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Effect of sewage sludge and digestate from anaerobic fermentation on the accumulation of cadmium (Cd), gallium (Ga), germanium (Ge), and rare earth elements (REEs) in soil and uptake by plants with different nutrition strategies

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This study investigates how sewage sludge and liquid digestate, as biosolid amendments, affect the mobility of cadmium (Cd), gallium (Ga), germanium (Ge), and rare earth elements (REEs) in soil, as well as their uptake by plants with differing nutritional strategies. Four species *Alyssum murale, Lupinus albus, Fagopyrum esculentum*, and *Carthamus tinctorius* were cultivated on unamended soil or soil amended with either sewage sludge or digestate. Shoot uptake of the essential elements P, Fe, Mn, Ni and of non-essential elements was evaluated alongside changes in ammonium-acetate-extractable (labile) element concentrations. For three species, root carboxylate exudation and rhizosphere acidification were also measured under variable phosphorus (P) supply conditions induced by the amendments. Both biosolids improved micronutrient availability across all species. However, increased shoot P concentrations were only observed in plants treated with sewage sludge. Digestate addition elevated total Ge (14.4%), labile Ga (178%), and labile REE (22%) concentrations in soil, while sewage sludge increased labile Cd (31%) and decreased labile REEs (18%) concentration. Neither amendment enhanced Ge uptake by plants. A higher proportion of labile Cd correlated with a higher Cd uptake in all tested plant species. However, the shoot net uptake of Ga and REE did not reflect their mobility in soil. More specifically, a higher Ga mobility in soil only increased Ga uptake in *F. esculentum*. *F. esculentum* acidified the rhizosphere and released fewer carboxylates under conditions of high P supply. Despite lower labile REEs concentrations in sewage sludge amended soil, *L. albus* and *F. esculentum* accumulated more REEs when the P supply was increased due to biosolids addition. The findings highlight that while Cd transfer is predictably linked to its labile soil pool, the uptake of Ge, Ga, and REEs depends on complex interactions between soil chemistry and plant-specific physiological traits responses to biosolid-derived nutrient inputs.

KEYWORDS

soil amendment, heavy metal, plant availability, metalloids, phytoextraction

1 Introduction

The global production of sewage sludge and digestate from anaerobic fermentation is rising due to increasing urban populations (Rékási et al., 2019). As the main solid by-products from urban wastewater treatment and anaerobic digestion, these biosolids contain high concentrations of essential plant nutrients. Hence, biosolids represent valuable secondary raw materials for fertilizer production and are widely applied as organic fertilizers or soil conditioners (Pecorini et al., 2020; Jama-Rodzeńska et al., 2021; Kanteraki et al., 2022; Marchuk et al., 2023). However, biosolids also contain a diverse range of potentially toxic metal (loid)s (Marotrao et al., 2021; Kowalik et al., 2022; Zaffar et al., 2023) that can accumulate in soil and crops, often limiting their use in agriculture (Sinha et al., 2023; Alengebawy et al., 2021; Marchuk et al., 2023). Among these, cadmium (Cd), lead (Pb), chromium (Cr), and mercury (Hg) are the most extensively studied (Marotrao et al., 2021; Kowalik et al., 2022; Marchuk et al., 2023). In contrast, less attention has been paid to germanium (Ge), gallium (Ga), and rare earth elements (REEs: La-Lu, Sc,Y) despite their increasing detection in soils and plant biomass (Wiche et al., 2017; Okoroafor et al., 2022). Given their widespread occurrence and growing recognition as emerging environmental pollutants (Turcotte et al., 2022; Akarsu et al., 2023), as well as the potential role of REEs as beneficial elements (Qvarforth et al., 2025), it is important to assess how biosolid applications affect the plant availability not only of nutrients and commonly monitored metal (loid)s, but also of Ge, Ga, and REEs.

The plant availability of a given element depends on the complex interplay between soil-associated factors and plant-associated factors (Wiche et al., 2018). Soil-associated factors govern the solubility and chemical speciation of elements in soil, including soil pH, Eh, organic matter content, cation exchange capacity, and the distribution of elements in labile mineral and organic soil phases (Sheoran et al., 2016). In this regard, biosolid application may increase the concentrations of mobile and labile-bound elements in the soil when the elements contained are released from the organic matrix and/or when soil-born elements are mobilized through changes in pH and the increase in dissolved organic carbon following biosolid application (Badewa et al., 2023). Besides morphological root traits, plant-associated factors include root-derived chemical changes in the rhizosphere and the plant's capacity for element uptake, transport and sequestration. The literature indicates large differences in the ability of plants to alter rhizosphere chemistry, to utilize a specific element pool (Lambers et al., 2015; Lu et al., 2020), and to cope with non-essential elements in the soil (Dillon and Courtney, 2023). With regard to metalloid accumulation and tolerance, the functional adaptations of plants include two basic physiological strategies: accumulators and excluders (Noor et al., 2022). The majority of plant species tolerate metals in the substrate by excluding the elements at the sites of uptake through restricting influx, promoting efflux, or extracellular complexation with organic ligands (Akram et al., 2024). In contrast, accumulators efficiently acquire/utilize elements from the soil and avoid element toxicity in the roots by rapidly transporting the elements to the shoots, where they are sequestrated in the leaf tissue. Specialized (hyper-) accumulators that evolved in metalliferous environments, of which *Thlaspi caerulenscens* and *Alyssum murale* are profoundly studied, accumulate more than 1,000 mg of Cd, Ni, and Zn in their shoot dry matter (van der Ent et al., 2013) but only when they are growing on soils with high metal mobility. These species rely on highly efficient transport and cellular hypertolerance mechanisms rather than alteration of rhizosphere chemistry (Pollard, 2023). In accumulator species from non-metalliferous soils, however, metal accumulation may appear as a side effect of functional traits related to P, Fe, and Mn acquisition (Monei et al., 2022), especially the release of carboxylates and acidification of the rhizosphere. Indeed, rhizosphere acidification has been linked with metal accumulation in different taxa from the Phytolaccaceae, Polygonaceae, Brassicaceae and Proteaceae (Kikis et al., 2024). Of these species, *Fagopyrum esculentum* unspecifically accumulates metals even when it is growing on soils with relatively low metal solubility, which has been attributed to rhizosphere acidification (Kreuzeder et al., 2018). In *Lupinus albus* and P-efficient species from the Proteaceae, Mn hyperaccumulation coincides with enhanced carboxylate release under conditions of P deficiency (Lambers et al., 2015; Lambers, 2022; Olt et al., 2022). Concomitantly, these species typically show low concentrations of non-essential elements (Martínez-Alcalá et al., 2013), likely as a consequence of element exclusion through extracellular complexation. Recently, relationships between carboxylate release and metal exclusion have been demonstrated for Al, Cd, Pb, and REEs (Wiche and Heilmeier, 2016; Wiche et al., 2023; Wiche and Pourret, 2023). Given that carboxylate release and rhizosphere acidification are regulated by the plant's nutrient status (Lambers, 2022; Wiche et al., 2023), it is reasonable that soil amendment with digestate or sewage sludge impacts the uptake of non-essential elements not only through a direct increase in potentially available element pools in the soil but also indirectly through the alteration of plant nutrition.

To date, studies on Ga uptake in plants are scarce, and no research has addressed how sewage sludge or digestate amendments influence the availability and plant uptake of Ge, Ga, and REEs specially when the nutritional status of the plants is altered through the addition of amendments. Therefore, the objectives of this study were to: i) determine the impact of digestate and sewage sludge amendment on ammonium acetate-extractable (labile) essential (P, Fe, Mn, Ni) and non-essential elements (Cd, Ge, Ga, REEs) in soil, ii) evaluate the uptake of Cd, Ge, Ga, and REEs in four plants with contrasting element acquisition strategies using the P-efficient Mn accumulator *L. albus* (Pearse et al., 2006), the phosphophile unspecific metal accumulator *F. esculentum*, the element excluder *Carthamus tinctorius* Ciaramella et al., 2022), and the specialized Ni hyperaccumulator *A. murale* (Wiche and Pourret, 2023) and iii) investigate how changes in essential and non-essential element pools in the soil following biosolid amendment influence the soil-plant transfer of Cd, Ge, Ga, and REEs in these species.

2 Materials and methods

2.1 Greenhouse experiment on the effect of biosolids

Alyssum murale (yellow tuft), *Lupinus albus* (white lupin), *Carthamus tinctorius* (safflower) and *Fagopyrum esculentum* (buckwheat) were cultivated on soil (unamended), and soil mixed with sewage sludge or digestate from anaerobic fermentation in the greenhouse. Each treatment within each plant species was fivefold replicated. The soil was collected from the campus of TU Bergakademie Freiberg and characterized as luvisol. The vegetation was stripped off, and 200 kg of topsoil (0–20 cm depth) was collected, homogenized and sieved (2 mm). The sewage sludge was obtained from the sewage treatment plant in Muldental, Germany. The catchment area of the sewage plant includes industrial areas. Hence, the concentrations of toxic elements frequently exceed the thresholds for sewage sludge and limits the use of the material for thermal treatment. The digestate was obtained from the biogas lab of the Institute of Thermodynamics, TU Freiberg and derived from mesophilic anaerobic fermentation batch experiments with cow dung and grass biomass. The digestate and sewage sludge were thoroughly mixed. Of each material, the soil and the biosolids, ten homogeneous samples were collected and stored in centrifuge tubes at 4°C before being analyzed. In total, sixty pots (volume 2 L) were filled with either 3 kg of a homogenous mixture of 1800 g soil and 1,200 g sewage sludge (40:60% sewage sludge: soil, fw), 2,550 g soil and 450 g digestate (15:85% digestate: soil fw), or unamended soil as a reference. The water contents of soil, sewage sludge and digestate were 16%, 77% and 94%, respectively. Thus, the amount of digestate and sewage sludge added corresponded to 3% and 24% on a dry matter basis. Here, a higher portion of sewage sludge was added due to the substantially higher labile P concentrations in digestate compared to the sewage sludge (Supplementary Material S1). The substrates were allowed to settle for 4 weeks. Shortly before the transplantation of seedlings, soil samples (5 g of soil, sampling depth 10 cm) were collected from five randomly selected pots within each treatment. The samples were stored in centrifuge tubes at 4°C before being analyzed.

Seeds of *A. murale* (origin: Ankara, Kizilcahamam) were provided by the Botanical Garden and Botanical Museum Berlin, and seeds of *C. tinctorius* (cv CT05 Calin) were provided by the Exsemine Company. Seeds of *F. esculentum* (cv Bamby) and *L. albus* (cv Feodora) were obtained from Bornträger GmbH. Seeds of *L. albus*, *F. esculentum, A. murale* and *C. tinctorius* were surface-sterilized (H_2O_2) and germinated on a wet filter paper in a Petri dish. One individual of the three-day-old seedlings was placed in the middle of the pots to obtain each species growing on each substrate in fivefold replication. The pots were incubated in a growth chamber with 65% relative humidity, 25°C average temperature and 600 μmol/m^2 s Photosynthetically active radiation (PAR) in a fully randomized design. The plants did not receive additional fertilizer; all pots were watered weekly with 200 mL of tap water over 6 weeks. After 6 weeks of plant growth, all plants were cut 1 cm above the soil surface. The shoot biomass was washed with deionized water, dried at 60°C, ground to a fine powder using an ultra-centrifugal mill (type ZM 1000, Retsch, Germany), and stored in centrifuge tubes at 4°C until being analyzed.

2.2 Collection of root exudates

A separate greenhouse experiment was designed for the determination of root exudates in cultivars of *L. albus* (cv. Feodora), *F. esculentum* (cv. Bamby), and *C. tinctorius* (cv. CT05 Calin) depending on P status. Seeds were surface-sterilized by washing with 0.5% sodium hypochlorite (NaOCl) for 3 min, followed by carefully rinsing with deionized water, and then allowed to germinate in Petri dishes in a growth chamber at 20°C. After germination, the seedlings of each plant species (one seedling per pot) were planted in 10 plastic pots (2 L total volume) filled with acid (HNO_3) washed quartz sand. The pots were incubated for 5 weeks with a 15 h photoperiod, 18°C–30°C, relative humidity of 65%, and an average photosynthetically active photon flux density of 600 μmol/m^2 s. During 5 weeks, all plants received weekly 200 mL of a 1/5 strength Hoaglands solution (Arnon and Stout, 1939) but with differing P concentrations. Specifically, half of the plants received a solution containing 100 μM KHPO4 together with the other nutrients (P100), while the other plants received 20 μM P (P20). After a cultivation period of 4 weeks, the mature plants were carefully removed from the sand by washing with tap water and transferred into glass beakers containing 100 mL of a 2.5 μM $CaCl_2$ solution, where they were let to stay for 30 min under the growth lamp and allowed to release carboxylates into the collection solutions (Neumann et al., 2009). Immediately after the collection of root exudates, the pH was measured, and 1 mL L^{-1} Micropur was added to prevent microbial decomposition of carboxylates (Oburger et al., 2013). Thereafter, the shoots and roots were separated, weighed, and dried for 24 h at 60°C.

2.3 Chemical characterization of biosolids and soils

Each sample of the unamended soil, digestate, sewage sludge, and soil amended with the biosolids was homogenized and split into two subsamples. One-half was dried at 105°C to determine total element concentrations and water content. The other half was left fresh for the determination of labile-bound elements, mineral nitrogen (N_{min}), soil pH, and DL-extractable phosphate. The dried samples were powdered in a boron carbide mortar. The ground samples (100 mg) were fully digested in a laboratory microwave (Ethos Plus MLS) with a mixture of HNO_3 and HF, according to Krachler et al. (2002). In all sample processing steps, certified reference soil samples GBW 07406 and GBW 07407 were used for quality control. The resulting solutions were stored at 4°C before being analyzed. For the determination of mobile/exchangeable and acid-soluble elements, the samples were extracted with 1 M NH_4^+-acetate, pH 5, for 24 h, according to Wiche et al. (2017). The resulting solutions were centrifuged, filtered (200 nm), and stored at 4°C before being analyzed. For analysis of mineral N (NO_3^- and NH_4^+), the substrate samples were extracted with deionized water and 1 mol/L KCl (1:10 extracts) and photometrically analyzed according to Bolleter et al., 1961 and Hartley and Asai (1963).

2.4 Determination of element concentrations and carboxylates

Ground plant samples (100 mg) were digested in a laboratory microwave (Ethos plus) with 1.9 mL of nitric acid (65% supra) and 0.6 mL of hydrofluoric acid (4.9% supra), according to Krachler et al. (2002). In all sample processing steps, certified reference plant samples NCS ZC73032 and NCS ZC73030 were used for quality control. The concentrations of P, Fe, Mn, Zn, Ni, Cd, Ga, Ge and REE in soil extracts and digestion solutions were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, xseries 2, Thermo Scientific and NexION 300D, Perkin Elmer), using 10 µg/L rhodium and rhenium as internal standards (Krachler et al. 2002; Monei et al., 2022). Calibration solutions ranging from 0.01 to 100 µg/L were prepared through suitable dilution of a multi-element stock standard solution (Merck). Accuracy verification was conducted by analyzing the certified reference materials GBW 07406, GBW 07407, NCS ZC73032, and NCS ZC73030 (LGC Standards); the results from soil analysis deviated by less than 16%, and the results from plant analysis by less than 12% from the certified values. Concentrations of acetate, malonate, fumarate, glutarate, malate, and citrate in the collection solutions were determined by ion chromatography equipped with suppressed conductivity detection (ICS-5000, Thermo Scientific). Organic anions were separated at 30°C on an IonPac® AS11-HC column (Thermo Scientific) using gradient elution with sodium hydroxide as eluent and a flow rate of 1.0 mL/min.

2.5 Data processing and statistics

Concentrations of light rare earth elements (LREEs) and heavy rare earth elements (HREEs) in the plant and soil samples were calculated as sums of La, Ce, Pr, Nd, Pm, Sm, Eu (LREEs) and Gd, Tb, Y, Ho, Er, Yb, Tm, Lu (HREEs) according to Tyler (2004). Based on LREE and HREE concentration the LREE/HREE-ratio was calculated to explore changes in REE fractionation depending on pant species and soil treatment (Wiche et al., 2023; Wiche and Pourret, 2023). All element concentrations reported were calculated on a dry-weight basis. Variance homogeneity, a model requirement of ANOVA, was checked using the Brown-Forsythe test. In the case of variance inhomogeneity, the data were transformed using log transformation to achieve variance homogeneity. Differences between element concentrations and contents in plants and soil were tested using a one-factor analysis of variance (ANOVA) followed by a Bonferroni post-hoc test (α = 5%). All statistical analyses were performed using SAS OnDemand for Academics.

3 Results

3.1 Physicochemical properties of the sewage sludge, digestate, and biosolids-amended soil

Sewage sludge and digestate had similar organic matter contents, whereas digestate had a higher pH and higher N_{min} concentrations (Table 1). The sewage sludge contained 137% higher total P concentrations than digestate, but most of P (99%) was present in relatively insoluble forms and could not be dissolved by NH_4^+-acetate (pH 5) (Supplementary Material S1). In comparison, 42% of P in the digestate was present in the mobile, exchangeable, and acid-soluble forms. In addition, the digestate contained higher total and labile concentrations of Mn, Zn, and Ni as well as of total Fe (Supplementary Material S1).

Concerning the potentially toxic elements, there were no differences in the total concentrations of Cd, Ge, LREE and HREE in sewage sludge and digestate (Supplementary Material S1). However, the concentration of Ga was roughly 5-fold higher in digestate than in sewage sludge. Concomitantly, the sewage sludge contained significantly higher concentrations of labile Cd, Ge, LREE, and HREE and was characterized by a higher LREE/HREE ratio (Supplementary Material S1). When added to the soil, sewage sludge increased soil organic matter contents, EC, and N_{min} significantly, whereas there was no effect of the addition of digestate (Table 1). We emphasize that the digestate had a higher water content than the sewage sludge (77% sewage sludge and 90% digestate) and was added at lower application rates, which impairs a direct comparison between the two treatments.

Nevertheless, compared to the unamended soil, the application of digestate increased soil pH, but this was not the case when sewage sludge was added (Table 1). The addition of both biosolids, the digestate and sewage sludge, respectively, did not alter the total concentrations of Cd and did not change the LREE/HREE ratio of the soil (Table 3). Moreover, the addition of digestate did not alter the total concentrations of the plant nutrients P, Fe, Mn and Zn (Table 2) or of the non-essential elements Ge, LREE, and HREE (Table 3). However, compared to the unamended soil, Ni concentrations were 53%, and Ga concentrations were 17% significantly higher when digestate was added (Table 2). In comparison, in the soil amended with sewage sludge, the total concentrations of P, Zn, and Ni were significantly higher than in unamended soil. At the same time, total Fe and Mn, Ge, Ga, LREE, and HREE concentrations were lower, whereas Cd concentrations, as well as LREE/HREE ratios, remained unchanged (Tables 2, 3).

Although total concentrations of P, Fe, Mn, and Zn remained unchanged, the addition of digestate led to a higher mobility of these elements in the soil (by 122, 115, 529, and 44%). The increase in total

TABLE 1 Physicochemical parameters of soil (unamended), sewage sludge, digestate, and mixtures of soil and sewage sludge (soil + SS) or soil and digestate (soil + DG), respectively. Mean ± standard deviation (n = 4–5). Organic matter content (OM), electrical conductivity (EC), pH in aqueous solution (pH H_2O), and mineral nitrogen (Nmin). Differences between means were identified by a one-way analysis of variance followed by a Bonferroni post-hoc test. Means with different letters are significantly different at (α = 5%).

Parameter	Soil	Sewage sludge	Digestate	Soil + SS	Soil + DG
OM (%)	8.0 ± 0.2a	70 ± 1d	66 ± 1c	18 ± 1b	8.1 ± 0.2a
EC (μS cm^{-1})	42 ± 6a	587 ± 35d	231 ± 64b	323 ± 7c	78 ± 2a
pH H_2O	6.5 ± 0.1a	7.2 ± 0.1c	8.2 ± 0.1d	6.6 ± 0.1 ab	6.9 ± 0.2b
Nmin (g kg^{-1})	0.12 ± 0.01a	1.3 ± 0.2b	2.5 ± 0.1c	0.24 ± 0.04d	0.10 ± 0.01a

TABLE 2 Total and NH_4-acetate extractable concentrations (mg/kg dw) of essential elements in soil (unamended), soil amended with digestate (soil + DG) and soil amended with sewage sludge (soil + SS). Mean ± standard error (n = 4–5). Concentrations within the same element fraction between different substrates were compared by a one-way analysis of variance followed by a Bonferroni post-hoc test. Means with different letters are significantly different at (α = 5%).

Element	Fraction	Soil	Soil + DG	Soil + SS
	mg/kg dw			
P	Total	745 ± 46b	889 ± 92b	4,006 ± 648a
	Labile	6.3 ± 0.6c	14 ± 4b	53 ± 6a
Fe	Total	31,465 ± 2718a	33,256 ± 4644a	28,870 ± 1647b
	Labile	4.7 ± 1.1c	10.1 ± 2.1a	7.2 ± 0.9b
Mn	Total	720 ± 44a	847 ± 68a	593 ± 29b
	Labile	17 ± 2c	107 ± 12a	60 ± 4b
Zn	Total	214 ± 32b	279 ± 62 ab	358 ± 41a
	Labile	9.1 ± 0.8c	13 ± 1b	22 ± 1a
Ni	Total	7.2 ± 0.8b	11 ± 2a	17 ± 4a
	Labile	0.10 ± 0.01b	0.18 ± 0.11b	0.69 ± 0.10a

Ni did not correspond with a higher Ni mobility. Similarly, the addition of digestate did not increase the mobility of Cd and Ge. However, the digestate significantly increased the mobility of Ga, LREE, and HREE, and it led to a higher LREE/HREE ratio in the NH_4^+-acetate-extractable element fraction. The sewage sludge increased the mobility of all considered plant nutrients, above all P, which showed a 741% higher mobility when sewage sludge was added. Moreover, the addition of sewage sludge increased the concentrations of NH_4^+-acetate-extractable Cd and Ga while the concentrations of mobile LREE, HREE, and the LREE/HREE-ratio decreased, resulting in LREE/HREE-ratios that were the lowest compared to all treatments (Table 3).

3.2 Carboxylate release in response to P-supply

Compared to plants growing under conditions of low P supply (20 μM P), all plants with high P supply (100 μM P) responded with increased shoot P concentrations (Table 4). Shoot P increased by 99%, 171%, and 195% in *L. albus*, *F. esculentum*, and *C. tinctorius*, respectively, indicating a more strongly pronounced effect in *F. esculentum* and *C. tinctorius* than in *L. albus*. All plants responded to increased shoot P supply with higher shoot biomass, except *F. esculentum*, which was characterized by marginal differences in shoot mass between the P treatments. The root mass of *L. albus* was higher when P supply was low, whereas the root mass of *C. tinctorius* declined. Neither *L. albus* nor *C. tinctorius* altered the pH of the exudate collection solutions. Irrespective of P supply, the collection solutions of *F. esculentum* showed the lowest pH values of all plant species tested and had significantly lower pH values under conditions of high P supply (Table 4). Low P supply increased the release of malonate and citrate in *F. esculentum* but did not alter the release of other carboxylates. Thus, there were no significant differences in the sum of carboxylates in this species. Similarly, in *C. tinctorius,* total carboxylate release remained unchanged in response to P supply. Here, only single components were affected in divergent ways, showing a lower release of malate, but a higher release of citrate when P supply was high. In contrast, *L. albus* responded to a high P supply with decreased malate release, whereas release of other compounds remained unchanged, leading to a net reduction of total carboxylate release by 60% in high P-supplied plants compared to P-deficient plants (Table 4).

3.3 Plant growth and shoot nutrient concentrations in plants treated with biosolids

Plants of *F. esculentum* developed the highest biomass, and those of *A. murale* the lowest (Table 5). The addition of digestate tended to decrease plant growth of all species except *C. tinctorius*; however, this effect was not statistically significant at α = 5%. In contrast, sewage sludge-treated plants of *F. esculentum*, *C. tinctorius*, and *L. albus* developed 88%, 158% and 82% higher biomass, respectively. The addition of digestate did not alter the shoot P concentrations of the tested plants. There were apparent effects on the concentrations of the trace nutrients Fe and Mn, which varied among plant species, whereas Zn and Ni remained unchanged. In *L. albus*, trace nutrient concentrations were unaffected by the addition of digestate. Notably, *L. albus* showed the highest shoot Mn concentrations (more than 1,000 mg/kg Mn) in both digestate-treated and unamended soil, (Table 5). Shoot Fe and Mn of *F. esculentum* tended to increase; however, this was not statistically significant due to high data variability. In the other species, the addition of digestate led to higher shoot Fe and Mn concentrations (except Fe in *C. tinctorius,*

TABLE 3 Total and NH_4-acetate extractable concentrations (mg/kg dw) of essential elements in soil (unamended), soil amended with digestate (soil + DG), and soil amended with sewage sludge (soil + SS). Mean ± standard error (n = 4–5). Concentrations within the same element fraction between different substrates were compared by a one-way analysis of variance followed by a Bonferroni post-hoc test. Means with different letters are significantly different at (α = 5%).

Element	Fraction	Soil	Soil + DG	Soil + SS
	mg/kg dw			
Cd	Total	1.8 ± 0.6ns	1.3 ± 0.4ns	1.9 ± 1.0ns
	Labile	0.62 ± 0.04b	0.75 ± 0.16 ab	0.81 ± 0.05a
Ge	Total	1.87 ± 0.05a	2.14 ± 0.27a	1.57 ± 0.24b
	Labile	0.0049 ± 0.0009 ns	0.0037 ± 0.0015ns	0.0036 ± 0.0002ns
Ga	Total	14.1 ± 0.2b	16.5 ± 2.1a	12.5 ± 0.1c
	Labile	0.0037 ± 0.0008c	0.0103 ± 0.0012a	0.0067 ± 0.0012b
LREE	Total	125 ± 3a	137 ± 16a	109 ± 4c
	Labile	0.39 ± 0.02b	0.45 ± 0.03a	0.28 ± 0.01c
HREE	Total	44 ± 2 ab	52 ± 6a	34 ± 7b
	Labile	0.16 ± 0.01b	0.18 ± 0.02a	0.12 ± 0.01c
LREE/HREE	Total	2.86 ± 0.06ns	2.82 ± 0.09ns	3.26 ± 0.70ns
	Labile	2.46 ± 0.02b	2.52 ± 0.04a	2.27 ± 0.04c

TABLE 4 Shoot P concentrations shoot mass, root mass, root carboxylate release (µmol/h), and pH of the collection solutions after 30 min of exudation time of plants cultivated in quartz sand treated with nutrient solutions containing 100 µM P or 20 µM P. Differences between P treatments within a specific plant species were compared by t-tests with Bonferroni adjustment. Means with different letters are significantly different at (α = 5%).

Treatment	Unit	*Lupinus albus*		*Fagopyrum esculentum*		*Carthamus tinctorius*	
		P100	P20	P100	P20	P100	P20
Shoot P	mg/g	2.74 ± 0.11a	1.38 ± 0.32b	5.39 ± 0.80a	1.99 ± 0.13b	4.33 ± 2.31a	1.47 ± 0.10b
Shoot mass	G	5.3 ± 0.4a	4.1 ± 0.8b	6.6 ± 2.1a	6.2 ± 0.2a	3.9 ± 0.5a	2.8 ± 0.4b
Root mass		4.0 ± 0.8b	5.4 ± 0.4a	5.4 ± 1.8a	4.9 ± 1.4a	5.3 ± 2.3a	2.3 ± 1.4b
pH		7.9 ± 0.1a	8.0 ± 0.2a	7.4 ± 0.1b	7.8 ± 0.1a	7.9 ± 0.1a	8.0 ± 0.1a
Acetate	µmol/h	0.59 ± 0.34a	0.58 ± 0.48a	<0.01a	<0.01a	<0.01a	<0.01a
Fumarate		0.06 ± 0.04a	0.05 ± 0.01a	<0.01a	<0.01a	0.03 ± 0.02a	0.02 ± 0.01a
Lactate		0.04 ± 0.01b	0.24 ± 0.11a	<0.01a	<0.01a	0.5 ± 0.1a	0.3 ± 0.2a
Malate		11.2 ± 8.5b	29.5 ± 3.4a	2.9 ± 0.9a	4.2 ± 2.9a	1.5 ± 0.3a	2.0 ± 1.5a
Malonate		<0.01a	<0.01a	1.0 ± 0.2b	1.8 ± 0.5a	<0.01a	<0.01a
Citrate		2.3 ± 0.6b	4.5 ± 1.4a	1.2 ± 0.1b	2.1 ± 0.5a	3.9 ± 1.6a	1.7 ± 0.8b
∑Carboxylates		13.9 ± 8.8b	34.8 ± 2.4a	5.2 ± 1.0a	8.1 ± 3.8a	6.1 ± 1.4a	4.9 ± 1.2a

which were lower), indicating an improved metal nutrient supply (Table 5).

Compared to the plants grown in unamended soil, the addition of sewage sludge strongly increased the concentrations of shoot P in all species. Shoot Mn concentrations were increased in *A. murale, F. esculentum* and *C. tinctorius* by 863%, 1,480%, and 700%, but not in *L. albus,* which exhibited a lower Mn concentration than plants grown in unamended soil (Table 5). In addition, the sewage sludge led to a higher Zn concentration in all species. In particular, sewage sludge-treated *A. murale* showed the highest shoot Zn concentrations of all plant species tested (more than 1,000 mg/kg Zn). Moreover, *A. murale* and *F. esculentum* responded to the sewage sludge treatment with 287% and 263% higher Ni concentrations compared to the plants grown in unamended soil. In contrast, shoot Ni remained unchanged in *C. tinctorius* and *L. albus*. Iron concentrations remained relatively unaffected in sewage

TABLE 5 Shoot biomass and concentrations of nutrients in plants cultivated on soil (unamended), soil amended with digestate (Soil + DG), and soil amended with sewage sludge (Soil + SS); mean ± sd, n = 4–5. Small letters show concentration differences among different substrates for the same plant species. Capital letters denote accumulation differences between plant species within same substrate. Significant differences were identified by one-factor analysis of variance (ANOVA) followed by a Bonferroni post-hoc test. Means with different letters are statistically significantly different at (α = 5%).

Species	Treatment	Biomass	P	Mn	Fe	Zn	Ni
		g	g/kg	mg/kg			
A. murale	Soil	0.41 ± 0.17C	2.4 ± 0.2bA	24 ± 4bB	78 ± 23bAB	107 ± 87b	1.6 ± 0.3b
	Soil + DG	0.14 ± 0.09B	2.5 ± 0.6b	224 ± 91 aB	252 ± 117 aA	173 ± 86bA	2.5 ± 1.4b
	Soil + SS	0.25 ± 0.16C	4.5 ± 0.9 aA	231 ± 36 aB	135 ± 57abA	1,071 ± 639 aA	6.2 ± 2.6 aA
	p-value	0.08	<0.01	<0.01	0.03	<0.01	0.03
F. esculentum	Soil	7.4 ± 1.0bA	2.5 ± 0.4bA	40 ± 22bB	31 ± 10bB	54 ± 10bB	1.1 ± 0.2b
	Soil + DG	3.6 ± 3.0bA	2.2 ± 0.7b	163 ± 159bB	100 ± 81bB	38 ± 16bB	1.3 ± 0.1b
	Soil + SS	13.9 ± 2.1 aA	6.9 ± 1.4 aA	632 ± 262 aA	132 ± 63 aA	482 ± 133 aA	4.0 ± 0.9 aA
	p-value	<0.001	<0.001	<0.001	0.04	<0.001	<0.001
C. tinctorius	Soil	1.2 ± 0.9bB	2.0 ± 0.4bAB	27 ± 5 cB	98 ± 75A	79 ± 13b	2.0 ± 1.3
	Soil + DG	1.8 ± 0.6abAB	2.3 ± 0.1b	82 ± 5bB	68 ± 49B	71 ± 10bB	2.7 ± 2.4
	Soil + SS	3.1 ± 1.5 aB	3.0 ± 0.5 aB	216 ± 22 aB	55 ± 10B	152 ± 25 aB	1.1 ± 0.3B
	p-value	0.05	<0.01	<0.001	0.4	<0.001	0.37
L. albus	Soil	2.7 ± 1.0bB	1.5 ± 0.2bB	996 ± 353 aA	79 ± 15abAB	48 ± 5b	2.4 ± 1.1
	Soil + DG	1.3 ± 0.5bB	1.9 ± 0.3b	1,412 ± 805 aA	103 ± 38 aB	42 ± 9bB	1.7 ± 0.3
	Soil + SS	4.9 ± 1.0 aB	2.5 ± 0.4 aB	404 ± 149bB	46 ± 7bB	94 ± 21 aB	1.8 ± 0.3B
	p-value	<0.001	<0.01	0.05	0.01	<0.001	0.45

sludge-treated plants, except in *F. esculentum* and *A. murale,* where Fe increased by 325% and 73%, respectively (Table 5).

3.4 Shoot accumulation of non-essential elements

Considering all treatments, *A. murale* showed the highest concentrations of the studied non-essential elements and *L. albus* the lowest (Table 6). The addition of digestate did not significantly alter Cd concentrations in the investigated species (Table 6). Germanium concentrations were generally very low and rarely exceeded 10 μg/kg. In *F. esculentum*, Ge was not detectable, irrespective of the treatment. Moreover, the digestate did not alter the concentrations of Ga, LREE and HREE in *F. esculentum* and *C. tinctorius*. However, in *A. murale*, Ge, Ga, LREE, and HREE concentrations were higher when digestate was added (Table 6). *Lupinus albus* did not show altered concentrations of Ge, Ga and HREE but exclusively showed a higher concentration of LREE.

When sewage sludge was added, all plants except *C. tinctorius* responded with substantially higher Cd concentrations. Further, *C. tinctorius* showed declining concentrations of all other investigated elements, while the concentrations remained unchanged in *A. murale. Fagopyrum esculentum* showed a higher concentration of Ga but did not show any changes in LREE and HREE concentrations. Finally, *L. albus* did not show changes in Ge, Ga and LREE concentrations; however, this plant species showed higher HREE concentrations when sewage sludge was added, indicating significant changes in LREE/HREE ratios (Figure 1).

Considering the shoot biomass and the herein quantified element concentrations, amounts of elements in the respective plant tissues and whole shoot contents were calculated (Figure 1). Plants grown in unamended soil of *F. esculentum* and *C. tinctorius* showed by far the highest net shoot uptake of Cd. Cadmium accumulation decreased in the order *F. esculentum* > *C. tinctorius* > *L. albus*, *A. murale*. A similar trend was observed when REE accumulation was compared among the species (Figure 1). Germanium was not detectable in *F. esculentum,* and there were no differences in Ga accumulation between *A. murale*, *C. tinctorius* and *L. albus*. Instead, the lowest Ga uptake was observed in *F. esculentum*.

The addition of digestate did not alter the accumulation of Cd in the investigated plant species, and *A. murale* did not show any changes in net shoot uptake of all investigated elements, including Cd. Similarly, *F. esculentum* did not show changes in LREE, HREE, and Ge uptake; however, a higher Ga uptake was observed (Figure 1). Conversely, in *C. tinctorius*, Ge and Ga accumulation declined following the addition of digestate. Lower Ge contents were also visible in *L. albus*, but Ga contents remained unchanged. In addition, the shoots of *L. albus* contained lower HREE contents, leading to significantly higher LREE/HREE ratios (Figure 1).

The addition of sewage sludge substantially increased the Cd uptake in all plants, regardless of the tested species (Figure 1). Germanium uptake was only affected in *C. tinctorius*, showing a lower content when sewage sludge was added, and Ga uptake was

TABLE 6 Concentrations of non-essential elements in plants cultivated on soil (unamended) soil amended with digestate (Soil + DG) and soil amended with sewage sludge (Soil + SS); mean ± sd, n = 4–5). Significant differences within the substrate were identified by one-factor analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test. Small letters show concentration differences among different substrates for the a same plant species. Capital letters denote accumulation differences between plant species within a same substrate. Means with different letters are statistically significantly different at (α = 5%).

	Treatment	Cd	Ge	Ga	LREE	HREE
		mg/kg	μg/kg	μg/kg	μg/kg	μg/kg
A. murale	Soil	0.6 ± 0.1bB	6 ± 3bA	18 ± 14b	198 ± 94bA	84 ± 34b
	Soil + DG	2.8 ± 2.1bA	22 ± 5 aA	92 ± 54 aA	858 ± 376 aA	280 ± 122a
	Soil + SS	10 ± 6 aA	8 ± 4b	45 ± 27bA	378 ± 265bA	152 ± 61 ab
	p-value	<0.01	<0.01	0.04	0.02	0.02
F. esculentum	Soil	1.1 ± 0.3bBC	<1B	7 ± 3b	237 ± 151A	93 ± 65A
	Soil + DG	0.8 ± 0.6bB	<1B	6 ± 4bB	204 ± 78B	82 ± 38B
	Soil + SS	7.1 ± 1.4aAB	<1	14 ± 4 aB	281 ± 139A	104 ± 57A
	p-value	<0.001	n.a	<0.01	0.68	0.84
C. tinctorius	Soil	4.7 ± 1.4A	12 ± 7 aA	11 ± 8	207 ± 152 aA	79 ± 48a
	Soil + DG	3.6 ± 1.0A	<1	4 ± 2B	96 ± 20abB	44 ± 12 ab
	Soil + SS	4.3 ± 1.0B	2 ± 1b	8 ± 2B	70 ± 22bB	35 ± 10b
	p-value	0.33	0.02	0.11	0.03	0.05
L. albus	Soil	0.09 ± 0.03bC	7 ± 4A	9 ± 1 ab	39 ± 13bB	18 ± 4b
	Soil + DG	0.16 ± 0.05bB	4 ± 1B	16 ± 6 aB	65 ± 22 aB	19 ± 5b
	Soil + SS	0.24 ± 0.07 aC	8 ± 7	8 ± 3bB	31 ± 10bB	26 ± 5a
	p-value	<0.01	0.70	0.05	0.01	0.04

only affected in *F. esculentum* following the addition of sewage sludge. With regard to REEs, sewage sludge did not alter LREE and HREE accumulation in *A. murale* and *C. tinctorius*. However, *F. esculentum* responded to the addition of sewage sludge with a higher accumulation of both LREE and HREE, but there were no changes in the LREE/HREE ratios. In contrast, in *L. albus*, HREE concentrations were higher compared to the plants grown in unamended soil, leading to a substantially lower LREE/HREE ratio.

4 Discussion

4.1 Differences in shoot element uptake among the tested plant species

The soil used in our study was characterized by moderate mobility of phosphorus and micronutrients (Table 2), elevated concentrations of Cd (Kabata-Pendias, 2004) and average earth crust levels of Ga, Ge, and REE (Table 3) (Kabata-Pendias, 2004; Wiche et al., 2017; Wiche et al., 2018). Of these elements, 33% of total Cd was present in mobile and exchangeable forms that can be readily absorbed by plant roots (Bali et al., 2020). In contrast, the proportion of labile Ge, Ga, and REE did not exceed 0.5% of the total concentrations. Based on results from NH_4^+-acetate extracts, the potential availability decreased in the order Cd > LREE > HREE > Ge, Ga (Table 3). This is in accordance with the findings of Tyler and Olsson (2001), who demonstrated that compared to Cd and REE, the solubility of Ge and Ga in soils is low, and their mobilization requires substantial changes in physicochemical soil properties. Consequently, shoot element contents decreased in the order Cd > REE > Ga, Ge (Table 6; Figure 1). However, there were significant differences between plant species in handling the mobile elements, depending on the plant's ability to take up and translocate the elements. The low accumulation of Ge (Figure 1) with equal (barely detectable) concentrations across all species (Table 6) can be attributed to its low solubility in the soil (Table 6) and/or inefficient uptake and root-shoot transport. The dicots tested do not have silicon transporters that are involved in Ge uptake (Nikolić et al., 2023; Kaiser et al., 2020). After Ge had been passively absorbed, the element was likely diluted by the biomass accumulation that decreased in the order *F. esculentum* > *L. albus* > *C. tinctorius* >> *A. murale* (Table 5). In contrast to Ge, the uptake of Cd, REE, and Ga is mediated by Zn, Fe, and Ca transporters (Shi et al., 2022), so differences in element uptake depend more strongly on processes related to plant nutrition. The shoot nutrient concentrations of plants grown in unamended soil (Table 5) indicated that all plants contained adequate P concentrations, except *L. albus*, which exhibited shoot P concentrations below the critical level of 2 g/kg (Lambers et al., 2013). Surely, the shoot P status might not sufficiently reflect P availability due to an altered P mobility within the plants (Zohar et al., 2024). Nevertheless, high Mn concentrations of *L. albus* (Table 5)

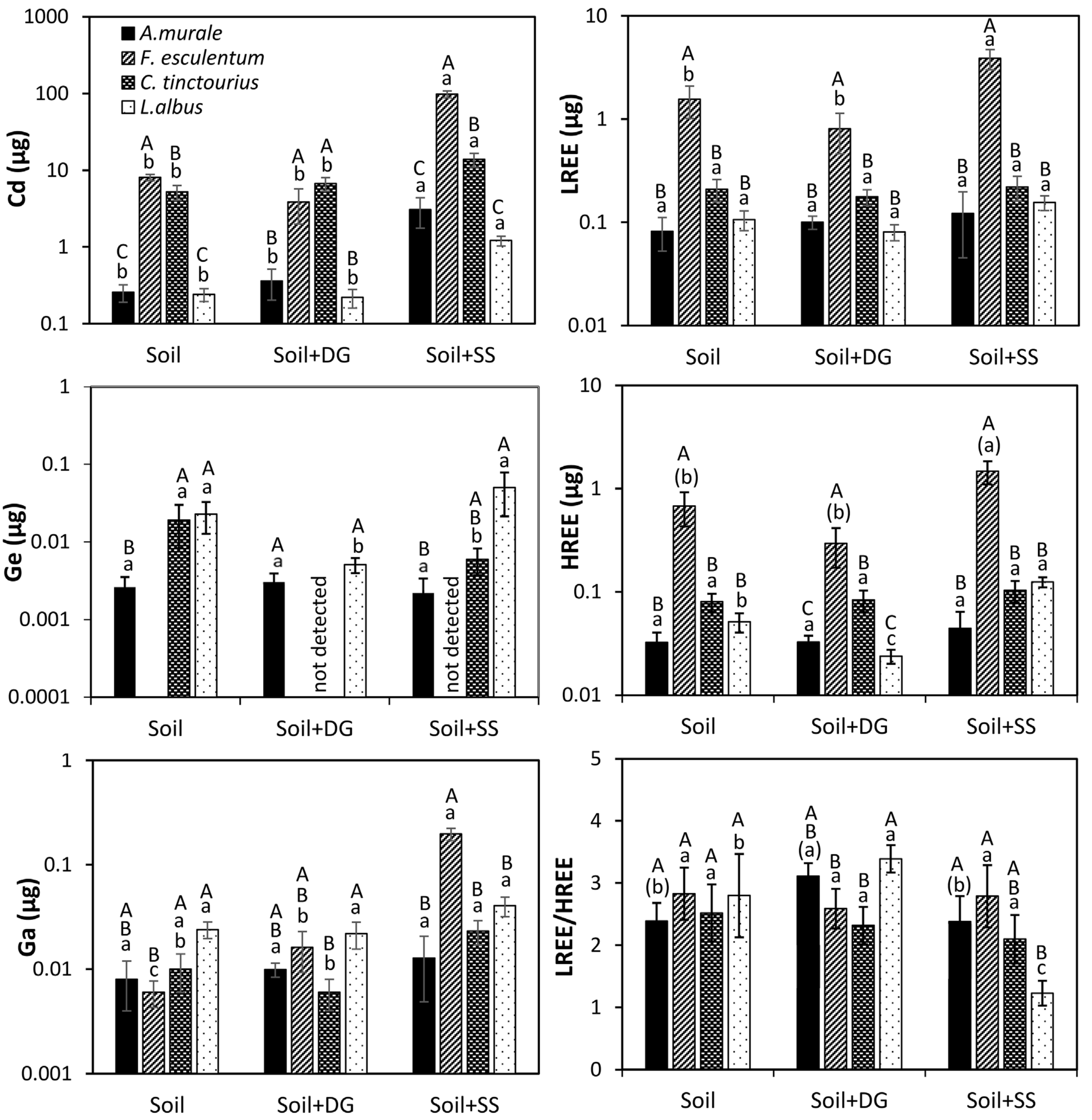

FIGURE 1
Effect of substrate properties on the accumulation of essential elements in the target plant species. Mean ± se (n = 4–5). Soil (S) unamended, soil + digestate (Soil + DG) and soil + sewage sludge (Soil + SS). Differences in shoot element contents were identified by one-way analysis of variance (ANOVA) followed by a Bonferroni test. Small letters show accumulation differences among different treatments within a specific plant species. Capital letters denote the accumulation differences of elements within a specific substrate among different plant species. Means with small letters (without bracket) different letters are statistically significantly different at (α = 5%). Small letters in brackets denote a statistical significance at (α = 10%).

clearly indicated that the plants released carboxylates under low P conditions (Lambers, 2022). Indeed, the lupin cultivar used in our study exhibited a substantially higher carboxylate release when P supply declined (Table 4).

Carboxylates and protons released by plant roots not only mobilize P and micronutrients, but also increase the solubility of an array of non-essential elements (Andresen et al., 2018) through dissolution, complexation, and ligand exchange (Kang and Peña, 2023; Wiche and Pourret, 2023). However, the mobile elements are not necessarily available for uptake when the elements are present as organo-metal complexes (Lee et al., 2021). Uptake systems predominantly shuttle ions through membranes, as demonstrated for Al, Cd, and REE (Wiche et al., 2023). Consequently, rhizosphere acidification, which predominantly mobilizes elements through dissolution and cation exchange, is likely an important plant trait related to accumulating non-essential elements in non-metalliferous environments, unlike carboxylate release. Overall, this explains the relatively low contents of non-essential elements in *L. albus* and the high contents in *F. esculentum* (Table 6; Figure 1). Possibly, the lupins efficiently mobilized the elements in the root zone, but chelation by carboxylates excluded them from uptake, whereas *F. esculentum* acidified the rhizosphere and translocated the absorbed elements to the shoots. High shoot Ga contents in *L. albus* could be a side effect of Mn hyperaccumulation and the upregulation of transition metal transporters under high Mn availability inflicted by P deficiency (Olt et al., 2022), given that Ga shares chemical similarities with Fe (Yandem and Jabłońska-Czapla, 2024). Concomitantly, Ga mobilization in the soil requires substantial changes in pH and/or the presence of high concentrations of carboxylates (Tyler and Olsson, 2001). We emphasize that information on differences in Ga accumulation in plant species is very scarce in the literature, and the elucidation of processes is fundamental in the face of soil pollution with this emerging pollutant (Shtangeeva, 2023). *Carthamus tinctorius* released minor amounts of carboxylates and did not acidify the rhizosphere (Table 4) as it has been previously described for P-inefficient phosphophilic species of the Brassicaceae (Lambers, 2022). Hence, this plant species accumulated only high amounts of Cd (Figure 1), which exhibited the highest mobility in the substrate (Table 3). Unfortunately, we did not analyze the carboxylate release of *A. murale*. Nonetheless, the literature indicates that *A. murale is* adapted to environments with high metal solubility (Bani et al., 2010) and relies on effective metal transport and internal detoxification rather than rhizosphere processes related to element mobilization and/or exclusion (Wiche and Pourret, 2023). Therefore, it is not surprising that the plants grown in unamended soil in our study exhibited relatively low Zn, Mn, and Ni concentrations (Table 5). The concentrations observed were two orders of magnitude lower than in plants from metalliferous environments (Bani et al., 2015; Van der Ent et al., 2021). Possibly, the plants suffered Ni deficiency, which in turn may have affected biomass development (Bani et al., 2015). In this plant species, high shoot concentrations of Ga, Ge, and REE might be related to processes of metal tolerance, but the resulting net element uptake was low due to the low biomass of this species.

4.2 Effect of digestate on plant nutrition and element accumulation in plants

All plants except *C. tinctorius* responded to the addition of digestate by accumulating less biomass. However, all plants showed higher micronutrient concentrations (Table 5), likely resulting from a higher proportion of labile nutrients in the digestate-amended soil (Table 2). A higher portion of labile P (Table 2) did not improve shoot P supply (Table 5), indicating metabolic changes or changes in root activity. Fedeli et al. (2023) investigated the effect of different forms of digestate on plant growth and demonstrated that solid digestate may have adverse effects, while liquid digestate improved plant growth. In our study, we observed growth inhibition following the addition of liquid digestate, except for *C. tinctorius*, where plant growth remained unchanged. Possibly, the digestate detrimentally altered the soil microbiome (Karimi et al., 2022), led to soil compaction (Caracciolo et al., 2022) or altered other physicochemical soil properties (Przygocka-Cyna and Grzebisz, 2018). Future studies should consider microbial communities changes depending on digestate composition. In this regard, the unaffected growth of *C. tinctorius* is particularly interesting (Table 4). *Carthamus tinctorius* develops extensive root systems (Montiel et al., 2020) and is described as a suitable species for crop production on marginal soils (Rosero et al., 2020), highlighting the role of species-specific traits in plant responses to soil additives like digestate. Given that concentrations do not necessarily reflect element uptake due to enrichment or dilution of elements in varying biomass, plant availability was evaluated by net shoot uptake (Figure 1). Changes in aboveground biomass are typically accompanied by changes in root biomass and altered metabolic activity as a stress response that, in turn, can affect element absorption (Shtangeeva, 2023). The present work concentrates on shoot content. In a future study, root content will be considered to clarify the total net uptake of plant biomass.

Nevertheless, we clearly observed that the digestate did neither affect labile Cd in the soil (Table 3) nor Cd uptake in the tested plants (Figure 1). A lower Ge uptake (Figure 1) but unchanged element mobility could derive from a lower Ge diffusion in the soil when the pH and soil organic matter (OM) raised following digestate addition (Table 1). Moreover, slightly higher concentrations of labile REEs in the substrate did not alter LREE and HREE uptake in *A. murale*, *C. tinctorius* and *F. esculentum*. The LREE/HREE ratios in *A. murale* reflected the higher LREE/HREE ratio of the digestate-treated soil (Table 3; Figure 1), suggesting that *A. murale* utilizes this element pool for uptake and translocates the elements to the shoots without discrimination. Although not statistically significant at $\alpha = 5\%$, the LREE/HREE ratio of *L. albus* exceeded the LREE/HREE ratio of *A. murale*, suggesting a discrimination of HREE relative to LREE through extracellular complexation with carboxylates (Wiche and Pourret, 2023) (Figure 1). In fact, *L. albus* showed a significantly lower HREE uptake, which cannot be explained by altered soil properties (Figure 1). Still, the digestate-treated lupins were P-deficient (Table 5) and released large amounts of carboxylates, as indicated by the high shoot Mn concentrations (Table 5). Non-significant results might be from low sample size so the validation remains field for future studies. Similarly, a higher Ga mobility in the digestate treatment increased Ga uptake only in *F. esculentum*, while *A. murale*, *L. albus,* and *C. tinctorius* did not respond to a higher portion of labile Ga in the substrate. Overall, this suggests that Ga and REE uptake and accumulation are predominantly controlled by plant physiological traits related to nutrition acquisition (Zohar et al., 2024) rather than by element mobility in the soil.

4.3 Effect of sewage sludge on plant nutrition and element accumulation in the plants

The sewage sludge-treated soil was characterized by a substantially higher mobility of all measured essential nutrients, as well as Cd and Ga (Tables 2, 3). Ge mobility was not affected by sewage sludge, and Ge uptake in plants tended to decrease (Figure 1), suggesting that the impact of biosolids on the soil–plant transfer of Ge is low. All plants except *A. murale* responded to the sewage sludge treatment with substantially higher biomass and all plants exhibited a luxury supply of P and micronutrients (Table 5). High Mn and Zn concentrations in *A. murale*, *F. esculentum*, and *C. tinctorius* likely derive from a higher portion of labile elements in the substrate in concert with higher root activity. All plants responded to the higher Cd mobility in the substrate with a higher Cd uptake (Figure 1). Still, the plants exhibited the same pattern of shoot Cd contents as the plants grown in unamended soil, supporting the application of NH_4^+-acetate-extracts for estimating overall Cd mobility in soil (Wang et al., 2024). However, species-specific physiological traits clearly govern the degree to which a plant can utilize this mobile element pool in the soil. Similarly, to the treatment with digestate, a higher Ga mobility in the substrate only affected shoot accumulation in *F. esculentum*. Moreover, the addition of sewage sludge resulted in unchanged LREE and HREE accumulation in *A. murale*, *F. esculentum*, and *C. tinctorius*. Possibly, the nutrients contained in the sewage sludge improved root growth, leading to a larger soil volume accessed by the roots. At the same time, *L. albus* exhibited a significantly lower LREE/HREE ratio that clearly resulted from a higher shoot uptake of HREE relative to LREE, which cannot be solely explained by altered soil chemistry (Table 3; Figure 1). Instead, it seems that the changes in nutrient supply following sewage sludge amendment altered root activity and element acquisition processes. In *L. albus*, Mn concentrations declined, suggesting a lower carboxylate release under conditions of high P supply (Lambers et al., 2015; Tables 4, 5). Given that HREEs form more stable complexes with carboxylates than LREEs (Wiche and Pourret, 2023), reduced carboxylate concentrations in the rhizosphere and apoplast may enhance HREE uptake and thus decrease the LREE/HREE ratio (Wiche et al., 2023). This demonstrates that the assessment of the effects of biosolids on the soil-plant transfer of Ge, Ga, and REE should include not only altered element concentrations in the soil but also physiological responses of the plant to altered nutritional status.

5 Conclusion

We demonstrated that both digestate and sewage sludge influence not only the mobility of nutrients in the soil but also the occurrence of Ge, Ga, and REE in plant-available forms. The physico-chemical properties and the spectrum of elements in sewage sludge and digestate may vary depending on their origin and the treatment technology. Moreover a longer experimental time would be necessary to explore the fate of elements in soil plant system in detail. Nevertheless, changes in total concentrations and element mobility assessed by NH_4^+-acetate extraction do not sufficiently explain element availability to plants, especially when it comes to the assessment of soil-plant transfer of Ga and REE. Of the investigated elements, Ge mobility and its uptake by plants were the least affected by sewage sludge and digestate, suggesting that the risk of soil-plant transfer is relatively low. We emphasize that digestate amendment increases the total Ge in the soil, which may accumulate in the soil over time and pose a risk once the element is released from the organic matrix. In contrast, mobility and plant-availability of Cd, Ga, and REEs were clearly affected depending on soil amendment and plant species. A higher portion of NH_4^+-acetate-extractable Cd in the soil resulting from the sewage sludge treatment increased Cd accumulation in all species, indicating that the plants utilized this mobile element pool during uptake. Consequently, the assessment of mobile/exchangeable elements seems to be a good proxy of soil-plant transfer for this element. However, plant-availability of Ga and REE was not directly reflected by the elements' mobility in the soil. Instead, it seems that the soil-plant transfer of Ga is governed by physiological traits involved in uptake, while the soil-plant transfer of REE additionally depends on the plant's nutritional status and below-ground functional traits related to phosphorus acquisition. This highlights the necessity for the evaluation of soil-plant transfer of Ge, Ga, and REEs in long-term field experiments, in which changes in soil chemistry are monitored in addition to physiological responses of species and genotypes to altered nutrient supply.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

NZ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review and editing. VL: Data curation, Formal Analysis, Methodology, Writing – original draft. AS: Writing – original draft. TA: Data curation, Formal Analysis, Writing – review and editing. OP: Data curation, Writing – review and editing. SF: Data curation, Writing – review and editing. PB: Data curation, Writing – review and editing. EV: Data curation, Writing – review and editing. MP: Data curation, Formal Analysis, Writing – review and editing. SW: Data curation, Formal Analysis, Writing – review and editing. EP: Writing – original draft. OW: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review and editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2025.1628175/full#supplementary-material

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