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RESEARCH ARTICLE

From waste to value: agromining of nickel, cobalt, and selenium from mine waste amended with sewage sludge

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Abstract

Background and aims The global demand for critical elements such as nickel, cobalt, and selenium is projected to increase significantly, driven by the transition to renewable energy. Mine wastes and sewage sludge, often enriched with these elements, represent untapped secondary resources. Agromining, an emerging phytotechnology that employs hyperaccumulator plants to extract metals from contaminated or enriched soils, offers a sustainable recovery method. The aim of this study is to provide a crucial proof-of-concept for the recovery of the critical elements nickel, cobalt, and selenium from these alternative resources through agromining.

Methods Mine wastes from three Italian mining sites were amended with sewage sludge at rates of 0, 15, and 30 kg m^{-2}. Three hyperaccumulator species targeting cobalt, (*Berkheya coddii*), selenium (*Astragalus bisulcatus*) and nickel (*Odontarrhena chalcidica*) were grown on these wastes. Biogeochemical characterization of the substrate was performed before and after agromining. In addition, plant biomass and metal accumulation were monitored.

Results Sewage sludge increased biomass across all species, especially in *A. bisulcatus*, but metal accumulation decreased by 33–60% for nickel, 0–41% for cobalt, and 67–74% for selenium. Furthermore, sewage sludge and agromining enhanced mine waste fertility and microbial diversity.

Conclusions Agromining of sewage sludge-amended mine wastes shows potential for sustainable recovery of selenium and cobalt. However, high sewage sludge application increased plant biomass, but concurrently reduced plant tissue metal concentrations. This study underscores the importance of optimizing sewage sludge / mine waste ratios for successful agromining.

Keywords Phytomining · Critical elements · Hyperaccumulator plants · Mined land rehabilitation · Soil microbial communities

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Background

Global consumption of metals and metalloids is increasing rapidly, driven by growing demand from the technological and renewable energy sector (Watari et al. 2020). Many of these elements, such as selenium (Se), cobalt (Co), and nickel (Ni), are classified as critical elements, since their supply is expected to be critically limiting in the near future. By 2050, the consumption of these elements is projected to increase five to ten-fold compared to current levels (Watari et al. 2020).

The transition to electric vehicles is a major factor driving the rising demand for Ni and Co. The two most common battery types, Nickel Cobalt Aluminium (NCA) and Nickel Cobalt Manganese (NCM), contain, on average, 30 kg of Ni and 8 kg of Co per unit. As demand continues to rise, a sharp increase in the prices for these elements is predicted in the coming years (Nkrumah et al. 2022). Selenium has a wide range of applications, from glass manufacturing, where it serves both as decolorizing and colouring agent (~50% of global demand), to technologies like photovoltaics, where it is valued for its semiconducting properties (Green et al. 2021; U.S. Geological Survey 2024). Additionally, selenium is vital for human and animal nutrition and is commonly supplemented in fertilizers and livestock feed, particularly in Se-deficient regions (Fordyce 2013).

The conventional mining of these elements is associated with significant environmental and social costs. Nickel is the most carbon-intensive metal, emitting approximately 51 kg of CO_2 per kg of metal, followed by cobalt with 28 kg CO_2 emitted per kg of metal (Ilunga Kabeya et al. 2018). Additionally, declining ore quality worldwide is causing the extraction of increasingly larger volumes of raw material (van der Ent et al. 2021). On average, only 1% of the ore material consists of valuable metal, while the rest is dumped as mine waste in the nearby areas, often causing serious soil and water contamination. Beyond environmental concerns, the large Co production in the Democratic Republic of Congo (DRC) (70% of global supply) poses important ethical issues due to widespread human rights abuses and prevalent child labour (U.S. Geological Survey 2024).

Given this situation, there is an urgent need to develop new green approaches for the exploitation of unconventional Ni, Co and Se resources, thereby reducing dependence on conventional mining and politically unstable countries. One promising strategy is agromining (also called phytomining) which can be considered by far the greenest approach to extract metals, even from sub-economic and secondary metal(loid) sources (van der Ent et al. 2015). This phytotechnology employs metal(loid) hyperaccumulator plants that selectively bio-concentrate target elements in their above-ground biomass, effectively removing them from the substrate (Corzo Remigio et al. 2020). Hyperaccumulators can concentrate target elements to levels over 1000-fold higher than those found in non-accumulators (van der Ent et al. 2013) (Reeves et al. 2018), producing metal(loid)-enriched biomass, commonly referred to as bio-ore, which can then be processed for metal recovery (van der Ent et al. 2018).

More than 700 plant species are known to be hyperaccumulators of various metals, and this number is increasing as explorations of metalliferous soils in remote areas continue. The majority of these species are Ni-hyperaccumulators (over 450 species), whereas Co and Se hyperaccumulators are much less represented (van der Ent et al. 2013; Reeves et al. 2018). The most widely studied temperate Ni-hyperaccumulator is *Odontarrhena chalcidica* (Janka) Španiel, Al-Shehbaz, D.A. German & Marhold, a biennial (occasionally perennial) plant of the Brassicaceae family, native to the Balkan region. This species is known to accumulate up to 22,400 mg kg^{-1} Ni in its aerial parts (from soil containing 39 mg kg^{-1} available Ni) (Rosenkranz et al. 2019), while producing substantial biomass yields that can reach over 9 tons ha^{-1} $year^{-1}$. With optimized soil fertilization, *O. chalcidica* can accumulate up to 166 kg Ni ha^{-1} $year^{-1}$ (Bani et al. 2015, 2024). Cobalt hyperaccumulators are primarily native to the Katangan Copperbelt in the DRC. *Haumaniastrum robertii* is the strongest known Co-hyperaccumulator, capable of reaching leaf concentrations of 4,000 mg kg^{-1} dry weight (DW), when growing in soil with 100–900 mg kg^{-1} available Co (Lange et al. 2017). However, to the best of our knowledge, the cultivation of this species on soil outside its natural environment has never been achieved, if excluding hydroponics (Ilunga Kabeya et al. 2018).

Due to its chemical similarity to Ni, Co can also be absorbed by Ni-hyperaccumulators when soil conditions are favourable (i.e., Co:Ni ratios > 1:10 and acidic pH). Under such conditions, *Berkheya coddii* Roessler, a Ni hyperaccumulator native to South Africa, has shown the ability to accumulate 2,000–5,000 mg kg-1 DW of Co in its leaves (Lange et al. 2017; Rue et al. 2020). *Astragalus bisulcatus*, the strongest known Se-hyperaccumulator, is a perennial herb from the Fabaceae family, native to the western United States. It can accumulate up to 15,000 mg kg^{-1} Se in its shoots (Galeas et al. 2007). Although agromining for Se has not yet been tested on large scale, biomass yields are estimated at approximately 10 t ha^{-1} $year^{-1}$, potentially resulting in Se recovery of ~ 70 kg ha^{-1} $year^{-1}$ (Corzo Remigio et al. 2020).

Agromining operations can become economically viable when the market value of the targeted elements is high. Estimates suggest that agromining could yield annual revenues of approximately 1,350 USD, 1,680 USD, and 1,100 USD per hectare for Co, Se, and Ni, respectively (Corzo Remigio et al. 2020). This approach has been primarily validated on metalliferous natural soils (e.g., serpentine soils), its application to secondary sources, such as mine waste and sewage sludge, has been poorly explored, despite showing promising potential (Tognacchini et al. 2020; van der Ent et al. 2021).

Mining and quarrying activities generate over 600 million tonnes of waste annually, accounting for about 25% of total waste production in the European Union (EU) (Kulczycka et al. 2020). These wastes often retain high levels of residual metals and are typically abandoned onsite after the extraction activities have ceased (van der Ent et al. 2021). Across Europe, over 31,000 km^2 of land is affected by mining activities, lying unproductive and in need of costly remediation. Sewage sludge is a byproduct of wastewater treatment process and contains appreciable amounts of N (2.5–3.4%), P (1.0–1.3%), K (0.2–0.4%), organic matter (20–25%) and metal(loid)s. In Europe, around 10 million tons of sewage sludge are produced each year and can be used as fertilizers in agriculture according to the EU directive 86/278/EEC (Ilunga Kabeya et al. 2018), incinerated or landfilled. More than one-third of sewage sludge is still sent to landfill or incinerated because of its high pollutant load, which makes it unsuitable for safe agricultural re-use. However, certain sewage sludge contaminants, despite their potential toxicity, are valuable metal(loid)s that may represent an opportunity rather than a problem. Selenium, Co, and Ni, can reach concentrations of 100, 300 and 1000 mg kg^{-1} DW, respectively, in sewage sludge derived from mixed urban and industrial wastewater (Bai et al. 2012).

Combining sewage sludge and mine waste to enhance plant growth is a promising approach that has already been explored for land reclamation. At a copper mine in Sweden, sewage sludge was successfully tested as a soil amendment, demonstrating benefits such as accelerated land reclamation and enhanced soil fertility, water retention and revegetation (Forsberg and Ledin 2006). Recent research also highlighted the potential for growing hyperaccumulators directly on sewage sludge. Four species targeting arsenic (As), Se, Ni and zinc (Zn) and cultivated on pure sewage sludge, produced 2–5 times more biomass compared to natural soils conditions and removed 6–27% of target metals within four months (Salinitro et al. 2022). In another study, the Zn-hyperaccumulator *Sedum alfredii* (*Sedum plunbizincincola*) accumulated 600 mg Zn kg^{-1} in its shoots and produced 3.4 ton ha^{-1} of biomass when grown on sewage sludge (Qiu et al. 2014). Investigating this approach could open new opportunities for recovering critical elements while simultaneously addressing environmental and waste management challenges.

The present study aims to provide a crucial proof-of-concept for the recovery of three critical elements, Ni, Co and Se, from alternative resources using agromining. To achieve this goal, the following specific objectives were pursued: i) to optimize an efficient agromining system for the extraction of Se, Co, and Ni from metal(loid) enriched mine waste and sewage sludge; ii) to characterize the plant-derived bio-ores to assess their potential downstream use; iii) to investigate the chemical and biological changes (i.e. bacterial diversity) in the substrate driven by sewage sludge addition to mine wastes and agromining practices.

To achieve the above-mentioned aims, our experimental approach consisted in mixing metal(loid) enriched mine wastes with different levels of sewage sludge; followed by the cultivation of the *O. chalcidica, B. coddii* and *A. bisulcatus* on those substrates to target Ni, Co and Se respectively. In the long term, the findings of this study may contribute to a substantial reduction in sewage sludge disposal volumes, while promoting the reuse of degraded mined lands as alternative sources of critical elements.

Methods

Mine waste and sewage sludge sampling

Mine wastes were collected from three mining sites in Northern Italy: Campello (CAMP), N45.9355552, E8.2385905, Mt. Prinzera, (PRIN) N44.6441354, E10.0791121, and Cave Nord (NORD) N44.54228, E11.2870564. The three sites were respectively enriched in Co, Se, and Ni. CAMP site is an old mine, closed in 1948, where the extraction and on-site processing of ores (flotation enrichment) have left behind mineral waste heaps rich in Ni (1,100 mg kg^{-1} DW) and Co (150 mg kg^{-1} DW) (Capitani and Ventruti 2018; Padoan et al. 2021). PRIN site, is a small quarry of ophiolitic material closed in 1991, characterized by bare sand and gravel heaps rich in Ni (1,200 mg kg^{-1} DW). NORD site, is an active sand quarry, simultaneously used as disposal site for demolition materials and smelter sands. Some of these waste materials may contain Se (up to 12 mg kg^{-1} DW) although its presence is highly variable. From each mine site, mineral waste was sieved at 2 cm in loco, and one cubic metre volume of sieved material was transported offsite. Sewage sludge was collected from 3 different wastewater treatment plants (WWTPs) of the Emilia-Romagna region, Italy. These will be referred to as CAS, SEC and FOR, to ensure anonymity, as requested by the multiutility company management. Each WWTP was characterized by an elevated concentration of a specific contaminant in the sludge. CAS is a small WWTP of 25,000 population equivalent (PE) and produces sludge characterized by high Ni levels (380 mg kg^{-1} DW). FOR is a medium-large WWTP of 450,000 PE and produces sludge enriched in Se (~50 mg kg^{-1} DW). SEC is a small WWTP of 20,000 PE and produces sludge characterized by the presence of Co (100 mg kg^{-1} DW). At each WWTP, 200 kg of sewage sludge were collected (water content ~ 75% w/w), then air dried and sieved at 2 cm to discard large aggregates.

Mesocosm setup

For the phytoextraction trial, 18 mesocosms were constructed ex situ at the Bologna Botanical Garden, Italy (N44.4991644, E11.3502627); six mesocosms for each mine wastes. This experimental design was chosen to gather data as similar as possible to field conditions, while having the possibility to monitor all trials constantly and simultaneously. Each mesocosm contained 0.2 m^3 of waste material, weighting between 280–400 kg m^{-3}. To allow for controlled comparison, each mesocosm was divided into two equal halves using a plastic septum. One half served as control, where spontaneous vegetation dynamics were allowed to proceed without intervention (Fig. 1). The other half was treated with sewage sludge at three different levels (0, 15, and 30 kg m^{-2}; Table 1) and each treatment was done in duplicate. All mesocosms were equipped with a micro-drip irrigation system and covered with an anti-hail net. Seeds of *Odontarrhena chalcidica* and *Berkheya coddii* were obtained from Econick (Lunéville, France), while *Astragalus bisulcatus* seeds were purchased from Prairie Moon Nursery (Winona, Minnesota, USA). The seeds were sown on a 1:1 sand:peat substrate and kept in a glasshouse at 20 °C under natural light conditions for the initial growing phases. At time zero (T0), one-month-old seedlings of *B. coddii, A. bisulcatus*, and *O. chalcidica*, hyperaccumulating cobalt, selenium, and nickel, respectively, were transplanted into the treated halves of the mesocosms. Planting density

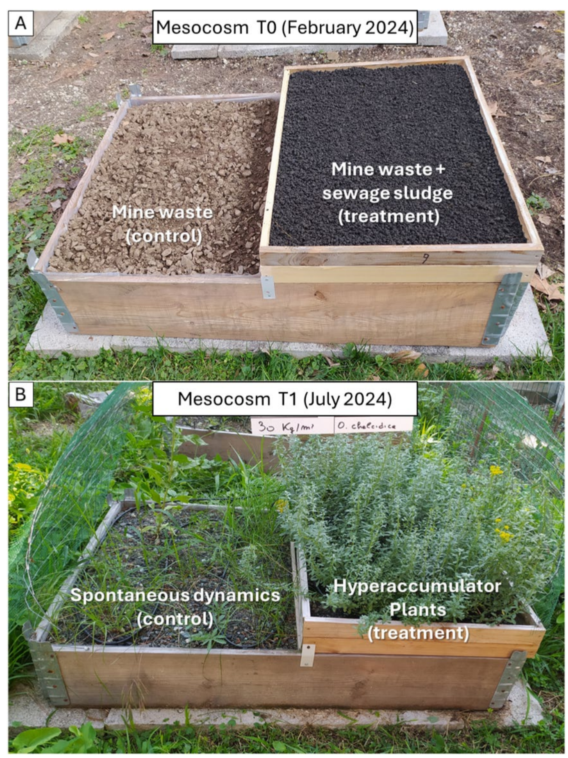

Fig. 1 Example of a mesocosm used in the SS30 treatment (30 kg sludge m^{-2}) on PRIN mine waste. The hyperaccumulator plant *Odontarrhena chalcidica* was cultivated in the right half of the mesocosm, while the left half (control) was left untreated to follow spontaneous dynamics. (**A**) Mesocosm set-up at the beginning of the experiment (T0); (**B**) Mesocosm at the end of the experiment, prior to biomass harvest (T1)

Table 1 Overview of the experimental conditions used in the agromining trial. Each mesocosm was divided in a treated-half (amended with 0, 15, 30 kg of sewage sludge m^{-2}) and a control-half that was left to spontaneaus vegetation dynamics. CAMP: Campello mine site; NORD: Cave Nord mine site; PRIN: Prinzera mine site; SS: Sewage sludge; SEC, FOR and CAS wastewater treatment plants abbreviations

Mine waste	SS origin	Target element	SS treatment	Hyperaccumulator species
CAMP	-	Cobalt	Control	-
CAMP	SEC	Cobalt	0 kg m^{-2}	*B. coddii*
CAMP	SEC	Cobalt	15 kg m^{-2}	*B. coddii*
CAMP	SEC	Cobalt	30 kg m^{-2}	*B. coddii*
NORD	-	Selenium	Control	-
NORD	FOR	Selenium	0 kg m^{-2}	*A. bisulcatus*
NORD	FOR	Selenium	15 kg m^{-2}	*A. bisulcatus*
NORD	FOR	Selenium	30 kg m^{-2}	*A. bisulcatus*
PRIN	-	Nickel	Control	-
PRIN	CAS	Nickel	0 kg m^{-2}	*O. chalcidica*
PRIN	CAS	Nickel	15 kg m^{-2}	*O. chalcidica*
PRIN	CAS	Nickel	30 kg m^{-2}	*O. chalcidica*

was standardized at 24 plants m^{-2} for all species (12 plants in each half-mesocosm). To assure uniform cover, any damaged or dead plant was replaced during the first two weeks. Plants were grown under field conditions from 1st March to 31st July and, to prevent drought stress during the summer, deionized (DI) water was supplied daily at a rate of 5 L m^{-2} using the automated irrigation system. After five months of growth, plants were harvested at flowering time (T1).

Mine waste and sewage sludge physico-chemical characterization

Four biological replicates for each substrate treatment were collected both before and after phytoextraction. Each replicated was constituted by two subsamples of ~ 50 g randomly collected within the mesocosm at 5 cm depth. Substrate samples were dried at 60° C until constant weight, sieved at 2 mm, then used for all the following analyses.

Particle size distribution in mine wastes was determined only at T0 by the hydrometer sedimentation method, using a dispersing solution of 4% w/v sodium hexametaphosphate (ISO 11277 2020). Ten grams of 2-mm-sieved mine waste were dispersed, shaken for 2 h, and transferred to a sedimentation cylinder. Aliquots of 10 ml were collected after 2 min, 12 min, and 20 h, dried at 105 °C, and weighed. Coarse sand was separated by wet sieving. Particle size fractions were calculated based on sedimentation times and mass differences and expressed as percentages of total dry soil weight (Table 2).

Mine waste and sewage sludge pH and electrical conductivity (EC) were measured in a 1:2.5 (w/v) soil:water suspension. Ten grams of 2-mm-sieved mine waste were mixed with 25 mL of water, shaken for 30 min at 30 rpm, and centrifuged at 3000 rpm for 5 min. The supernatant was filtered using Whatman 42 ashless filter paper (Whatman, UK) and analysed using a HI9814C GroLine Portable pH/EC/TDS meter (Hanna Instruments, UK). Total carbon (C) and nitrogen (N) were determined using an elemental analyzer UNICUBE (Elementar Analysensysteme, Langenselbold, DE). Cation Exchange Capacity (CEC) of mine wastes and sewage sludge was determined using $BaCl_2$ 10% w/v at pH 8.1 (Colombo and Miano 2015).

Total metal concentration in mine waste and sewage sludge was determined using monochromatic energy-dispersive X-ray fluorescence (μ-XRF) with Z-Spec JP500 instrument (Z-Spec Inc., New York) after drying substrate samples at 60 °C, sieving at 1 mm, and loading 0.5 g subsamples into custom XRF holders sealed with 6-μm polypropylene film.

Bioavailable Ni and Co were determined using the DTPA extraction method (Lindsay and Norvell 1978), whereas bioavailable selenium (Se) was extracted using the AB-DTPA method (Sharmasarka and Vance 1995). Three grams of 2-mm-sieved mine waste or sludge were extracted with 6 mL of DTPA or

Table 2 Mine waste and sewage sludge characterization at T0 and T1. CEC: cation exchage capapcity; EC: electrical conductivity; SS: Sewage sludge; SS0, SS15, SS30: 0, 15, 30 kg of sewage sludge m^{-2}; CAMP: Campello mine site; NORD: Cave Nord mine site; PRIN: Prinzera mine site; SEC, FOR and CAS wastewater treatment plants abbreviations. Data are the average of 4 biological replicates ± SD (*n* = 4). Different letters indicate statistically significant differences among samples after ANOVA/Kruskal–Wallis tests, followed by Tukey HSD/Dunn's tests, $p < 0.05$. Asterisk (*) indicate a significant difference for a ginven paramenter within the same treatment at T0 and T1 after Student's/Wilcoxon Rank Sum Tests ($p < 0.05$). Control (T1) and SS0 (T1) are both compared with SS0 (T0)

Mine waste grain size (T0)							
Mine site	Skeleton > 2 mm	Coarse sand 0.5–2 mm	Fine sand 0.063—0.5 mm	Coarse silt 0.02—0.063 mm	Fine silt 0.004—0.02 mm	Clay < 0.004 mm	
CAMP	76.0 ± 3.0 ^{a}	16.3 ± 0.5^{a}	4.8 ± 0.2^{a}	0.9 ± 0.1^{a}	1.2 ± 0.1^{a}	0.8 ± 0.1^{a}	
NORD	45.0 ± 2.0^{b}	42 ± 1^{b}	6.6 ± 0.2^{b}	1.3 ± 0.1^{b}	1.9 ± 0.1^{b}	3.3 ± 0.2^{b}	
PRIN	79.0 ± 1.0^{a}	14.1 ± 0.2^{c}	3.6 ± 0.2^{c}	0.9 ± 0.1^{a}	1.2 ± 0.1^{a}	1.3 ± 0.1^{c}	
Sewage sludge chemical analysis (T0)							
WWTP		C (%)	N (%)	C/N	CEC (meq/100 g)	pH	EC (dS/m)
SEC		22.8 ± 0.4^{a}	3.16 ± 0.06^{a}	7.2 ± 0.2	20 ± 1^{a}	6.55 ± 0.05^{a}	7.1 ± 0.1^{a}
CAS		27.9 ± 0.3^{b}	4.49 ± 0.03 ^{b}	6.2 ± 0.1	71 ± 3^{b}	6.81 ± 0.12^{ab}	20.3 ± 0.1^{b}
FOR		14.0 ± 0.1^{c}	1.36 ± 0.02^{c}	10.3 ± 0.2	63 ± 2 ^{c}	7.09 ± 0.03^{b}	4.1 ± 0.01^{c}
Mine waste chemical analysis (T0)							
Mine site	SS treatment	C (%)	N (%)	C/N	CEC (meq/100 g)	pH	EC (dS/m)
CAMP	SS0	0.81 ± 0.03	0.08 ± 0.01	10.4 ± 0.8	4.5 ± 0.7	5.94 ± 0.04	0.15 ± 0.01
	SS15	3.20 ± 0.09	0.43 ± 0.01	7.4 ± 0.3	6.3 ± 0.3	6.30 ± 0.04	1.8 ± 0.1
	SS30	6.5 ± 0.5	0.86 ± 0.03	7.5 ± 0.6	9.8 ± 0.4	6.44 ± 0.04	3.2 ± 0.2
NORD	SS0	2.6 ± 0.1	0.06 ± 0.01	48 ± 6	1.0 ± 0.1	7.49 ± 0.04	2.2 ± 0.1
	SS15	3.1 ± 0.2	0.20 ± 0.01	15 ± 1	1.7 ± 0.1	7.39 ± 0.03	2.6 ± 0.2
	SS30	6.2 ± 0.6	0.40 ± 0.02	16 ± 2	3.1 ± 0.1	7.25 ± 0.04	3.3 ± 0.2
PRIN	SS0	0.53 ± 0.03	0.06 ± 0.01	10 ± 1	5.3 ± 0.2	7.84 ± 0.06	0.16 ± 0.01
	SS15	3.8 ± 0.1	0.59 ± 0.04	6.5 ± 0.5	6.5 ± 0.2	7.12 ± 0.05	4.9 ± 0.3
	SS30	7.5 ± 0.3	1.18 ± 0.03	6.4 ± 0.3	11.6 ± 0.2	6.96 ± 0.09	8.6 ± 0.5
Mine waste chemical analysis (T1)							
Mine site	SS treatment	C (%)	N (%)	C/N	CEC (meq/100 g)	pH	EC (dS/m)
CAMP	Control	1.27 ± 0.07 *	0.12 ± 0.01 *	10.6 ± 0.8	7.5 ± 0.7 *	7.30 ± 0.04 *	0.23 ± 0.03 *
	SS0	1.4 ± 0.2 *	0.12 ± 0.02 *	11.0 ± 3.0	7.6 ± 0.7 *	7.31 ± 0.03 *	0.19 ± 0.01 *
	SS15	2.1 ± 0.3 *	0.24 ± 0.02 *	8.7 ± 1.0	16 ± 1 *	6.22 ± 0.06	1.3 ± 0.2 *
	SS30	4.8 ± 0.4 *	0.66 ± 0.02 *	7.4 ± 0.6	24 ± 2 *	6.5 ± 0.2	2.5 ± 0.1 *
NORD	Control	2.5 ± 0.1	0.11 ± 0.02 *	42 ± 5	2.5 ± 0.2 *	7.59 ± 0.09	1.6 ± 0.2 *
	SS0	2.5 ± 0.3	0.06 ± 0.01	43 ± 6	2.6 ± 0.2 *	7.52 ± 0.07	1.4 ± 0.1 *
	SS15	2.7 ± 0.2	0.08 ± 0.02 *	34 ± 7	4.5 ± 0.5 *	7.82 ± 0.06 *	1.58 ± 0.06 *
	SS30	3.54 ± 0.08 *	0.14 ± 0.01 *	25 ± 2	6.9 ± 0.1 *	7.55 ± 0.08 *	1.3 ± 0.1 *
PRIN	Control	0.90 ± 0.05 *	0.09 ± 0.01 *	10 ± 2	10.6 ± 0.3 *	8.27 ± 0.04 *	0.27 ± 0.04 *
	SS0	0.85 ± 0.03 *	0.08 ± 0.01	10 ± 1	11.1 ± 0.3 *	8.30 ± 0.08 *	0.24 ± 0.03 *
	SS15	2.5 ± 0.1 *	0.39 ± 0.03 *	6.5 ± 0.6	16.2 ± 1.0 *	7.01 ± 0.06	1.51 ± 0.07 *
	SS30	4.9 ± 0.5 *	0.8 ± 0.1 *	7 ± 1	26 ± 2 *	6.51 ± 0.05 *	2.27 ± 0.0 *6

AB-DTPA solution in 15 mL tubes, shaken for 2 h, and centrifuged at 10,000 rpm for 3 min. Aliquots of 2 mL clear supernatant were collected for analysis. The DTPA solution contained 14.92 g L^{-1} triethanolamine (TEA), 1.47 g L^{-1} $CaCl_2 \cdot 2H_2O$, and 1.97 g L^{-1} DTPA, adjusted to pH 7.3 with HCl. The AB-DTPA solution c^{-1} NH_4HCO_3 + 1.97 g L^{-1} DTPA, adjusted to pH 7.6 with HCl. Extracts were also analysed using Z-Spec

JP500 after being transferred into special XRF holders designed for liquids. Bioavailable metal concentrations were expressed as mg kg^{-1} DW of the extracted substrate.

Z-Spec XRF uses dual excitation at 17.48 keV and 4.5 keV to detect elements from Na (Z = 11) to U (Z = 92), with optimal sensitivity for Cu–Se and Hg–Tl–Pb and detection limits of 0.009–0.025 mg kg^{-1}. Each sample was scanned for 30 s in the appropriate analysis mode Soil or Water (Zhang et al. 2025). Certified reference materials (NIST SRM 2702 marine sediments, 2709a San Joaquin soil) and TraceCERT® multielement standard solution (SUPELCO, Bellefonte, PA, USA) were used for quality control. Recovery rates for Ni, Co and Se were ± 5% of the reference values for soils and ± 2% of the reference value for liquids. The substrate elemental concentrations are expressed as mg kg^{-1} dry weight (DW).

Plant biomass and elemental analysis

Above ground biomass was collected for analysis at T1 (31st July 2024) from four randomly selected plants per mesocosm, chosen by generating four random numbers between 1 to 12. From the same plants subsamples old leaves (first 5 cm of the main stem) and young leaves (last 5 cm of the main stem), were separately analysed for target element content, to gather insights on their plant-level distribution. To get insights on metal accumulation in plants naturally developing in the substrates, four replicates of wild plants (four plants per species in each substrate) were also collected for elemental analysis. Plant material was dried until constant weight at 60 °C, in a ventilated oven. Single plants were then weighed to calculate the biomass, and the young and old leaves previously removed were also added. The total biomass of wild plants growing in the control halves of the mesocosm was also calculated. All samples were ground to < 200 µm using an IKA A11 basic mill and 0.2 g of powdered material was loaded into custom XRF holders as described for the substrates. Each sample was scanned for 30 s in plant mode. Certified reference materials (NIST SRM 1570a spinach leaves, 1573a tomato leaves) were used for quality control, with recovery rates for Ni, Co and Se of ± 5% of the reference values. The plant metal concentrations are expressed as mg kg^{-1} dry weight (DW).

Metagenomic analysis of bacterial community

Substrate samples were collected at two time points: two replicates after mesocosm set-up (T0) and four to six replicates after plant harvest (T1). After each sampling, the substrates were freshly sieved at 2 mm and immediately stored at −80 °C until DNA extraction. Microbial DNA was extracted from 0.5 g of fresh substrate using the DNeasy PowerSoil Kit (Qiagen, Germany) following the manufacturer's protocol. DNA quantity and quality were quickly assessed using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. The full-length 16S rDNA gene (~ 1.5 kb), a widely used phylogenetic marker for bacterial taxonomic identification, was amplified using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). PCR products were purified using AMPure XP beads (Beckman Coulter, USA) and quantified prior to library preparation. Sequencing libraries were constructed using the Oxford Nanopore Technologies 16S Barcoding Kit (SQK-16S024) according to the manufacturer's instructions. Sequencing was carried out on a MinION Mk1B device using an R9.4.1 flow cell. Fast base calling and demultiplexing were performed using Guppy (v6.0.1). The reads were filtered by size using chopper retaining sequences in the range of 1200–1800 bp. The average Phred quality score was assessed using NanoPlot. Taxonomic classification of sequences was performed using kraken2 with SILVA database 138.2 SSU Ref NR 99.

Data analysis

All plant and soil variables were tested for homogeneity of variances using Levene's test and for normality using the Shapiro–Wilk test, implemented via the car package (https://cran.r-project.org/web/packages/car/). For a parametric variable, one-way ANOVA was performed followed by Tukey's HSD test for post hoc comparisons. For non-parametric data, the Kruskal–Wallis rank sum test was used, followed by Dunn's test for multiple comparisons using rank sums (dunn.test package: https://cran.r-project.org/web/packages/dunn.test/). To compare a single variable before and after phytoremediation, the Student's t-test (for parametric data) or the Wilcoxon rank-sum test (for non-parametric data) was applied, with statistical significance set at $p < 0.05$. All statistical and graphical analyses were conducted using R version 4.4.3, and datasets were compiled using Microsoft Excel for Microsoft 365 (Version 2404). Analysis of bacterial community was performed with vegan R package. PERMANOVA analysis were performed with adonis2 function, which was used to compare microbial communities among the treatments, with statistical significance set at $p < 0.05$. The biodiversity indices of Pielou evenness, Shanon diversity and Richness were calculated with diversity function and statistical significance were tested with one-way ANOVA and followed by Tukey's HSD test for post hoc comparisons.

Results

Physico-chemical properties of substrates before agromining

Granulometric analysis of mine wastes (Table 2) showed notable differences among the three mine sites. Overall, all substrates were predominantly composed of coarse material, referred to as skeleton (> 2 mm). The PRIN and CAMP sites exhibited the highest skeleton content (79% and 76% of the total, respectively), compared to the NORD site (45%). The remaining fine fraction (≤ 2 mm) was similar in particle size distribution between PRIN and CAMP, with 14–16% coarse sand and 3.6–4.8% fine sand. In contrast, the NORD site had a higher proportion of coarse sand (42%). Silt and clay fractions were generally low across all substrates, except for clay content in NORD (3.3%).

Chemical characterization of sewage sludge was conducted at T0, prior to mixing with the mine wastes (Table 2). CAS sludge (target element Ni) had the highest values of C (27.9%), N (4.49%), cation exchange capacity (CEC: 71 meq/100 g), and electrical conductivity (EC: 20.3 dS/m). In contrast, FOR sludge (target element Se) had the lowest C (14.0%) and N (1.36%) contents and a low EC (4.1 dS/m), with CEC (63 meq/100 g) comparable to that of CAS. SEC sludge (target element Co) showed the lowest CEC (20 meq/100 g), but intermediate values for all other parameters. All sludges had near-neutral pH values, ranging from 6.55 to 7.09.

Physico-chemical properties of substrates after agromining

Changes in substrate (mine waste amended or not with sewage sludge) chemical properties before and after agromining are reported in Table 2. Samples SS0 at T0 were compared with control at T1 (mine waste left to spontaneous dynamics) and SS0 at T1 (mine waste with hyperaccumulator plants). Overall, the addition of sewage sludge to mine wastes led to increases in C, N, CEC and EC, with effects being dose-dependent. Conversely, the C/N ratio decreased across all mine wastes following the addition of sewage sludge, with similar values observed in SS15 and SS30 treatments within each substrate group. The pH values of the three substrates were differently affected by sewage addition and agromining. In the CAMP substrate, C and N contents in the SS0 treatment increased from 0.81% to 1.4% and from 0.08% to 0.12%, respectively, from T0 to T1 and relative to the control at T1, with a relatively stable C/N ratio (10.4 to 11). CEC increased significantly across all treatments, while EC decreased by about 30% from T0 to T1, except in SS0 and control. The pH increased from 5.94 to 7.31 in SS0 and control but did not change in SS15 and SS30 treatments. In the NORD substrate, C content increased slightly from T0 to T1 (+2.6%) in SS0 and control, but decreased by approximately 50% in SS15 and SS30. At T1, N content declined by 55–65% in SS15 and SS30 compared to T0. As observed for CAMP, CEC increased more than double, while EC declined by a similar proportion across treatments from T0 to T1. The pH remained stable in SS0 and control, but increased slightly in SS15 and SS30 (~7.3 to 7.6). In the PRIN substrate, C increased moderately from 0.53% to 0.85% in SS0 and control at T1, while declining sharply (~−35%) in SS15 and SS30. A similar pattern was observed for N (−34%). The C/N ratio remained almost unchanged from T0 to T1. CEC substantially increased in all treatments (by 2 to 2.5 folds) from T0 to T1. EC increased slightly in SS0 and control, but decreased up to threefold in SS15 and SS30. pH showed minor fluctuations without clear trends.

Overall, the bioavailable fractions (DTPA- or AB-DTPA-extractable) consistently decreased after agromining (T1) across all three metals in SS15 and SS30 treatments. Total Co and Ni concentrations remarkably increased in SS15 and SS30 at T1 (Figures. 2a, c), whereas Se concentration was relatively stable in the NORD substrate (Fig. 2B). At T0, bioavailable metal fractions were generally highest in pure sludge (pure SS), followed by SS30, SS15, and SS0 treatment. According to this order, in the CAMP substrate, the DTPA-Co fractions were 62%, 37%, 31%, and 0.7%, respectively (Fig. 2a); for NORD, AB-DTPA-Se fractions were 20%, 21%, 23%, and 11%, respectively (Fig. 2b), and for PRIN, the DTPA-Ni fractions were 47%, 13%, 13%, and 0.7%, respectively (Fig. 2c). Supplementary Tables S1 and S2 report total and bioavailable concentrations of Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Hg, and Pb at T0 and T1 in sludge and mine wastes. Similar to the trends already found for the three target elements, the total concentrations of other elements significantly increased in SS15 and SS30 at T1, while the available fractions generally decreased.

Microbial community structure

The relative abundance of bacterial taxa in mine wastes at collection time (control T0) and after sludge treatments and agromining (T1) is illustrated in Fig. 3. PERMANOVA statistical analysis showed significant differences in microbial community

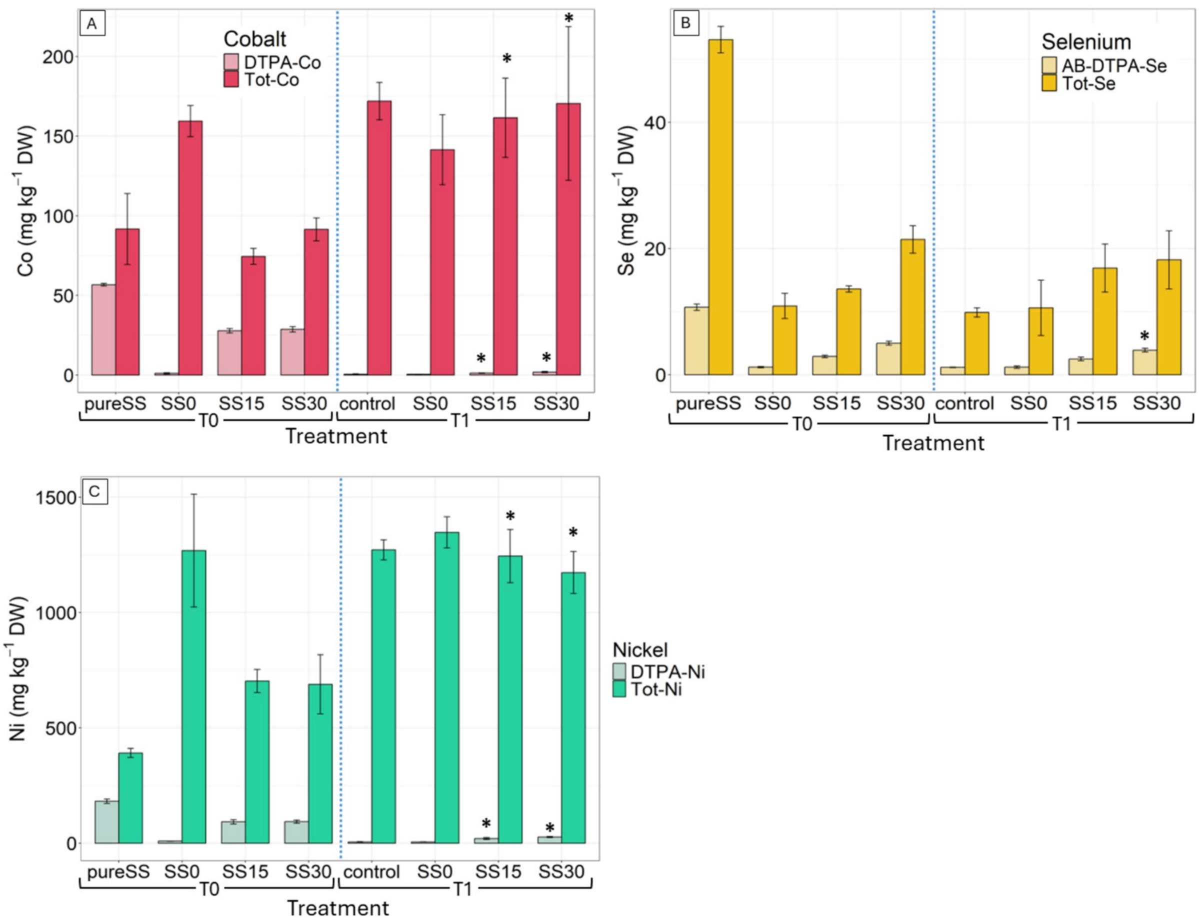

Fig. 2 Total and bioavailable metal(loid) content in the growing substrate. (**A**) Total and DTPA-extractable Co in CAMP mine waste at T0 and T1 under different treatments. (**B**) Total and AB-DTPA-extractable Se in NORD mine waste at T0 and T1 under different treatments. (**C**) Total and DTPA-extractable Ni in PRIN mine waste at T0 and T1 under different treatments. SS: sewage sludge; SS0: 0 kg SS m^{-2}, SS15: 15 kg SS m^{-2}, SS30: 30 kg SS m^{-2}. Data are the average of 4 biological replicates for each treatment ($n = 4$). Asterisks (*) indicate significant differences within the same treatment at T0 and T1 for total or bioavailable metal(loid)s content after Student's/Wilcoxon Rank Sum Tests ($p < 0.05$). PureSS was characterized at the beginning of the experiment and has no term of comparison in T1. SS0 (T1) and control (T1) are both compared with SS0 (T0)

structure among treatments in CAMP ($F = 1.70$, $R^2 = 0.40$, $p = 0.0367$), NORD ($F = 3.64$, $R^2 = 0.58$, $p = 0.0005$) and PRIN ($F = 1.93$, $R^2 = 0.39$, $p = 0.0477$). Overall, at T0, Actinobacteria and Alphaproteobacteria were consistently among the most abundant classes across all sites. The more complex community was observed in CAMP and PRIN mine wastes, which displayed the higher count of classes, including Planctomycetes and several minor taxa. In contrast, the NORD mine waste exhibited lower complexity, dominated by Bacilli and Actinobacteria, suggesting a more selective environment.

After analyzing each site individually before and after agromining, the following trends emerged. In CAMP mine waste, the community at T0 was complex, including Actinobacteria, Thermoleophilia, Bacteroidia, Anaerolineae, and Bacilli (Fig. 3a). Community complexity remained largely unchanged

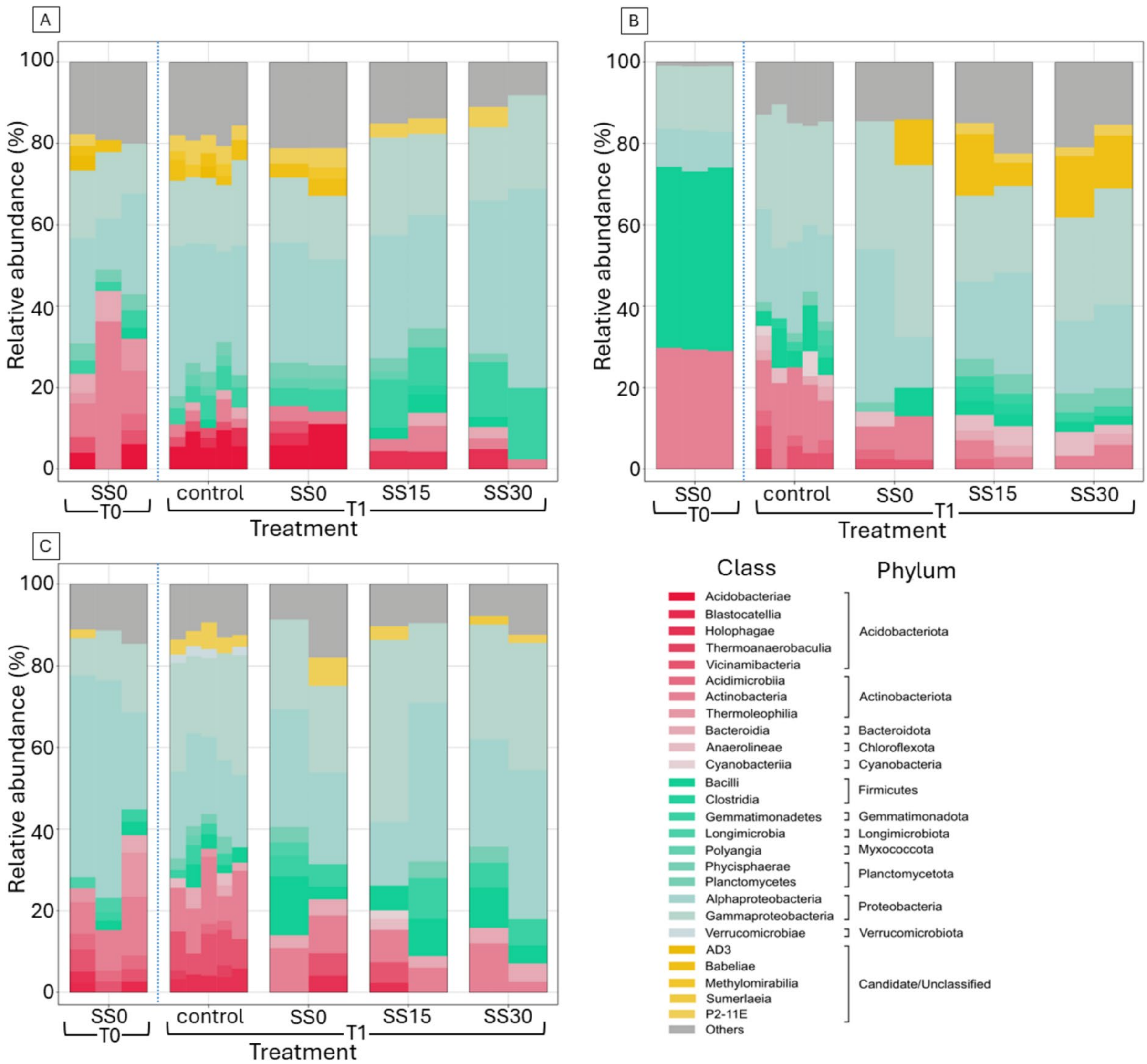

Fig. 3 Bacterial community structure at class and phylum level. (**A**) Bacterial community in CAMP mine wastes at T0 and T1 under different treatments. (**B**) Bacterial community in NORD mine wastes at T0 and T1 under different treatments. (**C**) Bacterial community in CAMP mine waste at T0 and T1 under different treatments. SS: sewage sludge; SS0: 0 kg SS m^{-2}, SS15: 15 kg SS m^{-2}, SS30: 30 kg SS m^{-2}. Each column shows the variability of 2 to 5 biological replicates ($n = 2$–5). SS0 (T1) and control (T1) are both compared with SS0 (T0)

in the control, but in SS15 and SS30 treatments at T1 a shift toward Alphaproteobacteria and Gammaproteobacteria dominance was observed, accompanied by an overall reduction in complexity. In NORD mine waste, the community at T0 was poor and dominated by Bacilli by approximately 40% (Fig. 3b). At T1, the control exhibited a more balanced and complex community, with emerging minor classes (e.g., Bacteroidia, Anaerolineae, Clostridia, Cyanobacteriia) becoming more prominent, while the relative abundance of Bacilli simultaneously declined. In mine wastes treated with sewage sludge at varying doses, a notable increase in the abundance of Alphaproteobacteria, Gammaproteobacteria and AD3 was observed, accompanied by a decrease in Actinobacteria. In the PRIN substrate, bacterial complexity increased from control T0 to T1, with greater representation of Bacteroidia, Anaerolineae, P2-11E, and Verrucomicrobia. Across SS0, SS15, and SS30 treatments, Acidobacteriota gradually declined, progressively replaced by Alphaproteobacteria and Gammaproteobacteria. A consistent pattern of sewage sludge influence on bacterial community was observed across all substrates, characterized by a marked stimulation of classes belonging to phylum Proteobacteria, particularly Alpha- and Gammaproteobacteria, which together accounted for over 50% of the total abundance. Conversely, members of the Acidobacteriota (e.g. Acidobactertiae) and Actinobacteriota (Actinobacteria) phyla declined in abundance.

The alpha-diversity metrics varied among all sites. However, they were mainly influenced by the characteristics of the original mining site (Supplementary Table S4). The alpha-diversity indices of Richness, Shannon diversity and Pielou Evenness after collection were lowest in NORD (26, 1.305, 0.401 respectively) compared to CAMP (65.3, 2.659, 0.636 respectively) and PRIN (47.7, 2.171, 0.565 respectively). The results observed in T1 varied among substrates. In the least diverse NORD, the significant increase of alpha-diversity indices was noted, but no differences were observed between SS treatments and control. In CAMP the biodiversity tended to decrease as an effect of sludge treatment, which was the most statistically significant for Shannon diversity and Pielou evenness for SS30 variant. In PRIN no significant changes in alpha-biodiversity were observed.

Plant biomass, metal(loid) accumulation and yields

Sludge treatments significantly affected both biomass and metal(loid) accumulation across the three plant species: biomass generally increased with higher sewage sludge application, whereas metal(loid) accumulation exhibited an inverse trend (Fig. 4). The *B. coddii* biomass was enhanced from 1.9 to 5 g DW plant^{-1} upon sewage sludge application at SS15 compared to SS0, but slightly declined to 4 g DW plant^{-1} at SS30 (Fig. 4A). *Astragalus bisulcatus* showed ninefold biomass increase across treatments, rising from 1.2 to 8.2 to 11.2 g DW plant^{-1} in the SS0, SS15 and SS30 treatments, respectively (Fig. 4B). Similarly, the biomass of *O. chalcidica* increased progressively from SS0 (7.7 g DW plant^{-1}) to SS15 (10 g DW plant^{-1}) to SS30 (14.4 g DW plant^{-1}, Fig. 4C).

Cobalt accumulation remained stable between SS0 and SS15 (~250 mg kg^{-1} DW) but significantly dropped at SS30 (148 mg kg^{-1} DW; Fig. 4D). In *A. bisulcatus*, Se concentrations decreased fourfold from SS0 to SS15 (198 to 55 mg kg^{-1} DW) with no further reduction at SS30 (Fig. 4E). *O. chalcidica* showed a consistent decline in nickel accumulation across all treatments, with values of 6,150, 4,110 and 1,940 mg kg^{-1} DW, in SS0, SS15 and SS30 respectively (Fig. 4F). Metal(loid)s accumulation in old and young leaves was also measured in these hyperaccumulators (Fig. 5). Overall, a general decreasing trend in accumulation was observed with sludge addition. Cobalt consistently accumulated more in old leaves, with concentrations remaining stable at approximately 600 mg kg^{-1} DW in SS0 and SS15, and 355 mg kg^{-1} DW at SS30. In *Berkheya coddii,* young leaves contained Co levels ranging from 134 to 8 mg kg^{-1} DW, following the same trend as the old leaves (Fig. 5A).

Selenium showed an opposite pattern, with old leaves containing very low Se concentrations (78 to 32 mg kg^{-1} DW in SS0 to SS30), whereas young leaves accumulated much higher Se levels: 958 mg kg^{-1} DW at SS0; 357 mg kg^{-1} DW at SS15, 431 mg kg^{-1} DW at SS30 (Fig. 5B). Similarly to Se, Ni accumulation was substantially higher in young leaves compared to old ones, and both exhibited a clear decline in Ni concentration with increasing sludge application. The Ni concentration was 10,600 mg kg^{-1} DW at SS0; 5,400 mg kg^{-1} DW

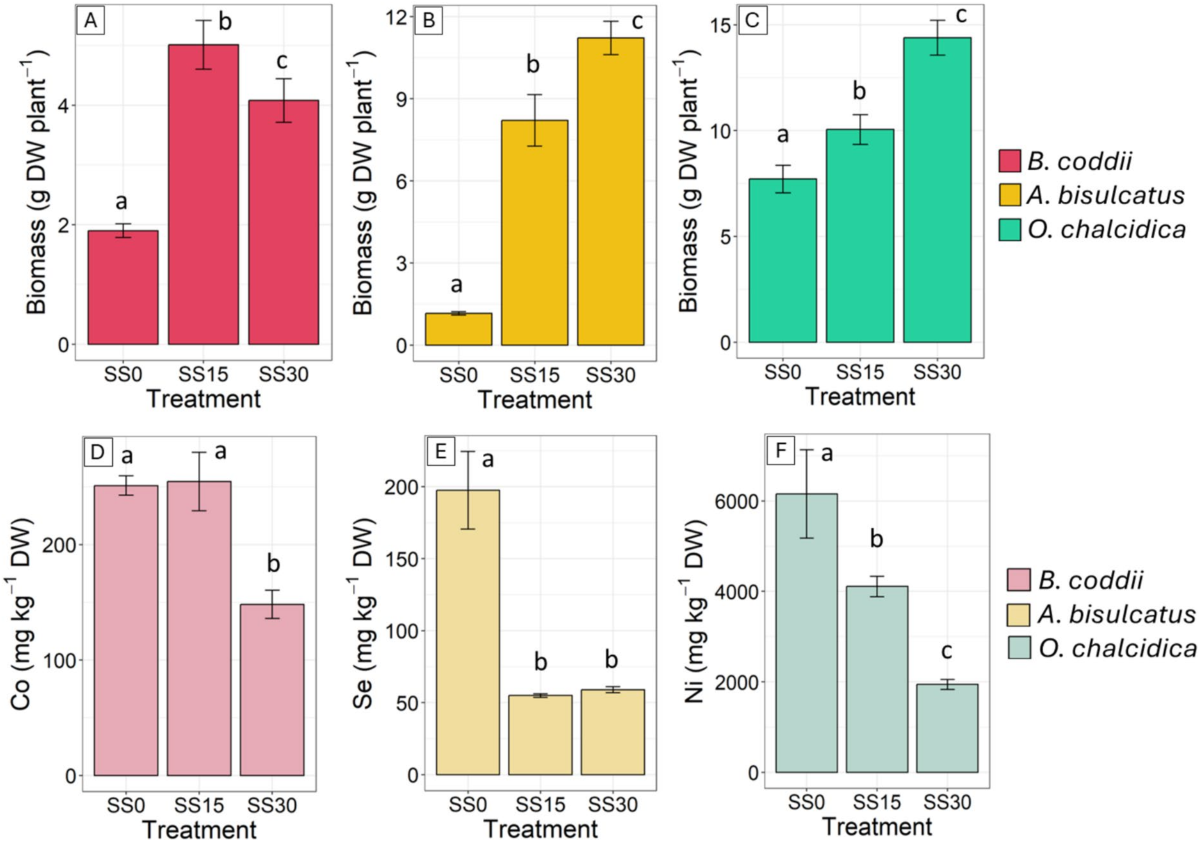

Fig. 4 Plant biomass and metal(loid) accumulation. (**A**) *Berkheya coddii* biomass in different treatments on CAMP mine waste. (**B**) *Astragalus bisulcatus* biomass in different treatments on NORD mine waste. (**C**) *Odontarrhena chalcidica* biomass in different treatments on PRIN mine waste. (**D**) Cobalt accumulation in *Berkheya coddii* under different treatments. (**B**) Selenium accumulation in *Astragalus bisulcatus* under different treatments. (**C**) Nickel accumulation in *Odontarrhena chalcidica* under different treatments. SS: sewage sludge; SS0: 0 kg SS m^{-2}, SS15: 15 kg SS m^{-2}, SS30: 30 kg SS m^{-2}. Data are the average of 4 biological replicates for each treatment ($n=4$). Different letters indicate statistically significant differences among treatments within the same substrate after ANOVA/Kruskal–Wallis tests, followed by Tukey HSD/Dunn's tests, ($p<0.05$)

at SS15 and 3,000 mg kg^{-1} DW at SS30 in young leaves (Fig. 5C), while reaching 7,600, 2,300 and 1,000 mg kg^{-1} DW in the same treatments in old leaves.

In supplementary table S3 the accumulation of other elements (over the target element) is reported for each species and treatment. Some common trends among all species are the reduction of Cr and Fe concentrations (3 to 30-fold and 0.5 to onefold, respectively) with the increase of sludge treatment. Conversely, a generalized increase of Ca accumulation (10–30%) can be noted from SS0 to SS30 treatment in *B. coddii* and *O. chalcidica*, but not in *A. bisulcatus* where its level was unchanged across treatments.

The estimated biomass (in g DW m^{-2}) and annual metal(loid) yield (mg m^{-2}) for the three hyperaccumulator species cultivated on mine wasted amended with SS were estimated (Table 3). As expected, the biomass production increased in all species with the increase of sludge dose applied. The most marked effect was observed in *A. bisulcatus*, which exhibited a ninefold biomass increase, from 14 in SS0 to 130 g DW m^{-2} in SS30. *O. chalcidica* and *B. coddii* showed only about a twofold increase in biomass from SS0 to SS30 (Table 3). Estimated Co yields reached the highest level under SS15 treatment (15 mg m^{-2}), whereas lower values were found in SS30 and SS0 (7.2 and 5.7 mg m^{-2} respectively). For Se, the yield

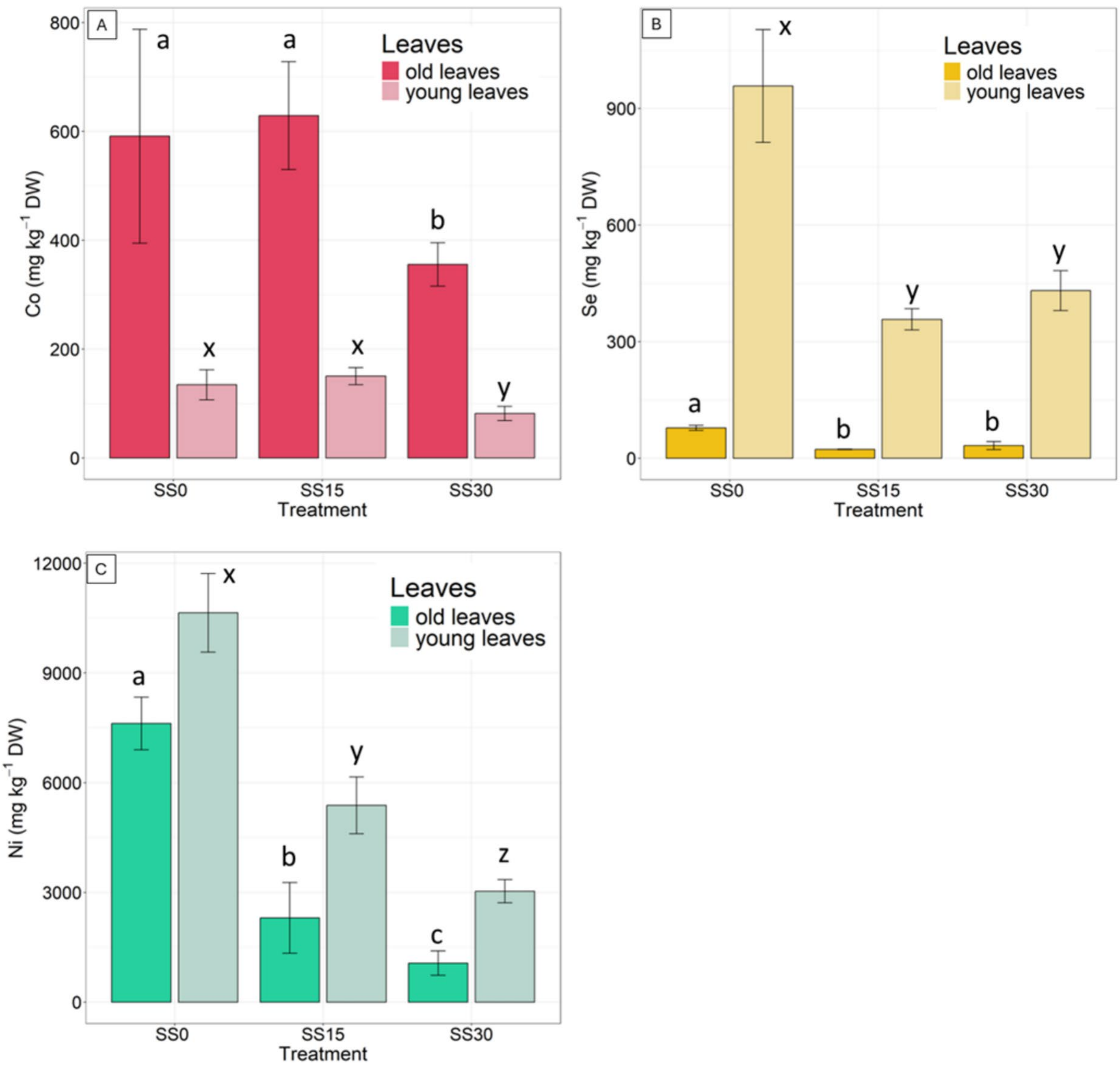

Fig. 5 Metal(loid) accumulation in old and young leaves of the three hyperaccumulators. (**A**) *Berkheya coddii* (Co) growing on CAMP mine waste; (**B**) *Astragalus bisulcatus* (Se) growing on NORD mine waste. (**C**) *Odontarrhena chalcidica* (Ni) growing on PRIN mine waste. SS: sewage sludge; SS0: 0 kg SS m^{-2}, SS15: 15 kg SS m^{-2}, SS30: 30 kg SS m^{-2}. Data are the average of 4 biological replicates for each treatment ($n=4$). Different letters (a-c: old leaves; x–z: young leaves) indicate statistically significant differences among treatments after ANOVA/Kruskal–Wallis tests, followed by Tukey HSD/Dunn's tests, $p<0.05$)

consistently increased with sludge application, corresponding to 2.8, 5.4, 7.9 mg m^{-2} in SS0, SS15 and SS30 respectively. The opposite was observed for Ni yields, which declined from 600 mg m^{-2} in SS0, to 500 in SS15 and 340 mg m^{-2} in SS30.

The control halves of the mesocosms that were left to spontaneous dynamics harboured a rich and diverse flora at T1: 38 species in PRIN substrate, followed by NORD (21 species) and CAMP (12 species) (Supplementary Figures S1, S2, S3). Overall, spontaneous plant species behaved as excluders, limiting metal uptake. The average concentrations of target metals in the biomass of the spontaneous flora were 12 mg Co kg^{-1} in CAMP, 6 mg Se kg^{-1} in NORD, and 12 mg

Table 3 Hyperaccumulator biomass production and estimated Co, Ni and Se yields. SS: Sewage sludge; SS0, SS15, SS30: 0, 15, 30 kg of sewage sludge m^{-2}; CAMP: Campello mine site; NORD: Cave Nord mine site; PRIN: Prinzera mine site. Data are the average of 4 biological replicates ± SD ($n=4$). Data in gray, does not refer to the element of interest mined from that specific subtrate (Co in CAMP, Se in NORD, Ni in PRIN) and were not included in the statisitical analysis. Different letters indicate statistically significant differences among treatments within the same mine waste after ANOVA/Kruskal–Wallis tests, followed by Tukey HSD/Dunn's tests, $p<0.05$

Mine site	Treatment	Biomass (g DW m^{-2})	Co (mg m^{-2})	Ni (mg m^{-2})	Se (mg m^{-2})
CAMP	SS0	20 ± 1 [a]	5.7 ± 0.2 [a]	300 ± 20	0.03 ± 0.02
CAMP	SS15	60 ± 5 [b]	15 ± 2 [b]	30 ± 1	0.06 ± 0.02
CAMP	SS30	50 ± 4 [b]	7.2 ± 0.6 [c]	18 ± 2	0.05 ± 0.02
NORD	SS0	14.0 ± 0.7 [a]	0.01 ± 0.01	0.09 ± 0.02	2.8 ± 0.4 [a]
NORD	SS15	100 ± 10 [b]	0.20 ± 0.05	0.60 ± 0.10	5.4 ± 0.1 [b]
NORD	SS30	130 ± 10 [b]	0.23 ± 0.01	1.6 ± 0.1	7.9 ± 0.3 [c]
PRIN	SS0	90 ± 8 [a]	2.6 ± 0.4	600 ± 90 [a]	0.10 ± 0.01
PRIN	SS15	120 ± 8 [b]	4.8 ± 0.3	500 ± 30 [b]	0.17 ± 0.02
PRIN	SS30	170 ± 10 [b]	9.3 ± 0.3	340 ± 20 [c]	0.09 ± 0.01

Ni kg^{-1} Ni in PRIN. The potential annual removal of the target elements in a "no management scenario" was limited to: 1 mg Co m^{-2} for the CAMP substrate, 1.5 mg Se m^{-2} for NORD, and 2.3 mg Ni m^{-2} for PRIN.

Discussion

Effect of sewage sludge addition on mine waste physico-chemical properties

The CAMP and PRIN mine wastes investigated in this study were mostly composed of coarse material (~78% skeleton, Table 2), derived by the parent rock material embedding the ore. These materials were discarded during ore processing and abandoned on-site after mine closure (van der Ent et al. 2021). The NORD mine waste originates from a completely different source, as indicated by its different particle size distribution, due to the site's ongoing use for the disposal of various inert materials. The higher sand percentage of the NORD mine waste is derived from the grinding of demolition rubble and smelter sands, whereas the higher clay content is attributed to the presence of excavation soil. Compared to similar wastes previously investigated for Ni-agromining, such as those from the Përrenjas mine in Albania (Osmani et al. 2018), the mine wastes examined in this study exhibit excessively coarse granulometry, which can hinder optimal plant growth when used without amendments. Furthermore, their silt and clay contents account for only 1–3%. In contrast, the Përrenjas mine waste contained up to 20% silt and clay content (Osmani et al. 2018), allowing for better water and nutrient retention capacity.

Sewage sludge application mostly impacted sludge chemical properties, significantly increasing the CEC across all substrates by a factor of two to three in high sewage sludge (SS30) treatments compared to unamended mine wastes (SS0) at T0 (Table 2). This increase was likely due to a higher abundance of organic colloids introduced by the sludge. The resulting enhancement in CEC improves the ability of the substrate to retain and exchange essential nutrients, thereby supporting plant growth. Similarly, N and C concentrations were substantially elevated in the SS30 treatments compared to SS0 at T0 (Table 2). Following agromining, a 30–50% decrease in N concentration was observed in SS15 and SS30 treatments across all substrates, indicating rapid nitrogen cycling likely driven by denitrifying bacterial activity (Bani et al. 2024) and assimilation into plant biomass.

The total concentration of metal(loids) was monitored during the experiment mainly because of the high degradability of the sludge. Notably, in the CAMP and PRIN substrates, the addition of sewage sludge at T0 led to a temporary reduction in total cobalt (Co) and nickel (Ni) concentrations in the SS15 and SS30 treatments compared to SS0 (Fig. 2). This reduction was attributed to a "dilution effect", as the sludge contained relatively lower metal concentrations than the mine wastes. Similar observations were reported by Forsberg and Ledin (2006), supporting the view that applying metal-enriched sewage sludge on mined land is generally safe, considering the high background metal levels at these sites. However, this did not apply to Se in the NORD substrate, where the Se concentration in the sludge (53 mg kg^{-1} DW) exceeded that of the substrate (10 mg kg^{-1} DW; Fig. 2b). The observed increase in total Co and Ni after agromining (T1) in the SS15 and SS30 treatments may be explained by sludge degradation by microbial activity, which reduced the overall substrate mass, especially organic matter, thereby concentrating the residual metals (Figs. 2A, C). This trend was not observed in NORD, where *A. bisulcatus* plants were apparently very efficient in removing the excess Se released by sludge decomposition (Fig. 2B). In addition, *A. bisulcatus* is capable of producing volatile forms of Se, which contribute to Se losses from the substrates (Statwick et al. 2016). In general, sludge addition combined with agromining seems to reduce the pool of readily available metals in the substrates at T1 compared to T0, particularly for Ni (fourfold decrease) and Co (15-fold decrease; Fig. 2A, C) in SS15 and SS30 treatments. A similar reduction of the DTPA-available Ni (−30%), after 3 months of Odontarrhena corsica cultivation on serpentine soil, was also observed by Paul and Chaney (2024). In addition to plant uptake, the reduction in available metals may also be attributed to the complexation of Ni, Co, and Se by the organic matter introduced through the sludge, which promotes metal retention or fixation, and consequently decreases metal bioavailability (Ernst 2005; Dinh et al. 2019).

Effect of sewage sludge and agromining on microbial communities

Mine waste materials present a harsh environment for microorganisms due to their low nutrient availability and high concentrations of toxic metals. Nevertheless, such conditions create selective pressure that favors the development of specialized microbiomes adapted to metal stress and resource limitation. Among these, some microorganisms are chemolithoautotrophs that utilize metals as electron donors and energy sources, enabling them to persist in nutrient-poor environments despite generally constrained metabolic activity (Newsome et al. 2021; Odisi et al. 2023; Zhang S et al. 2025). Although microbial processes in these settings are typically limited by the scarcity of organic substrates, studies have shown that microbial communities can adapt and even thrive under metal stress when sufficient organic matter is supplied, as observed with sewage sludge amendments. This organic input stimulates the growth of copiotrophic and heterotrophic microbes adapted to rapidly exploit readily available carbon sources (Newsome et al. 2021; Sircan et al. 2025; Stone et al. 2023. The addition of sewage sludge to these mine wastes significantly reshaped their microbial community composition, influenced mine waste properties and enhanced metal accumulation by hyperaccumulator plants growing on these substrates, factors that collectively have important implications for agromining efficiency.

The CAMP and PRIN sites, having been closed for decades, have undergone pedogenetic processes, unlike the NORD site, where fresh waste is continuously deposited or removed. This difference is reflected in the initial alpha-diversity of the bacterial communities at T0, which was higher in the mine wastes of the older, weathered PRIN and CAMP sites, and lower in the younger NORD substrate (Fig. 3). A similar trend was reported at Nanshan Iron Mine in eastern China, where microbial diversity and evenness progressively increased in waste deposits aged between 0 to 40 years (Zhang et al. 2021). The addition of sewage sludge and subsequent agromining resulted in contrasting shifts in bacterial diversity: it increased in substrates with initially low diversity (e.g., NORD) driven by the input of organic compounds and nutrients from sewage sludge, but decreased in substrates with initially high diversity. In general, high sludge levels favoured the selection of Alphaproteobacteria and Gammaproteobacteria. These groups are commonly associated with C and sulfur (S) cycling, as they are heterotrophic or chemolithoautotrophic sulphur-oxidizers. In sludge-amended mine wastes, organic C and S compounds are particularly abundant and serve as substrates by these microorganisms (Yousuf et al. 2014). Additionally, the selection of classes within the Proteobacteria phylum may be also partly explained by their copiotrophic behaviour, as they thrive on environments rich in easily available C and nutrients (Fierer et al. 2007).

The proliferation of these populations likely accelerated the degradation of sewage sludge. In addition, horizontal gene transfer (HGT) may have contributed to these dynamics. The introduction of sewage sludge likely brought copiotrophic microbes carrying distinct genetic traits, creating opportunities for gene exchange with resident communities (Aminov 2011; Yang et al. 2024). Moreover, copiotrophs often exhibit lower carbon use efficiency, with a greater proportion of carbon respired as CO_2 rather than incorporated into microbial biomass (Stone et al. 2023; Yang et al. 2025). This is consistent with the observed decline in carbon content between T0 and T1 in the SS15 and SS30 treatments.

Copiotrophs exhibit high metabolic rates and efficiently decompose labile organic matter. This rapid decomposition releases nutrients quickly, enhancing soil fertility and stimulating microbial activity (Hu et al. 1999; Stone et al. 2023). Consequently, an increased abundance of copiotrophic microorganisms in soil can promote short-term organic matter turnover and nutrient availability, boosting plant productivity (He et al. 2025; Stone et al. 2023).

The proliferation of AD3 and Verrucomicrobia under SS15 and SS30 treatments was also expected, as these groups are believed to be free-living heterotrophs implicated in organic matter degradation (Bergmann et al. 2011). In contrast, the abundance of Actinobacteriota and Acidobacteriota, often classified as oligotrophic taxa, decreased as an effect of sludge treatment. These microorganisms, adapted to low-nutrient environments, tend to be outcompeted by copiotrophs under nutrient-enriched conditions. By consuming the available nutrients and being fast-growing, copiotrophs reduce niche space for slow-growing oligotrophs (Fierer et al. 2007; Kalam et al. 2020; Bao et al. 2021).

Plant biomass, metal(loid) accumulation and yields

As previously cited, metal complexation on the organic matter helps explain the observed trade-off between increased biomass and reduced metal concentration in plant shoots, with higher sewage sludge treatment (Fig. 4). While improved plant nutrition and organic matter promoted greater biomass production, the concurrent immobilization of metals by the organic compounds pool limited their uptake (Ernst 2005). Linked to this factor, bigger plants might have been subjected to the "dilution" of metal(loids) in greater biomass. The generally lower accumulation rates in sludge-amended substrates align with previous agromining experiments carried out on sewage sludge (Salinitro et al. 2022). In the latter study, *A. bisulcatus* and *O. chalcidica*, targeting Se and Ni, showed an accumulation of these elements to 165 and 870 mg kg^{-1} DW compared to 50 and 2000 mg kg^{-1} DW found in the present study at SS30 (Fig. 4A, 4C). These values are markedly lower than those reported for *A. bisulcatus* growing on seleniferous soils, reaching 5,000–10,000 mg Se kg^{-1} DW (Galeas et al. 2007; Statwick et al. 2016) and for *O. chalcidica* growing in serpentine soil with up to 8,400–14,100 mg Ni kg^{-1} DW (Bani et al. 2015).

Beyond metal immobilization driven by organic matter, several additional factors may have contributed to the limited hyperaccumulation of the three target elements. Substrate pH (Table 2) is a key determinant of metal bioavailability, with the uptake of many divalent cations like Ni, Co, and Zn decreasing as pH increases (Pérez-Esteban et al. 2014). Cobalt accumulation in *B. coddii*, for example, is known to be optimal under acidic conditions (Rue et al. 2020) as also demonstrated by our study. More in general, substrate pH is a key determinant of metal bioavailability, with the uptake of many divalent cations like Ni, Co, and Zn decreasing as pH increases (Pérez-Esteban et al. 2014). However, this general rule does not always apply to hyperaccumulator plants as recently demonstrated by Risse et al. (2023) who observed higher Ni accumulation in *O. chalcidica* growing in soil with higher soil pH. They also hypothesized that the alkalinization of subtracted might be driven by plants root exudates, and this would be consisted with what we observed for *O. chalcidica* growing on unamended mine waste (PRIN SS0, Table 2). In the case of Se, elemental antagonisms may also play a role in restricting Se uptake by plants. For instance, Se and P are both taken up via phosphate transporters, leading to their competition for the uptake when P levels are particularly high as in the case of sewage sludge (~1.3%) (Salinitro et al. 2022; Zhang and Chu 2022). Additionally, the limited root development of temperate hyperaccumulator species in moist conditions may further constrain metal uptake. This has been shown for Odontarrhena corsica, where an increase in soil moisture in pot experiments led to reduced root growth and lower Ni accumulation (Paul and Chaney 2024).

The spatial distribution of accumulated elements revealed higher Co concentrations in older leaves of

B. coddii (Fig. 5A) compared to younger ones. This pattern, observed across all treatments, suggests limited phloem mobility of Co. These results are in accordance with previous findings, where *B. coddii* showed preferential Co accumulation in basal leaves (Rue et al. 2020). The average Co concentration in *B. coddii* grown on unamended mine waste (SS0) was approximately 250 mg kg^{-1} DW, which is within the same order of magnitude as values reported under optimal soil conditions (600 mg kg^{-1} DW; Rue et al. 2020).

In contrast, Ni and selenium (Se) were primarily translocated to and accumulated in younger leaves (Fig. 5B, C). This trend is supported by studies on Odontarrhena lesbiaca and Noccaea caerulescens, where Ni concentrations were significantly higher in young, actively growing tissues compared to old ones (Deng et al. 2016; Adamidis et al. 2017). Similarly, the preferential accumulation of Se in young plant parts has been consistently reported in most studied Se-hyperaccumulator species, including *A. bisulcatus*, indicating substantial phloem mobilization of this element between organs (Freeman et al. 2006; van der Ent et al. 2023).

For Co and Se, the substantial increase in plant biomass under SS15 and SS30 treatments compensated for the lower metal concentrations in shoots by increasing the overall metal yields (Table 3). Even when considering the best treatment for each species in terms of annual metal yields (SS15 for *B. coddii*, SS30 for *A. bisulcatus*, and SS0 for *O. chalcidica*) the yields extrapolated in the present study are lower than those estimated by other authors. For example, Corzo Remigio et al. (2020) estimated Se and Co yields between 2.5–70 and 25 kg ha^{-1}, respectively, based on pot and hydroponic data, versus only 0.08 and 0.15 kg ha^{-1} estimated in the present study (Table 3). Field data collected in comparable 1-m^2 plot conditions (Bani and Echevarria 2019) extrapolated annual Ni production using *O. chalcidica* between 13 and 145 kg ha^{-1}, whereas our study recorded only 6 kg ha^{-1}. However, our yields align more closely with a similar study on Ni extraction from galvanic sludge (Tognacchini et al. 2020), which reported estimated yields of approximately 1.5 kg ha^{-1} Ni.

As expected hyperaccumulator species were more efficient than spontaneous colonizing plants in accumulating the target elements, as the latter behaved predominantly as metal excluders (Osmani et al. 2018). Estimated annual Ni, Co and Se yields in mg^{-1} m^{-2} were 250-, 7- and 2-folds higher in dedicated metal crops compared to spontaneous vegetation, at SS0 conditions.

Conclusions

The current study provides novel insights into the feasibility of Ni, Co, and Se agromining from mine wastes amended with varying rates of sewage sludge. Our findings demonstrate that mine wastes amended with sewage sludge can support robust growth of *O. chalcidica*, *B. coddii*, and *A. bisulcatus,* enabling the recovery of Ni, Co, and Se, albeit at lower levels compared to natural metalliferous soils. In general, the reduced metal accumulation efficiency observed in SS15 and SS30 treatments underscores the importance of optimizing amendment rates to balance plant growth and metal accumulation. The SS application rates used in this study were found to be excessively high, leading to strong metal immobilization in the substrate and decline of plant Ni, Co and Se uptake. In the long term, this approach holds promise for the reduction of SS disposal volumes, while transforming neglected mine lands into economically viable "metal farms." Improvements in soil fertility parameters and increased microbial diversity, suggest that this strategy may also positively contribute to mined land reclamation.

Future research should focus on optimizing the sewage sludge:mine waste ratio, potentially reducing it by an order of magnitude (~ 1–3 kgm^{-2}, in line with its use as fertilizer on agricultural lands), to balance plant biomass production and the bioavailability of target metals. Additionally, the long-term implementation of such practices will rely on the willingness to the revise the current legislative framework (EU directive 86/278/EEC), which often prohibits the application of metal-contaminated sludge on soil. A deeper understanding of the fate of non-targeted elements (e.g., Cr) present in sludge and an economic viability analysis of agromining on waste material at larger scale, also needs further investigation.

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Author's contributions Mirko Salinitro contributed to the study conception, design and material preparation. Sampling was performed by Mirko Salinitro and Elio Padoan. Experimental set up and management was performed by Mirko Salinitro, Benedetta Montanarini and Annalisa Tassoni. Data collection and analysis was performed by Mirko Salinitro. Plant and soil analyses were performed by Mirko Salinitro, Michela Schiavon and Elio Padoan. Bacterial bioinformatics was performed by Marcin Musiałowski, Klaudia Dębiec-Andrzejewska. Antony van der Ent and Mark Aarts provided the resources and supervised the study. The draft of the manuscript was written by Mirko Salinitro. All the authors revised and edited the final version.

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Data availability All the datasets generated during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interest The authors declare no competing interests.

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