with intrinsically low HM concentrations, which causes soil contamination [1-2]. Soils are the main sink for heavy metals and they can finally reach animals and humans through the food chain [3]. Heavy metal toxicity depends on several factors such as metal speciation, concentration, soil composition, and pH, as well as plant species [4]. The latter factor is very important as

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plants are the first-hand bond between soil chemical composition and the food chain [5-6]. Hence, clean-up of HM-contaminated soil is very important for the negative impact on ecosystem reduction and ensuring food quality and safety. Conventional methods such as *in situ* vitrification, soil incineration, soil flushing, stabilization and solidification, electro-kinetic methods, and excavation are more suitable for point-source contamination [3, 7]. But it is difficult to apply the aforementioned remediation methods in the case of diffuse contamination when HM concentrations are low or moderate (but still exceeding threshold values) and affected territory is vast [8]. A lot of effort is put forth in order to develop efficient, cost-effective, and environmentally friendly methods for HM clean-up.

Phytoremediation, a clean-up method incorporating plants and associated soil microorganisms, is considered a gentle approach for reclamation soil quality and properties. It is cost-effective and can be carried out *in situ* without ecosystem perturbation [9-10]. Certain plant species are capable of accumulating substantial amounts of metals, e.g., > 1000 mg $kg^{-1}$ of Cu, Co, Cr, Ni, or Pb and > 10 000 mg $kg^{-1}$ of Mn or Zn, and are considered to be hyperaccumulators [11]. On the other hand, hyperaccumulators usually develop low biomass and are slow-growing [8, 12-13]. Such properties indicate that numerous vegetation cycles should be applied for soil clean-up. Relatively low metal solubility in soil is one of the factors limiting metal uptake by non-hyperaccumulating plants [14]. The addition of mobilizing agents to the contaminated soil can facilitate the release of a metal to a soil solution where plants can easily take it up [15-16]. There is a variety of HM mobility-intensifying agents that are fully described [13, 15, 17]. The HM mobilization enables the possibility of growing high biomass-yielding plants instead of smaller hyperaccumulators [3, 18].

Ethylenediaminetetraacetic acid (EDTA) is a versatile and the most often used chelating agent; its effectiveness has been proven with high biomass-yielding plants such as corn (*Zea mays* L.), sunflower (*Helianthus annuus* L.), Indian mustard (*Brassica juncea* L.), Chinese cabbage (*Brassica rapa* L.), tobacco (*Nicotiana tabacum* L.), and white bean (*Phaseolus vulgaris* L.), etc. [10, 12-13, 18]. Nonetheless, EDTA is hardly biodegradable, thus it can leach into groundwater and produce further environmental damage [10]. Environmentally friendlier chelating agents such as ethylenediaminedisuccinic acid (EDDS) [3], cyclohexane-1, 2-diaminetetraacetic acid (CDTA), and methylglycinediacetic acid (MGDA), etc., were recently proposed for inducing HM removal from soil [10, 12, 18].

An abundance of published papers indicates that chelate-enhanced phytoextraction is no more a novelty. On the other hand, in the new European Union member states like Lithuania, remediation of contaminated soil is still slow. It was approved by the Ministry of the Environment that HM-contaminated soil should be excavated and additionally treated ex-situ or landfilled [19]. Phytoextraction is still at the experimental stage in Lithuania, thus scientific studies leading from bench-scale to pilot-scale experiments performed with the native plant species and local soils are necessary to ensure public acceptance and effectiveness of a chosen clean-up method. In the case of our research, rapeseed (*Brassica napus* L.) was grown. It was stated by Wenzel et al. [20] that various hydroponic studies and vegetative experiments suggest the potential of Brassica species to uptake increased amounts of heavy metals. This crop is being widely grown all over the country and agrotechnological aspects are well-known.

The rapeseed plant is deeply-rooted and such a trait is important for any of the phytoremediation technologies, as plants can only contact HM from their root zone [10]. Furthermore, summer and winter rapeseed is considered to have the highest potential for biodiesel production in Lithuania. As the production of biofuels is encouraged by various political and economic means, there is a demand to grow more rapeseed, which consequently increases the demand for suitable land [21]. Thus marginal lands, including the ones with HM contamination, receive more attention. [22-23] Unlike valuable seeds, *B. napus* straw is considered as field residue and usually is left on soil surface [24]. Nonetheless, summer rapeseed produces more straw biomass from hectare in comparison to such crops as wheat (*Triticum* spp. L.), triticale *(× Triticosecale* Wittm. ex A. Camus), or barley (*Hordeum vulgare* L.) and thus can be used not only for cattle bedding or fodder, but for bioenergetic purposes as well [25]. As plants grown on soil with HM concentrations exceeding threshold values cannot be used for food and animal feeding purposes, rapeseed straw alternatively could be used for energy recovery through incineration or anaerobic digestion.

Our study was aimed at evaluating the effects of two HM-mobilizing chelants, EDTA and EDDS, on HM extraction from contaminated soil by rapeseed as a potential bioenergy crop. Growth parameters of the plants and its capacity to accumulate Cd, Cu, and Zn in different plant parts (roots, stems and leaves, pods, seeds) grown in contaminated soil from former septic drain fields in Lithuania were evaluated by a greenhouse pot experiment.

## Material and Methods

### Soil Sample Collection

Contaminated soil was taken from the former septic drain fields in Molainiai, Panevėžys, Lithuania. Wastewater was pumped into this area from households and several industrial companies that did not have their own wastewater treatment facilities in the 1960s. Due to the fact that these companies were involved in some heavy industry processes such as tin-dipping or galvanization, various amounts of HM occurred in the wastewater and later in the soil. A composite soil sample was pooled from the sub-samples taken at three different spots (0-0.2 m depth) where, according to previous investigations [26-28], from one to four heavy metals were exceeding maximum permissible concentration (MPC) values. Soil was taken using a plastic shovel, sieved through a 20 mm mesh screen, homogenized, and brought to a laboratory for further experiments.

Uncontaminated (without known contamination) soil was collected from the agricultural field of Aleksandras Stulginskis University experimental station (mid-Lithuania) and was used as a control for comparing the obtained results. This soil was also mixed, sieved to pass through a 20 mm mesh size screen, and thoroughly homogenized.

Both soils were analyzed with a particle size analyzer (Mastersizer 2000) using the wet type of dispersion for soil type identification. Primary analytical characterisation was performed in order to define pH, electrical conductivity, macronutrients (NPK), and heavy metals, including Cd, Cu, and Zn.

### Pot Experiment

Both contaminated and uncontaminated soils were separately divided into subsamples and placed into plastic buckets of 26 L volume. A hundred seeds of summer *B. napus* cultivar Fenja were seeded into each bucket. The pot experiment was carried out under greenhouse conditions where the temperature was kept at 25±2ºC and tapwater was used to maintain the stable moisture content. The experiment was implemented in triplicate. Throughout the 13 weeks of the experiment plants were thinned on three occasions until 21±3 plants in each bucket were left for the application of chelating agents. Solutions with chelants EDDS and EDTA were watered on soil in the buckets (avoiding contact with plants), twice using a total dose of 3 mmol kg$^{-1}$ of wet soil weight (assuming that the active soil layer with the densest roots is 0.15 m deep). Chelant solutions were applied with a three-day interval and plants were harvested 11 days after the second application. Pods with seeds were cut using scissors and shucked; stems with leaves were separated from the roots. In order to identify HM toxic effect on plant growth, vegetative parameters of *B. napus* (including dry weight, stem height, root length, and weight of 100 seeds) were recorded after harvesting the plants. At least three randomly selected plants from each bucket were measured for this purpose. The weight of 100 seeds was obtained by calculating randomly picked seeds from rapeseed plants of each bucket.

### Analytical Design

Separate parts of air-dried rapeseed plants were ground into fine powder using a laboratory grinder mill. Aliquots (0.503±0.005 g) of plant samples were wet-digested with 2 ml of 30% $H_2O_2$, 5 ml of concentrated $HNO_3$, and 1 ml of deionized water (added step-wise). Breakdown of the biomass was performed using a microwave digestion oven (CEM Mars 5), where Teflon bombs with samples were heated for 10 minutes at 195ºC. After cooling and diluting up to 100 mL with deionized water, heavy metal concentration in the digestate was determined using inductively coupled plasma atomic absorption spectrometry (Perkin-Elmer Optima 8x00 ICP-OES spectrometer). Reference material was analyzed to verify the reliability of the results.

### Data Evaluation

Concentrations of accumulated Cd, Cu, and Zn in different parts of *B. napus* were calculated as mg kg$^{-1}$ in the dry matter and are presented as averages ±standard deviation. Significance level was calculated using t-test (two-sample analysis assuming unequal variances when p = 0.05). Plant capacity to accumulate HM from the soil and rapeseed potential to be used for phytoremediation purposes were evaluated by calculating bioconcentration factor (BCF) using Equation (1) [9], and translocation factor (TF) using Equation (2) [29]:

$$BCF = C_{roots} \div C_{soil} \tag{1}$$

$$TF = C_{aboveground\ plant\ part} \div C_{roots} \tag{2}$$

…where $C_{roots}$ is metal concentration in roots, $C_{soil}$ is metal concentration in the soil where the plant was grown, and $C_{aboveground\ plant\ part}$ is metal concentration in a single aboveground plant part (i.e., stem with leaves, pods, or seeds).

## Results and Discussion

### Soil Properties and Heavy Metal Content

Both soils were identified as sandy loams. Analytical characteristics of two analyzed soils including averages, standard deviations, and methods used for each assay are given in Table 1. Maximum permissible and background concentrations (BC) for Cd, Zn, and Cu in the soil are also presented. Both soils were characterized as slightly alkaline with low electrical conductivity. Uncontaminated soil contained 2.4 times higher total nitrogen content, while contaminated soil held 1.7 and 2.6 times more mobile potassium (K) and phosphorus (P), respectively. Heavy metals in the uncontaminated soil did not exceed Lithuanian threshold values as indicated in the Hygiene Standard [30]. Cadmium content in the contaminated soil was 10 times higher than MPC and 207 times higher than the background concentration for sandy loams. Zinc concentration in contaminated soil was close to the limit value, but did not exceed it; however, its concentration was 12 times higher than BC. Copper concentration in contaminated soil was 3.4 times higher than MPC value and 42 times higher than BC.

### Vegetative Parameter of *B. napus* Plants

Average vegetative parameters of rapeseed grown on contaminated and uncontaminated soils are presented in Table 2. Taking into account that chelates were applied

Table 1. Analytical characteristics of soil samples.

| Analytes | Contaminated soil | Uncontaminated soil | MPC[b] | BC[c] sandy loam | Method |
|---|---|---|---|---|---|
| pH | 8.0±0.2 | 7.4±0.2 | - | - | ISO 1039:2005 |
| EC, (μS) | 290±5 | 245±5 | - | - | Potentiometric method |
| Total nitrogen, mg $kg^{-1}$ | 6.5±1.1 | 15.9±1.5 | - | - | Spectrometric flow method |
| $P_2O_5$, mg $kg^{-1}$ | 921±55 | 348±23 | - | - | Egner-Reihem Domingo method |
| $K_2O$, mg $kg^{-1}$ | 227±14 | 127±8 | - | - | |
| Cd, mg $kg^{-1}$ DW[a] | 31.10±4.98 | 0.34±0.05 | 3 | 0.15 | ISO 11047:1998, method B |
| Zn, mg $kg^{-1}$ DW | 288±36 | 41±5 | 300 | 26 | ISO 22036:2008 |
| Cu, mg $kg^{-1}$ DW | 339±56 | 13.8±2.3 | 100 | 8.1 | ISO 11047:1998, method A |

[a] DW: Dry weight
[b] MPC: Maximum permissible concentration in soil according to Lithuanian Hygiene Standard [30]
[c] BC: Background concentration in sandy loam soils according to Lithuanian Hygiene Standard [30]

only 11 days before harvesting, we assume that the application of EDTA and EDDS did not significantly affect final vegetative parameters of *B. napus*. Therefore, while evaluating biomass yield, the plants were not differentiated as grown on soil into treated with chelates and untreated ones. Rapeseed plants grown on contaminated soil were slightly taller, had longer main roots, and produced more aboveground biomass on a dry matter basis. However such differences were insignificant ($0.2 < p < 0.8$). Average weight of 100 seeds from plants grown on the contaminated soil was greater by 1.4 times compared to the weight of 100 seeds from plants grown on uncontaminated soil ($p = 0.03$). Furthermore, the weight of seeds from contaminated soil was slightly above the standard weight [31] for rapeseed cultivar Fenja, whereas the weight of the seeds from uncontaminated soil was below the standard by 24%.

We observed throughout the vegetative experiment that the development of plants grown on contaminated soil advanced by at least five days compared to those grown in the control soil. In addition, during an unexpected attack of thrips rapeseed plants from contaminated soil were more resistant and very few plants in total were infected. Thrips caused more damage to plants grown on uncontaminated soil as from five to 10 plants in each bucket had signs of serious infection. However, the colour of leaves and stems of plants grown on uncontaminated soil was dark green with bluish shade; meanwhile, plants grown on contaminated soil were light green and often had several yellow leaves.

## Accumulation of Heavy Metals

Average concentrations in rapeseed parts and standard deviations of accumulated HM are presented in Figs 1-3. Increased Cd, Cu, and Zn concentrations were detected in *B. napus* plants grown on contaminated soil. In cases when plants were grown on contaminated soil, Cd concentration in roots was higher by 62 times, in stems and leaves by 24 times, and in pods and seeds by 10 times more than the same parts of a plant grown on uncontaminated soil. The highest Cd concentrations in plants from contaminated soil were detected in the roots, and in uncontaminated soil in stems and leaves. Concentrations of Cu in the roots was higher by 27 times, in stems and leaves by 12 times, in pods by four, and in seeds by two times in comparison to their counterparts from the uncontaminated soil. In both cases, the highest concentrations were recorded in the roots. Differences between Zn accumulation in plants grown on contaminated and uncontaminated soil were the smallest among the three analyzed elements. Concentrations of Zn in roots and stem with leaves from plants grown on contaminated soil were higher by seven times than in plants from uncontaminated soil; while in pods and seeds such concentrations were almost the same.

Table 2. Average vegetative parameters of *B. napus* grown in contaminated and uncontaminated soil.

| Soil | DW of stem, leaves and roots per plant, g | Stem height, m | Length of the main root, m | Weight of 100 seeds, g | Weight deviation from Standard[d], % |
|---|---|---|---|---|---|
| Uncontaminated | 0.89 ± 0.12 | 0.54 ± 0.064 | 0.10 ± 0.015 | 0.28 ± 0.04 | -24.3 |
| Contaminated | 0.98 ± 0.16 | 0.59 ± 0.083 | 0.12 ± 0.022 | 0.39 ± 0.05 | +5.4 |

[d] Weight standard – A hundred seeds of rapeseed cultivar Fenja by standard weigh 0.37 g [31]

## Chelant Application

Soil treatment with chelants did not offer the desired effect on HM mobility enhancement. Accumulation of Cd by *B. napus* plants is presented in Fig. 1. The addition of EDTA and EDDS even lowered the accumulation of Cd in comparison to chelant-free contaminated soil. Significantly reduced concentrations were detected in the roots: by 49% when soil was treated with EDTA ($p = 0.004$) and by 48% when treated with EDDS ($p = 0.007$). When EDTA was applied the average accumulated concentration in stems and leaves declined by 8.6% and in seeds by 22%, while Cd accumulation in pods increased by 14%. Application of EDDS decreased Cd concentration in pods by 10% and in seeds by 20%, while concentrations in stems and leaves increased slightly – by 5.2%. However, these differences were statistically insignificant ($0.13<p<0.5$).

Copper accumulation in rapeseed biomass is presented in Fig. 2. The chelant-induced Cu accumulation pattern was very similar to Cd. After the treatment, the average Cu concentration in roots decreased by 59% using EDTA ($p = 0.008$) and by 78% using EDDS ($p = 0.01$). Average Cu concentration in stems and leaves decreased by 31% and in seeds by 19% (when EDTA-treated soil is compared to a chelant-free but contaminated soil). When soil was treated with EDDS, the average Cu concentration in stems and leaves declined by 14% and in seeds by 10%. In both cases, Cu concentration in pods increased by 8.2% using EDTA and by 32% using EDDS ($p = 0.01$).

Fig. 3 reveals the accumulation of Zn in *B. napus* plant parts. Average Zn concentrations in roots after using EDTA decreased by 41% ($p = 0.01$), in stems and leaves by 10%, and in seeds by 9.4%. When EDDS was applied, concentrations in roots decreased by 59% ($p = 0.009$), in stems and leaves by 9%, and in seeds by 16% in comparison to chelant-free contaminated soil. Here, as well as in the case of Cu uptake, both chelates increased Zn accumulation in pods: by 32% using EDDS and by 61% when EDTA was used.

Uncontaminated soil treatment with chelants had a very similar pattern on HM uptake by rapeseed plant parts as in the case of contaminated soil. Metal uptake after chelant treatment in most cases was lower in comparison to chelant-free uncontaminated soil. When chelants were applied, pods accumulated higher amounts of all metals (except Cu) under the EDTA treatment.

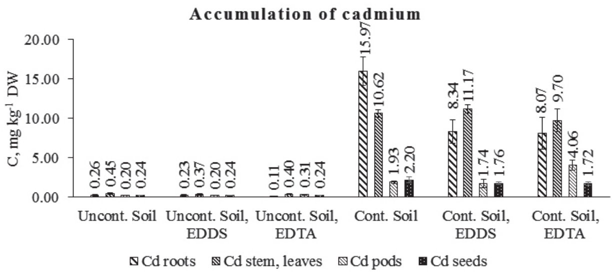


Fig. 1 Cadmium accumulation in *B. napus* plant parts.

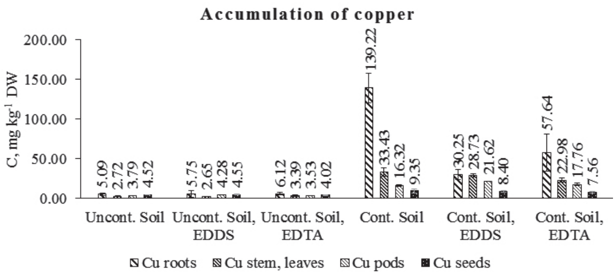


Fig. 2 Copper accumulation in *B. napus* plant parts.

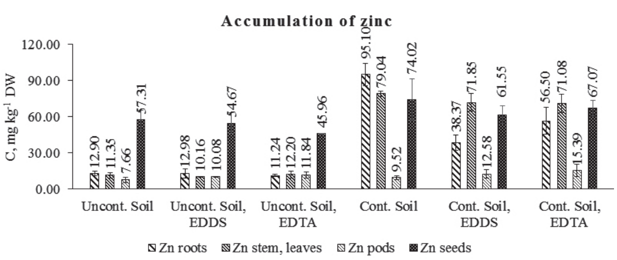


Fig. 3 Zinc accumulation in *B. napus* plant parts.

## Bioconcentration and Translocation

Fig. 4 presents calculated BCF values for rapeseed. The bioconcentration factor shows the ratio between metal concentration in plant roots and metal concentration in the soil. Plants displaying BCF value more than unity are considered suitable for phytoremediation [32]. According to the results obtained during this research, *B. napus* cannot be counted as a hyperaccumulator due to rather low BCF values.

The translocation factor is a ratio between metal concentrations in aboveground parts and metal concentrations in the roots; therefore, it shows plant capacity to translocate contaminants from roots to shoots. Results of TF calculated for each *B. napus* plant part are presented in Table 3. Translocation of HM from root zone to aboveground parts is essential for the phytoextraction process. Thus, TF values, greater than unity can be considered as an indicator of plant suitability for phytoextraction. In the case of Cd, the most intensive translocation was to stems and leaves in plants from uncontaminated soil, although total Cd concentrations

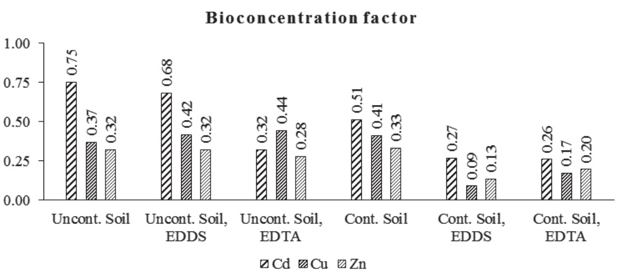


Fig. 4 Heavy metal Bioconcentration factor for *B. napus* plant.

Table 3. Heavy metal translocation factor in different *B. napus* plant parts.

| Soil, chelant application | Cd | | | Cu | | | Zn | | |
|---|---|---|---|---|---|---|---|---|---|
| | Stems and leaves | Pods | Seeds | Stems and leaves | Pods | Seeds | Stems and leaves | Pods | Seeds |
| Uncontaminated soil | 1.76 | 0.78 | 0.93 | 0.53 | 0.74 | 0.89 | 0.88 | 0.59 | 4.44 |
| Uncontaminated soil, EDDS-treated | 1.60 | 0.86 | 1.01 | 0.46 | 0.74 | 0.79 | 0.78 | 0.78 | 4.21 |
| Uncontaminated soil, EDTA-treated | 3.63 | 2.89 | 2.17 | 0.55 | 0.58 | 0.66 | 1.09 | 1.05 | 4.09 |
| Contaminated soil | 0.66 | 0.12 | 0.14 | 0.24 | 0.12 | 0.07 | 0.83 | 0.10 | 0.78 |
| Contaminated soil, EDDS-treated | 1.34 | 0.21 | 0.21 | 0.95 | 0.71 | 0.28 | 1.87 | 0.33 | 1.60 |
| Contaminated soil, EDTA-treated | 1.20 | 0.50 | 0.21 | 0.40 | 0.31 | 0.13 | 1.26 | 0.27 | 1.19 |

were comparably low. Copper is known to have a strong capability to hold against the transport to aboveground parts under both deficiency and excess circumstances [33], likely explaining why none of the TF values for Cu were above unity. Zinc, in contrast, was intensively transported to seeds – especially in the case of uncontaminated soil. Such observations confirm the importance of this microelement for plant growth and reproduction.

Results showed that the former septic drain fields in Molainiai, central Lithuania, are contaminated with a varying amount of heavy metals, with cadmium presenting the greatest risk. Concentrations of Cu and Zn exceeded background concentrations for both metals as well as maximum permissible concentrations in the case of Cu. Despite the general Cu tolerance among plant species and genotypes, this metal is regarded as very toxic [4]. Zinc is not considered a highly phytotoxic element, and agricultural soils often face Zn deficiency [5].

The pot experiment lasted 91 days while, according to Zadoks scale, normal *B. napus* vegetation in the field lasts for 70-89 days [34]. It was assumed that the duration of the pot experiment was sufficient to prove possible negative HM effects on plant growth. It is often emphasized that excess HM in the growing media negatively affects plant development [4, 12, 35]. Nonetheless, *B. napus* plants during the pot-experiment showed very little signs of HM toxicity except for the yellowish colour of leaves – a feature that can also be attributed to nitrogen deficiency. In general, plants from contaminated soil at the end of the vegetation produced more biomass and larger seeds, as well as exposed higher resistance to pests in comparison to plants grown on uncontaminated soil. Similar findings were obtained by Marchiol et al. [36] and Brunetti et al. [37], as both studies indicate that rapeseed exhibited diminutive symptoms of toxicity when grown on HM-contaminated soil under greenhouse conditions. Ghnaya et al. [38] tested the resistance of four *B. napus* cultivars to Cd and Zn stress and concluded that the response depends on both cultivar and metal.

During our experiment *B. napus* plants grown on contaminated soil accumulated HM in concentrations, which according to Kabata-Pendias [33] and Alloway [5] can be regarded as abnormal or phytotoxic. Even so, our results coincide with those of the previously mentioned Marchiol et al. [36] and Brunetti et al. [37] in that rapeseed behaves as a Cd-, Cu-, and Zn-tolerant plant and has the ability to accumulate several metals simultaneously. Rapeseed displayed high TF values for Cd and Zn, showing intensive transport of these metals from roots to the aboveground biomass as a green light for the phytoextraction application, but rather low BCF values revealed that rapeseed is not suitable enough for this purpose. On the other hand, plants tolerant of metal-contaminated soil and maintaining rather low total concentrations in the aboveground parts can be considered as excluders and used as a tool for erosion reduction and decrease metal leaching to groundwater in contaminated areas [32, 39].

Our experiment revealed that the application of chelants in almost all cases increased concentrations of all the analyzed metals in rapeseed pods. Although pods have a low mass in comparison to other plant parts and usually are treated as a waste product, it cannot be ignored that chelant treatment increases metal accumulation in this plant part and thus has to be handled properly, although in general, soil treatment with strong EDTA and EDDS chelants did not increase the uptake of HM by *B. napus* plants as was expected initially. In most cases metal concentrations in different plant parts, and roots in particular, were even lower than in plants from chelant-free soil. According to Evangelou et al. [18] and Baraud and Leleyter et al. [40], EDTA is one the most effective HM-mobilising agents and its success in mobilizing metals varies from none up to 200-fold. However, some researchers have demonstrated the negative effect of the EDTA application on Pb accumulation using tumbleweed (*Salsola tragus* L.) [41]. Tome et al. [42] obtained non-significant differences on metal uptake by plants after the chelant application as well. A structural EDTA isomer – EDDS – is produced by numerous microorganisms found in soil environment, therefore exhibits much higher biodegradability. Meers et al. [43] used a dose of

1.6 mmol $kg^{-1}$ and found that uptake in sunflowers of Zn increased by 1.7 times, Cu by 4.1, Cd by 1.3. and Ni by 2.8 times. Grcman et al. [44] treated Chinese cabbage with a multiple EDDS dose of 4 times 10 mmol $kg^{-1}$ and obtained a 3-fold increase in Cd and Zn and 10.3-fold in Pb uptake. Wang et al. [45] treated maize (*Zea mays* L.) with a single EDDS dose of 3 mmol $kg^{-1}$ and obtained a 2.7-fold increase in Cu uptake.

It is unclear why the EDDS treatment was unsuccessful in our case as no supporting references regarding ineffective use of this chelant were found. Wenzel et al. [20] specifies that chelate enhancement in HM-contaminated soil, especially for woody plants, should be very limited and other environmentally friendly technologies should be developed instead. Decreased uptake of heavy metals in soil treated with chelants during our experiment can be an indication of HM leaching. Inefficient HM uptake can also arise due to the inability of plant roots to accumulate chelate complexes or due to the lack of some specific carriers that transport metal ions to the aboveground parts. Low HM uptake in plants treated with mobilizing agents can be due to short exposure time between chelant applications and harvesting, which in our case was 14 days.

Meers et al. [43] indicate that unduly low metal uptake was observed in sunflowers after 7-day exposure with EDTA and EDDS. Chelants, EDTA in particular, can initiate HM off-side migration and leaching to groundwater as plants are able to uptake only a scant fraction of mobilized metals. It is worth mentioning that EDTA is used not only to aid phytoremediation, but is found in many domestic (cosmetics, washing powder) and food products as well as in industrial processes and agriculture [46]. According to Oviedo and Rodriguez [47], due to its immense applications, EDTA is one of the most abundant anthropogenic compounds in surface water bodies in Europe. And although Alkorta et al. [12] and Evangelou et al. [18] state that off-side migration and HM leaching are treated as unimportant side effects and are often neglected, some countries in the EU and the United States of America do not use EDTA for soil clean up [48].

Heavy metal concentrations exceeding national or EU threshold values limit soil usage so that no feed or fodder crops can be grown. On the other hand, high biomass-yielding plants can be cultivated as a source of renewable energy. So far, there are no specific legislative documents regarding cultivation of such plants on HM-contaminated soil. However, various regulations are faced when dealing with post-treatment residues: ash, slag, or digestate [8]. Due to the fact that agronomic technologies for *B. napus* cultivation allow for separate harvesting of seeds and straw, it is possible to use different conversion technologies for different plant parts, i.e., combustion of straw biomass and production of biodiesel from seeds. In general, a holistic approach – including not only occupation of contaminated land and removal of toxic metals, but also plant cultivation, harvesting, and post-harvest technologies – is gaining ground [8, 49-50]. Nevertheless, more insight is required in order to predict the fate of HM during various conversion technologies.

## Conclusions

Soil treatment with chelants did not increase the uptake of analyzed heavy metals. It was difficult to distinguish a pattern in HM mobility changes both for EDTA and EDDS treatment, but it was clear that in general accumulated HM amounts after chelant treatment were lower in comparison to chelants-untreated soil. Only in the case of pods did the application of both chelants increase the accumulation of all the analyzed metals. However, the impact on overall metal extraction would not be substantial assuming that pod mass is very small compared with the total rapeseed biomass.

The bioconcentration factor was below unity for all metals in all cases, indicating that *B. napus* is not sufficient to accumulate HM from the soil. Nonetheless, rapeseed tolerance to the studied metals suggests that this plant species could be considered as an excluder and help to reduce soil erosion and HM leaching. and contribute to both remediation and energy recovery processes. Moreover, decreased uptake of heavy metals in soil treated with chelants during our experiment can be an indication of HM leaching and points to the environmental limitations when using chelant-enhanced phytoextraction.

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