Chemosphere 273 (2021) 128483

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Assessment of biodegradable chelating agents in the phytoextraction of heavy metals from multi–metal contaminated soil

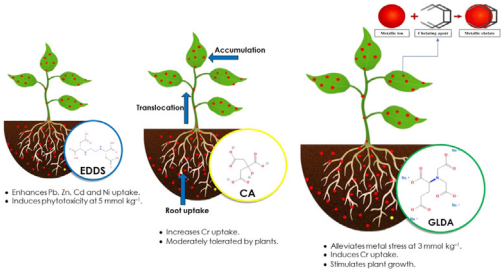
Ivan Diarra [a], Krishna Kumar Kotra [b], Surendra Prasad [a, *]

[a] School of Biological and Chemical Sciences, Faculty of Science, Technology and Environment, The University of the South Pacific, Laucala Campus, Suva, Fiji
[b] School of Biological and Chemical Sciences, Faculty of Science, Technology and Environment, The University of the South Pacific, Emalus Campus, Port Vila, Vanuatu

HIGHLIGHTS

- Brassica spp. can tolerate and accumulate a wide range of heavy metals.
- Phytoextraction by Brassica spp. is limited in multi-contaminated soils.
- EDDS induces high metal bioconcentration compared to CA and GLDA.
- GLDA alleviates heavy metal stress in plants and enhances plant growth.
- Metals are predominantly accumulated in roots with poor translocation to shoot (<1).

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 15 April 2020
Received in revised form
25 September 2020
Accepted 27 September 2020
Available online 4 October 2020

Handling Editor: X. Cao

Keywords:
Phytoextraction
Remediation
Heavy metals
Biodegradable chelating agents
Pacific Islands

ABSTRACT

A pot incubation experiment under natural conditions was designed to investigate the effects of three biodegradable chelating agents, namely; the [S,S]-isomer of ethylenediamine disuccinate (EDDS), citric acid (CA), and tetrasodium N,N-Bis(carboxymethyl)-L-glutamate acid (GLDA), on two plant species (*Brassica juncea* and *Brassica rapa*) in terms of plant foliar growth, dry matter yield, and heavy metal (HM) accumulation. Both plant species exhibited diminished growth and symptoms of phytotoxicity under HM stress. The application of EDDS and CA affected plant foliar growth, biomass production, and led to the development of chlorotic lesions on leaves. EDDS and CA also decreased the shoot length by 38.5% and 45.2% in *B. juncea*, and 60.1% and 100% in *B. rapa*, respectively. In contrast, GLDA relieved HM stress by significantly increasing plant growth ($P > 0.05$) and was shown to be well tolerated (tolerance index [TI]; *B. juncea* = 99% and *B. rapa* = 123%). Among both plants, *B. juncea* displayed the ability to accumulate a wider range of HMs at higher concentrations. Amongst the three chelators, EDDS induced the highest bioconcentration (BCF) of Pb (2.45), Zn (2.68), and Cd (3.36) while CA achieved better results for Ni (4.01) and Cr (1.45). However, the current results showed that even with the application of chelating agents, HMs were predominantly accumulated in roots and translocation factor was generally <1. The findings of this investigation emphasize that chelate–assisted phytoextraction with *Brassica* spp. is highly limited in multi–metal settings, making it an unsuitable option for severely contaminated sites.

1. Introduction

Anthropogenic activities continue to be a major source of recalcitrant toxicants such as heavy metals (HMs) in the

* Corresponding author.
E-mail address: prasad_su@usp.ac.fj (S. Prasad).

https://doi.org/10.1016/j.chemosphere.2020.128483
0045-6535/

environment. Their inherent inorganic nature and persistence to degradation has especially made these toxic HMs pervasive and difficult to contain. As such, their environmental proliferation has become prevalent even in some small and developing island nations of the South Pacific (Diarra and Prasad, 2020), clearly suggesting that the problem is indeed universal. Regional bodies such as the Secretariat of the Pacific Regional Environmental Programme (SPREP) and studies conducted over the last 3 decades have highlighted the extent of the problem and the long–term potential threats to the region (Morrison and Munro, 1999; Dewailly et al., 2008; Park et al., 2013; Imo et al., 2014; Chandra et al., 2015; Maeaba et al., 2019; Diarra and Prasad, 2020). In lieu of the numerous challenges facing the adoption and deployment of conventional HM remediation techniques, including high cost and disruptive nature (Khalid et al., 2016; Roy Chowdhury et al., 2018), there has been growing research interest into sustainable plant–based remediation alternatives such as phytoextraction.

Phytoextraction involves the utilization of specialized and highly adapted hyperaccumulators to absorb, transport and accumulate HMs in the biomass of harvestable organs. Hyperaccumulators refer to the plants growing on native soils which can concentrate >10 mg g^{-1} (1%) Mn or Zn, >1 mg g^{-1} (0.1%) As, Co, Cr, Cu, Ni, Pb, Sb, Se or Tl, and >0.1 mg g^{-1} (0.01%) Cd in their aerial organs, without suffering phytotoxic damage (Verbruggen et al., 2009). Plant selection criteria is the most critical factor determining the viability and success of phytoextraction, thus, hyperaccumulators with excellent tolerance and bioaccumulation of a wide range of HMs are prioritized. The *Brassicaceae* family contains the highest number of hyperaccumulator genera (11) and species (90) (Anjum et al., 2012), representing approximately 25% of all known hyperaccumulators (Rascio and Navari–Izzo, 2011). Phytoextraction assessments have reported on their excellent HM accumulation due to their intrinsic tolerance for HMs, relatively high aboveground biomass production and production of root exudates (Quartacci et al., 2009; Szczygłowska et al., 2011; Mourato et al., 2015). An evaluation of the phytoextractive capacity of five *Brassica* spp. revealed that the levels of Zn, Cu, Ni, and Pb was; *B. juncea* (130.7, 52.2, 5.9 and 56.8 mg kg^{-1}), *B. campestris* (194.9, 34.9, 19.1 and 22.3 mg kg^{-1}), *B. carinata* (145.1, 46.3, 19.5 and 66.5 mg kg^{-1}), *B. napus* (148.9, 64.7, 14.1 and 37.8 mg kg^{-1}) and *B. nigra* (119.2, 62.6, 16.7 and 27.9 mg kg^{-1}), respectively (Purakayastha et al., 2008).

However, natural phytoextraction is limited by poor HM bioavailability within the rhizosphere – which depends on soil pH and clay content, cellular tolerance to HMs, soil nutrient levels, and HM selectivity (Evangelou et al., 2007; Chibuike and Obiora, 2014). To overcome some of these limitations, researchers have turned to chemically assisted phytoextraction (also referred to as chelate–enhanced phytoextraction) to increase HM solubility and bioavailability. Synthetic aminopolycarboxylates (APCAs) such as ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) are among the most efficient chelating agents and have served as the standard in phytoextraction research for several decades due to their strong metal affinity (Meers et al., 2005b). EDTA forms highly stable chelate complexes with almost every polyvalent metal cation (Schmidt and Brauch, 2006; Hart, 2011), and has been highly effective in phytoextraction studies (Lesage et al., 2005; Kim and Lee, 2010; Guo et al., 2019). Nonetheless, concerns have arisen over the persistence of EDTA in the environment as stable HM–chelate complexes due to their long half–lives and poor degradability (Oviedo and Rodríguez, 2003). Microorganisms can transport free EDTA, but not HM–EDTA complexes, into cells for metabolism (Lewis et al., 2020), which has led to concerns over the risks of HM leaching and possible ecotoxicity (Sillanpää, 1997; Barona et al., 2001; Lanigan and Yamarik, 2002). These challenges are driving the search for suitable biodegradable and natural alternatives with equal or even superior HM chelating efficiency compared to synthetic APCAs.

Ethylenediamine disuccinate (EDDS) is also a low–toxicity chelator with strong chemical affinity for HMs and low residual risks (Yang et al., 2013). Structurally, EDDS has two chiral centers, and as such three stereoisomers; the enantiomeric [R,R] and [S,S] isomers, and the achiral meso [R,S] isomer. The [R,S] and [R,R] stereoisomers are less biodegradable, whereas the [S,S] stereoisomer has been shown to be effectively biodegraded even in highly polluted soils (Takahashi et al., 1997; Vandevivere et al., 2001). In a phytoextraction study, Meers et al. (2005b) described a high degree of biodegradability for [S,S]–EDDS with half–lives ranging from 3.8 to 7.5 days, contingent on the application rate, while Schowanek et al. (1997) reported that close to 96% of [S,S]–EDDS was mineralized (degraded) within one month. Studies evaluating the performance of EDDS in phytoextraction have reported on its effectiveness in enhancing the uptake of several HMs (Meers et al., 2005b; Epelde et al., 2008; Zhao et al., 2015). Produced from a naturally occurring amino acid, L–glutamic acid N,N–diacetic acid, tetrasodium salt (also referred to as tetrasodium glutamate diacetate, $C_9H_9NO_8Na_4$) (GLDA) is a novel chelating agent that is readily biodegradable with a high level of solubility over a wide pH range and thus, considered an eco-friendly alternative to synthetic APCAs. The product consists only of L–GLDA, as the D–form is not biodegradable. Kołodyńska (2013) reported that over 60% of the L–GLDA degrades within 28 days. Although its use in phytoextraction has been limited, several researchers including Mai et al. (2019) have recommended further efforts to explore GLDA–induced phytoextraction due to its many attributes and promising results obtained from activation tests. Few researchers have reported on its efficacy in inducing high HM uptake in plants (Wei et al., 2015; Ning et al., 2019; Wang et al., 2019).

In contrast to the use of costly APCAs, phytoextraction can also be improved through the use of low molecular weight organic acids (LMWOAs) such as citric acid, oxalic acid, malic acid, and acetic acid. LMWOAs can act as ligands binding HMs to form organometallic complexes in various stoichiometry and structures at low to moderate stability. Citric acid (CA) in particular has been reported to possess the strongest HM complexing ability among LMWOAs (del Mundo Dacera and Babel, 2006; Jean et al., 2008; Ding et al., 2014), displaying high biodegradability as well as complexation stability even in multi–metal settings without increasing the risks of leaching (do Nascimento et al., 2006; Ding et al., 2014). CA has a short half-life (2–6 days) and has achieved a cumulative degradation of 80% within 14 days (Brynhildsen and Rosswall, 1997).

Although phytoextraction studies with *Brassica* spp. have shown promising results in mono–metal settings, the synergistic effects of these plants and chelating ligands in multi–metal conditions have been far less explored. This is relevant since contaminated sites have been reported to often contain a mixture of several inorganic elements and/or organic compounds (Förstner, 1995), thereby requiring hyperaccumulators with the tolerance and capacity to bioaccumulate multiple HMs. Thus, this study was executed in order to (i) compare the effects of EDDS, GLDA, and CA on the phytoextraction of HMs in two hyperaccumulator species (*B. juncea* and *B. rapa*), and (ii) investigate the effects of HMs and chelators on plant growth and phytotoxicity, translocation and bioconcentration.

Table 1
Physicochemical properties of the original and metal-spiked soil (n = 3).

Parameters		Original soil (unspiked)	Spiked soil	Recovery (%)
Soil texture		Clay loam	Clay loam	–
Particle Density (g cm^{-3})		3.58	3.58	–
Organic Matter (%)		7.60	7.40	–
Carbon (%)		4.43	4.30	–
pH (H_2O)		6.38	6.87	–
pH (KCl)		6.18	6.84	–
Electrical Conductivity (mS s^{-1})		2.06	7.98	–
Water Holding Capacity (%)		18.00	18.00	–
Cation Exchange Capacity (meq $100g^{-1}$)		24.53	19.08	–
Elemental concentrations (mg kg^{-1})	Fe	22856.00 ± 932.31	23100.7 ± 750.00	–
	Mg	314.84 ± 101.8	321.72 ± 18.96	–
	Mn	70.26 ± 16.1	72.66 ± 4.72	–
	Cd (Cd granules)	2.62 ± 0.67	44.70 ± 7.00	84.21
	Cr ($Na_2Cr_2O_7 \cdot 2H_2O$)	47.45 ± 5.76	159.00 ± 24.70	111.55
	Cu ($CuCl_2 \cdot 2H_2O$)	72.58 ± 1.53	264.80 ± 8.40	96.10
	Ni ($NiCl_2 \cdot 6H_2O$)	40.25 ± 10.69	143.90 ± 16.60	103.63
	Pb ($Pb(NO_3)_2$)	110.35 ± 9.45	312.60 ± 18.90	101.12
	Zn ($ZnCl_2$)	34.80 ± 1.95	128.50 ± 12.90	93.74

Metal salts used for spiking are provided in brackets.

2. Materials and methods

2.1. Study area

The present study was conducted in the city of Suva, located on the Southeast coast of the island of Viti Levu, in the Republic of Fiji. As the national capital and centre of commercial and economic activity in the country, the city is exposed to high levels of HMs from several land–based industrial activities like metal fabrication and construction, paint manufacturing, petroleum storage and garment manufacturing, food processing and fish cannery as well as the activities of bottling plants (Arikibe and Prasad, 2020). Previous investigations conducted on soil, sediments and dust samples from the city have highlighted the presence of HMs arising from these anthropogenic sources (Naidu and Morrison, 1994; Chandra et al., 2015; Maeaba et al., 2019). As such, this study employed a multivariate analysis to determine the levels of HMs and some Natural Source Elements (NSE) in the study area (Table 1). NSEs including Fe, Mn, and Mg are considered crucial in the remediation process due to their role in HMs uptake and plant–soil interactions.

2.2. Soil sampling and characterization

A total of 14 sites with close proximity to industries, residential areas and centers of large commercial activities within the greater Suva area were selected for surface soil sampling (Fig. 1). At each site, surface soils (0–15 cm) were retrieved and placed in airtight Ziploc bags to make a single composite sample. After homogenization and air drying for 7 days, the composite soil sample was disaggregated and sieved through a 2 mm sieve to remove larger rock fragments and unwanted debris. For HM–spiked treatments, equal volumes of a HM solution prepared with seven analytical grade reagents (Table 1), was applied to the soil and incubated for 4 weeks to allow for equilibration of the added HMs in the soil matrix. The major soil parameters before and after HM spiking and incubation are presented in Table 3. The soil pH and electrical conductivity (EC) were 6.38 and 2.06 mS s^{-1} in the original soil, while in the spiked soil, both parameters increased to 6.87 and 7.98 mS s^{-1}, respectively. The soil texture was clay loam while soil particle density was 3.58 cm^{-3} and. The soil cation exchange capacity (CEC) in the original soil was 24.5 meq 100 g^{-1}, but decreased to 19.1 meq 100 g^{-1} in the HM-spiked soil due to metal addition and organic matter supplementation. The soil organic matter (SOM) and carbon contents were approximately 7.5% and 4.4%, respectively.

2.3. Plant growth and experimental design

Plant growth was carried out under natural conditions at The University of the South Pacific, Suva, between February and July 2019. The environmental conditions during plant growth were as follows; average relative humidity was 86%, average air temperature was 29.3 °C, average light intensity was 6156 Lumens, and day length was approximately 12.5 h. Plant species (*B. juncea* and *B. rapa*) were grown in triplicates following a randomized complete block design. 1.0 ± 0.005 kg of the soil were transferred into a series of plastic pots (depth = 15.7 cm, diameter = 12.4 cm) with bottoms completely sealed to prevent leaching of the mobilized HMs and nutrients. All pots were maintained at 70% of the soil water holding capacity. Commercial varieties of *B. juncea* (locally called *Sarso*) and *B. rapa* (*Pak choy*) seeds were germinated in trays after sterilization in H_2O_2 solution (2%, v/v) for 15 min and soaked in deionized water

Table 2
Analytical conditions and procedure for BCR sequential extraction.

Steps	Fraction	Procedure
Step 1	Water-soluble state	20 mL of deionized water was added and shaken for 16 h to obtain the supernatant
Step 2	Acid-soluble state	20 mL of acetic acid (HOAc, pH 2.8) was added and shaken for 16 h to obtain the supernatant. (The soil samples need to be washed with deionized water after each step)
Step 3	Reducible state	20 mL of $NH_2OH \cdot HCl$ (0.5 M, pH 2) was added and shaken for 16 h to obtain the supernatant.
Step 4	Oxidizable state	10 mL of H_2O_2 was added to the samples and heated at 85 °C for 1 h; 5 mL H_2O_2 (30%, pH 2) was added again and the samples were heated at 85 °C for 1 h, then NH_4OAc (1 M, pH 2) was added and shaken for 16 h to obtain the supernatant.
Step 5	Residual state	Digestion with $HCl–HNO_3–HF–HClO_4$ for 7 h.

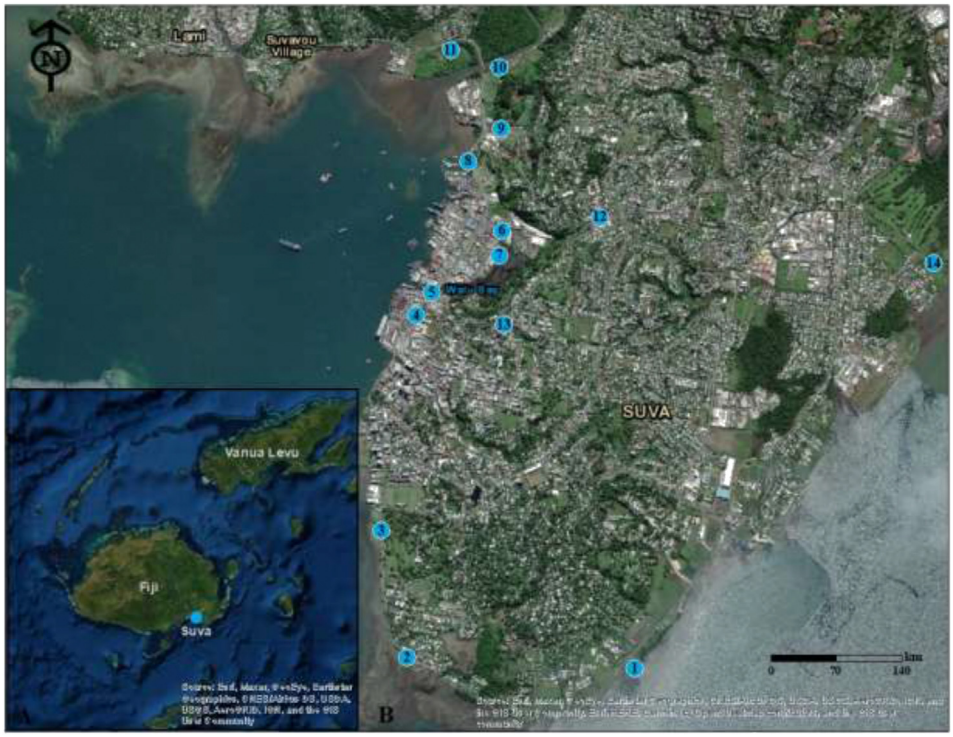


Fig. 1. Map of the study area and location of sampling stations.

Table 3
Photosynthetic pigment content, dry matter (DM), and tolerance index (TI) for *B. juncea* and *B. rapa* under different chelating agent treatments.

Plants	Parameters	Treatments				
		NC	PC	EDDS	CA	GLDA
B. juncea	Chl *a* (mg g^{-1})	48.20 ± 3.22	17.60 ± 4.84	11.50 ± 1.18	13.60 ± 1.75	22.40 ± 3.56
	Chl *b* (mg g^{-1})	30.10 ± 1.39	11.30 ± 3.95	5.80 ± 0.63	6.30 ± 1.12	13.08 ± 1.57
	Total Chl (mg g^{-1})	78.60 ± 9.29	29.30 ± 3.80	17.80 ± 3.66	19.45 ± 1.81	34.80 ± 2.90
	Chl *a*:*b*	1.60	1.56	1.98	2.16	1.72
	$Shoot_{DM}$ (g)	0.77 ± 0.28	0.45 ± 0.02	0.27 ± 0.11	0.37 ± 0.19	0.52 ± 0.16
	$Root_{DM}$ (g)	0.30 ± 0.18	0.27 ± 0.05	0.13 ± 0.08	0.28 ± 0.26	0.20 ± 0.08
	$Total_{DM}$ (g)	1.07 ± 0.46	0.73 ± 0.07	0.41 ± 0.19	0.65 ± 0.43	0.72 ± 0.25
	TI (%)	–	68	56	89	99
B. rapa	Chl *a* (mg g^{-1})	53.60 ± 7.43	13.10 ± 0.66	12.40 ± 0.94	18.20 ± 2.69	25.80 ± 2.41
	Chl *b* (mg g^{-1})	41.90 ± 5.21	8.60 ± 1.55	9.20 ± 0.89	10.70 ± 1.94	18.30 ± 3.53
	Total Chl (mg g^{-1})	94.70 ± 12.54	22.40 ± 3.37	21.10 ± 1.03	28.40 ± 2.83	42.70 ± 2.40
	Chl *a*:*b*	1.28	1.52	1.35	1.70	1.41
	$Shoot_{DM}$ (g)	0.97 ± 0.33	0.59 ± 0.01	0.51 ± 0.02	0.43 ± 0.02	0.83 ± 0.09
	$Root_{DM}$ (g)	0.59 ± 0.03	0.40 ± 0.11	0.19 ± 0.05	0.15 ± 0.03	0.38 ± 0.02
	$Total_{DM}$ (g)	1.56 ± 0.34	0.99 ± 0.10	0.70 ± 0.06	0.59 ± 0.01	1.22 ± 0.10
	TI (%)	–	64	71	59	123

Chl = chlorophyll, $Total_{DM}$ = Total dry matter; $Shoot_{DM}$ = Shoot dry matter; $Root_{DM}$ = Root dry matter.
All data except TI and Chl *a*:*b*, are expressed as Mean ± SD (n = 3).

(DW) overnight. One week after emergence, the seedlings were transplanted into the respective pots and eventually thinned to one per pot. Five treatment groups were evaluated namely; i) negative control (original soil with no HM addition); ii) positive control (HM–spiked soil [Cd, Cr, Cu, Ni, Pb, Zn]); iii) EDDS treatment (HM–spiked soil + 5 mmol kg^{-1} EDDS); iv) CA treatment (HM–spiked soil + 10 mmol kg^{-1} CA); and v) GLDA treatment (HM–spiked soil + 3 mmol kg^{-1} GLDA). Chelate treatments were applied one week before harvesting while for the control groups, equal volume of DI water was applied. Since studies have reported that fertilizer application did not significantly affect HM uptake by plants (Meers et al., 2005b), NPK fertilizer was applied at a rate of 100 mg kg^{-1} of soil weekly to each pot to avoid nutrient deficiencies. All plants were grown for five weeks, which is the optimal growth period for *Brassica* spp. to reach maturity (Corley and Mutiti, 2017).

Plant growth was monitored during the entire duration of the experiment to observe the effects of HMs and chelators on plant growth and development. This was achieved by measuring the longest plant leaf and shoot in each pot at the end of every week from transplanting to harvest. Foliage was routinely monitored visually for signs of disease or stress, colour changes, and pests.

After the growth period, plants were thoroughly washed in ultrapure DI water and separated into aerial parts (stem, leaves, flowers) and roots. Plants dry weights and biomass production was recorded after oven–drying at 70 °C for 48 h.

2.4. Soil and plant elemental analysis

A modified Community Bureau of Reference (BCR) five–step sequential extraction process (Wang et al., 2019) was employed to determine the pseudo–total soil HM content and the effects of chelating agents on the morphological distribution of HM fractions in the experimental soils. The specific steps and morphology definitions are presented in Table 2. The sequential extraction experiment was performed using a 1 g soil sample. After each extraction step, the extracted suspension was centrifuged at a speed of 4000 rpm for 20 min, and the residual supernatant was filtered through a 0.45 mm membrane. The residue was washed with DW three times before the next extraction step. The obtained filtrate was transferred to a 50 mL centrifuge tube and stored at 4 °C until further analysis.

Plants were digested according to USEPA method 3050B (USEPA, 1996). Approximately 0.5 g of dried and crushed plant tissue were digested with 15.6 M HNO_3 (10 mL) and 12 M HCl on a hotplate (LABEC Australia) at 95 ± 5 °C for 15 min. After cooling, ultrapure DI water (2 mL) and H_2O_2 (30% v/v, 3 mL) were added, and the solution was heated until effervescence subsided. The solution was then cooled, filtered (Whatman no. 41 filter paper) and diluted to 100 mL in a volumetric flask using ultrapure DI water. HM concentrations in extracted samples were analyzed using FAAS (PerkinElmer PinAAcle AAS 500).

2.5. Chlorophyll analysis

Photosynthetic pigment levels in plants were estimated using Arnon's method (Arnon, 1949). Leaf samples collected from each treatment were crushed in a mortar and pestle to which, acetone (80%, 20 mL) and $MgCO_3$ (0.5 g) powder was added. After refrigeration at 4 °C for 4 h, the sample was centrifuged at 10,000 rpm for 10 min. The supernatant was made up to the mark with 80% acetone in a 100 mL volumetric flask and the absorbance of the solution was estimated using a spectrophotometer (LAMBDA 365) at 645 and 663 nm wavelengths against the solvent. The concentration of the photosynthetic pigments was calculated as mg g^{-1} dry weight of the sample. Leaf chlorophyll (Chl) content was calculated using Eqs. (1)–(3) where Chl *a* and Chl *b* are the chlorophyll *a* and chlorophyll *b* contents, and A is the absorbance at particular wavelength.

$$\text{Total Chl Content}\left(\text{mg L}^{-1}\right) = (A_{645} \times 20.2) + (A_{663} \times 8.3) \quad (1)$$

$$\text{Chl } a \text{ content}\left(\text{mg L}^{-1}\right) = (A_{663} \times 12.7) - (A_{645} \times 2.69) \quad (2)$$

$$\text{Chl } b \text{ content}\left(\text{mg L}^{-1}\right) = (A_{645} \times 22.9) - (A_{663} \times 4.68) \quad (3)$$

2.6. Phytoremediation efficiency of plants

Generally, three key factors determine the HM accumulation efficiency of plants, namely; biomass production, bioconcentration factor (BCF) and translocation factor (TF). The tolerance index (TI) was used to evaluate the ability of the plants to grow and produce sufficient biomass in the presence of added HMs and chelating agents. TI was calculated as the ratio of dry matter (DM) of plants in treatment pots to control plants and expressed as a percentage, as shown in Eq. (4) (Wilkins, 1978).

$$\text{TI}(\%) = \frac{\text{DM}_{\text{Treatment plants}}(\text{g})}{\text{DM}_{\text{Control plants}}(\text{g})} \times 100 \quad (4)$$

BCF is an index which describes the ratio of HM concentration in plant tissues to that in the soil, and is a measure of the ability of a plant to accumulate HMs and was calculated using Eq. (5), where C_{plant} and C_{soil} represent the HM concentrations in plant and soil, respectively.

$$\text{BCF} = \frac{C_{plant}\left(\text{mg kg}^{-1}\right)}{C_{soil}\left(\text{mg kg}^{-1}\right)} \quad (5)$$

In contrast, the TF, describes an ability of a plant to translocate HMs from roots to the aboveground biomass (shoots, stems, leaves and flowers) and was calculated using Eq. (6), where C_{shoots} and C_{roots} represent the HM concentrations in shoots and roots respectively.

$$\text{TF} = \frac{C_{shoots}\left(\text{mg kg}^{-1}\right)}{C_{roots}\left(\text{mg kg}^{-1}\right)} \quad (6)$$

2.7. Quality control and statistical analysis

All chemicals were of analytical reagent grade and ultrapure DI water (Millipore 18.2 MΩ cm at 25 °C) was used for solution preparation and dilution. Reagent blanks, method blanks and certified reference material (CRM023-50G, Sigma Aldrich) were used to test the reliability of analytical procedures while stock metal solutions (Merck, Germany) were used for instrument calibration. All soil and plant samples were analyzed in triplicates. Data were statistically analyzed in SPSS® version 25 (IBM) reported as mean ± standard deviation (SD). Individual treatments (NC, PC, EDDS, CA and GLDA) were assigned indicator variables in a categorical factorial design to determine the effects of each treatment and potential interactions and analyzed by ANOVA. When a significant difference was observed between treatment means, multiple comparisons were made using the Bonferroni post hoc test. The correlation coefficient (r) was computed to test the relationship between plant growth parameters and HM concentrations. All tests were considered significant at the 5% confidence level ($\alpha = 0.05$).

3. Results and discussion

3.1. Plant growth and biomass production

3.1.1. Visual symptoms and leaf chlorophyll content

The HM–spiked treatments exhibited stunted growth and leaf chlorosis in comparison to the control. The application of chelating agents had a strong effect on the physical health of all plants. EDDS and CA both resulted in severe yellowing and drying of all plant leaves and stems. In addition, leaves developed severe white patches. In contrast, GLDA had a less noticeable impact on the plant's physical appearance for much of the research duration. Comparatively, plants in the GLDA treatments had larger and greener foliage, similar to those in the negative control (NC) treatment. This is supported by the findings of (Wang et al., 2019) which reported growth promotion in amaranth plants after GLDA addition. This is attributed to the rapid degradation of GLDA, which

Table 4
Bioconcentration and Translocation factors for *Brassica juncea* and *Brassica rapa* metal uptake.

Metals	BCF									
	B. juncea					*B. rapa*				
	NC	PC	EDDS	CA	GLDA	NC	PC	EDDS	CA	GLDA
Cr	0.16	0.64	1.44	1.45	1.25	0.16	0.68	1.06	1.24	1.26
Zn	1.03	1.57	2.68	2.59	2.53	1.07	1.83	2.62	2.38	2.62
Cd	0.85	1.93	3.36	2.78	2.45	0.92	2.17	3.65	3.01	3.43
Pb	1.70	1.57	2.45	2.25	2.03	1.72	1.28	1.74	1.52	1.58
Ni	4.03	2.17	3.69	4.01	3.24	4.00	2.29	2.90	2.94	2.04
Cu	2.13	0.53	0.87	0.77	0.77	1.22	0.59	0.62	0.47	0.44
	TF									
Cr	0.33	0.28	0.44	0.33	0.43	0.23	0.32	0.37	0.39	0.40
Zn	0.61	0.41	0.56	0.40	0.44	0.58	0.62	0.48	0.33	0.41
Cd	0.20	0.28	0.65	0.45	0.50	0.19	0.24	0.26	0.33	0.35
Pb	0.71	0.72	0.68	0.78	0.99	0.61	0.49	0.51	0.55	0.53
Ni	0.33	0.39	0.48	0.23	0.60	0.31	0.25	0.30	0.26	0.34
Cu	0.41	0.52	0.46	0.44	0.47	0.53	0.52	0.47	0.44	0.41

only persist in the soil for a short time and therefore has a limited impact on the soil properties. In addition, a main by−product of GLDA is Ammonia (NH_3), which stimulates plant growth.

Chlorophyll is the main photosynthetic pigment in plants and can reflect abiotic stress on plants. It was clear that HM concentrations in the soil greatly inhibited the production of chlorophyll (Table 4). The total chlorophyll content (total Chl) in *B. juncea* decreased by 62% between control (78.6 mg g^{-1} DW) and HM−spiked treatment (29.3 mg g^{-1} DW), while in *B. rapa*, the reduction was 76%. Similarly, *B. juncea* and *B. rapa* plants in both the EDDS and CA treatments had a significant decline in total chlorophyll (34.7% and 22.7%), whereas in the GLDA treatment, *B. juncea* total Chl increased by 27.3%. In accordance, both Chl *a* and Chl *b* contents were maximum in control plants and lowest in the EDDS treatment. Kumar et al. (2012) suggested that HMs interfered with the biosynthesis of chlorophyll, either through the direct inhibition of enzymes or through the substitution of the central Mg^{2+} ion. Khan et al. (2019) reported that increased uptake of Cd, Cr, Cu, Ni, and Pb by *P. hybrida* L resulted in a reduction in Chl *a*, Chl *b*, total Chl, and carotenoid content, while noting a significant increase in biochemical stress indicators, including MDA, H_2O_2 content, and electrolyte leakage.

3.1.2. Plant biomass production and tolerance index

Presented in Table 4 are the dry matter (DM) yields of the roots and aboveground tissues (stem, leaves, and flowers) of *B. juncea* and *B. rapa*. DM production showed that the general effects of chelating agents were in the order EDDS > CA > GLDA for both plant species, with very few exceptions. The highest DM for both plants occurred in the NC treatment with 1.55 g and 1.06 g for *B. rapa* and *B. juncea,* respectively. The total DM decreased in the HM−spiked treatment by 33.6% and 36.5% for *B. juncea* and *B. rapa*, respectively. However, this significant reduction was significantly higher in shoots than in roots, revealing that HMs had a greater impact on aerial biomass production. Root biomass for *B. juncea* did not differ significantly between HM−spiked and unspiked treatments, whereas in *B. rapa,* root biomass decreased by 32.2%. Plant exposure to high concentrations of non−redox reactive metals induces oxidative stress and inhibits physiological processes such as photosynthesis, respiration, transpiration rates, N−metabolism and mineral nutrition, cell elongation and decrease in biomass (Morkunas et al., 2018). The data from plant growth and biomass revealed that GLDA had no significant inhibitory effect on plant growth and in some cases, increased DM production significantly. When compared to the control, both plants tolerated GLDA well with a TI of 99% and 123% for *B. juncea* and *B. rapa*, respectively. In contrast, the addition of both EDDS and CA treatments resulted in the lowest DM yield for both plant species. In the present study, 10 mmol kg^{-1} CA also showed a mild negative effect on the DM and plant growth of both *Brassica* spp. in contrast to studies which have shown that the same concentration of CA markedly improved the growth of *B. napus* under Cr stress (Afshan et al., 2015) and *B. juncea* grown in Pb contaminated soil (Bouquet et al., 2017). This variance in results is attributed to the presence of soil HM combinations in the present study which exacerbated plant stressors. It has been suggested that HM combinations inhibit plant biomass as a result of synergistic and antagonistic effects suggesting metal crosstalk at the uptake site (Kutrowska et al., 2017). Although few studies have reported no DM loss or even improved plant growth due to EDDS application at 5 mmol kg^{-1} (She et al., 2014), an overwhelming number have reported declines in DM (Cheng et al., 2012) and increase in leaf necrosis (Yeh and Pan, 2012). The HM−EDDS complex can enter the roots via the Casparian strip, where it is quickly transported to the shoots; the toxic effects of EDDS may damage the physiological root barriers and cause a decrease in plant biomass (Wang et al., 2009). Although few studies on the effects of GLDA on plant growth have been reported, the consensus among available studies is a general promotion of growth and alleviation of HM stress.

3.1.3. Effects of heavy metals and chelators on plant foliage

B. juncea plants showed little variation in growth between treatments (Fig. 2). At harvest, plants in the unspiked treatment (NC) had a larger average leaf length (100.7 mm) compared to those in the HM−spiked treatment (91.7 mm), EDDS (83 mm), CA (84.7 mm), and GLDA (89.7 mm) treatments, respectively. *B. rapa* leaf length was highest in NC and GLDA treatments at 123.8 and 121.3 mm, respectively. The effects of chelators on plant leaf length was negligible, except for plants in EDDS treatment. *B. juncea* leaves in the EDDS treatment grew by only 4.6 mm after chelator application compared to 20.2 mm, and 19.6 mm for CA, and GLDA plants, respectively. In contrast, chelate addition increased the average leaf length in CA and GLDA treatments by 17.4% and 14%, respectively. The shoot growth trend in both plants showed a clear disparity between the unspiked and HM−spiked treatments. The unspiked treatment displayed the highest average shoot length until harvest. HM spiking had a significant reduction in shoot length with a 12% and 14% decrease in *B. juncea* and *B. rapa*, respectively. This is attributed to the damage of transport system by HMs which interferes with nutrient transport leading to cell death (Kumar et al., 2012). *B. juncea* shoot length decreased by 38.5% and 45.2% in the EDDS and CA treatments, whereas the GLDA treatment showed an increase of 10.6%. The addition of chelators decreased the shoot length of *B. rapa* by 60.1%, 100%, and 82.4% in the EDDS, CA, and GLDA treatments, respectively, when compared to the control (PC). In a recent study comparing GLDA, EDDS, and CA in Cd phytoextraction, Wang et al. (2019) noted that, except for EDDS, both GLDA and CA at 2.5 mmol kg^{-1} improved plant biomass. Under Cd stress, CA has been shown to improve *B. juncea* growth by reducing oxidative damage, enhancing the activities of the antioxidant enzymes such as ascorbate (AsA) and glutathione (GSH), while increasing phytochelatin (PC) content (Mahmud et al., 2018).

3.2. Soil heavy metal distribution

The modified BCR method divides the HMs in the soil into five forms, in which the water−soluble, acid−soluble, and reducible forms are readily bioavailable for absorption by plants than the last

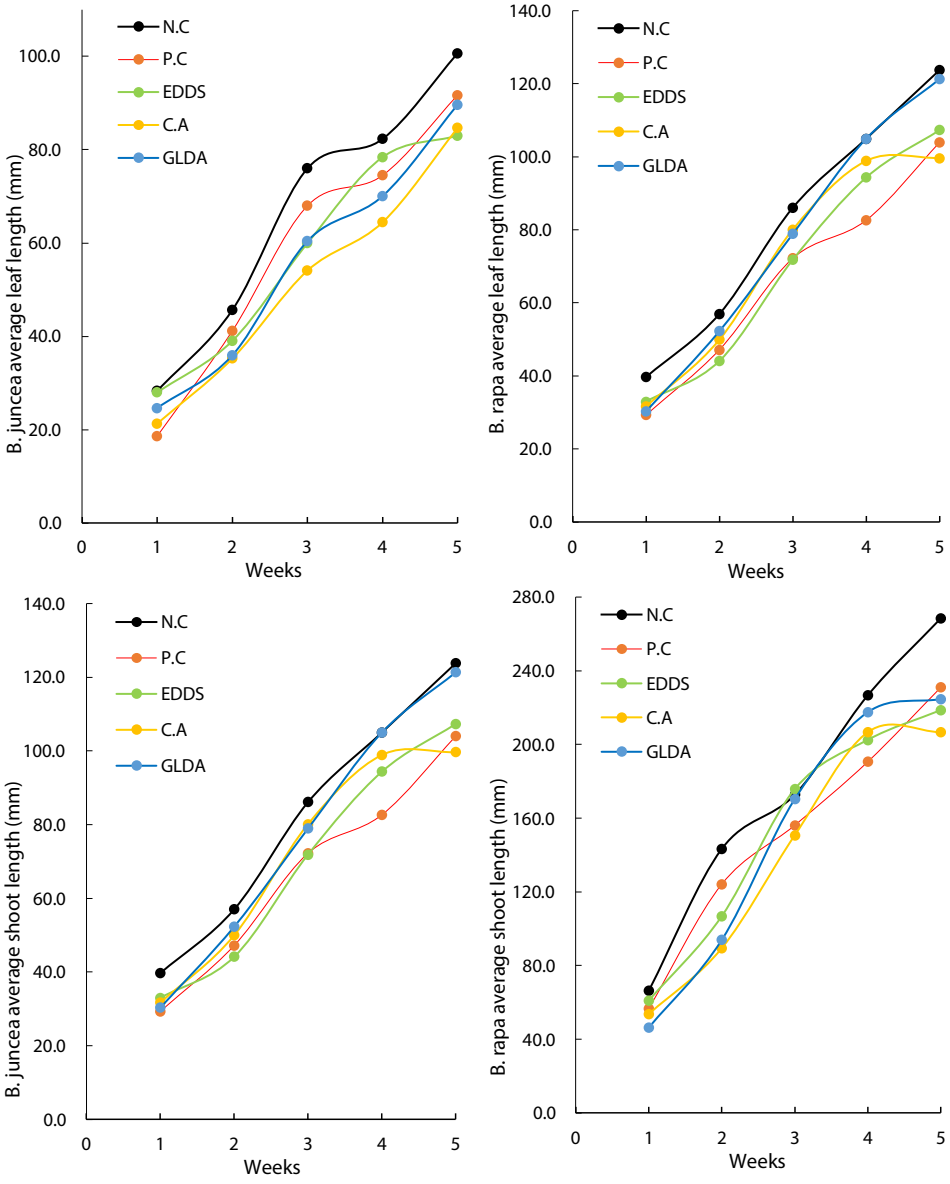


Fig. 2. Average leaf length in (A) *B. juncea* and (B) *B. rapa* during the growth period, Average shoot length in (C) *B. juncea* and (D) *B. rapa* during growth period. (NC: Negative control, PC: Positive control, EDDS: Ethylenediamine-N,N′-disuccinic acid, CA: Citric acid and GLDA: Tetrasodium N,N-Bis(carboxymethyl)-L-glutamate) acid).

two fractions (oxidizable and residual) which have less availability (Álvarez et al., 2002). Under natural conditions, the order of the various morphological contents is: reducible > oxidizable > resi dual > acid−soluble > water−soluble forms (Rauret et al., 1999). Generally, HMs in the unspiked soil followed the above order with both the water−soluble and acid−soluble fractions constituting <20%, whereas in the HM−spiked soil, bioavailability increased and both fractions made up ≥ 40%. HMs in uncontaminated soils and sediments are mainly immobile as they are bound to silicates and to primary minerals, while HMs in contaminated samples are bound to other phases and as such, have greater mobility (Rauret, 1998).

Fig. 3 provides the average percentages of HM fractions obtained by means of BCR sequential extraction method. Significant mobilization in soil HM fractions were achieved following the application of EDDS, CA and GLDA. Among the three chelators, EDDS provided the highest mobilization of Pb and Cd (87.2% and 70.2%), while GLDA mobilized the highest fractions of Cr and Zn (72.1% and 62.4%). In sequential extractions, EDDS has been shown to extract HMs almost exclusively from the exchangeable, mobile, and Mn−oxide fractions (Tandy et al., 2004). The present results showed that CA application released the highest soil fractions of Cu and Ni at 75.5% and 79.5%, respectively. This is corroborated by Wuana et al. (2010) who reported significantly high degrees of decontamination for Cu and Ni by batch soil washing with CA. Fractionation patterns have revealed that CA preferably targets HMs associated with the exchangeable and reducible fractions, and, to a lesser extent, part of HMs bound to the SOM; while recording little or no effect on the redistribution of the residual HM forms (Wuana et al., 2010).

3.3. Plant metal uptake

3.3.1. Cr uptake

Generally, Cr uptake in the unspiked soil was low owing to the limited bioavailable fractions in the soil matrix. Cr is a non−essential plant nutrient and therefore is not required for most

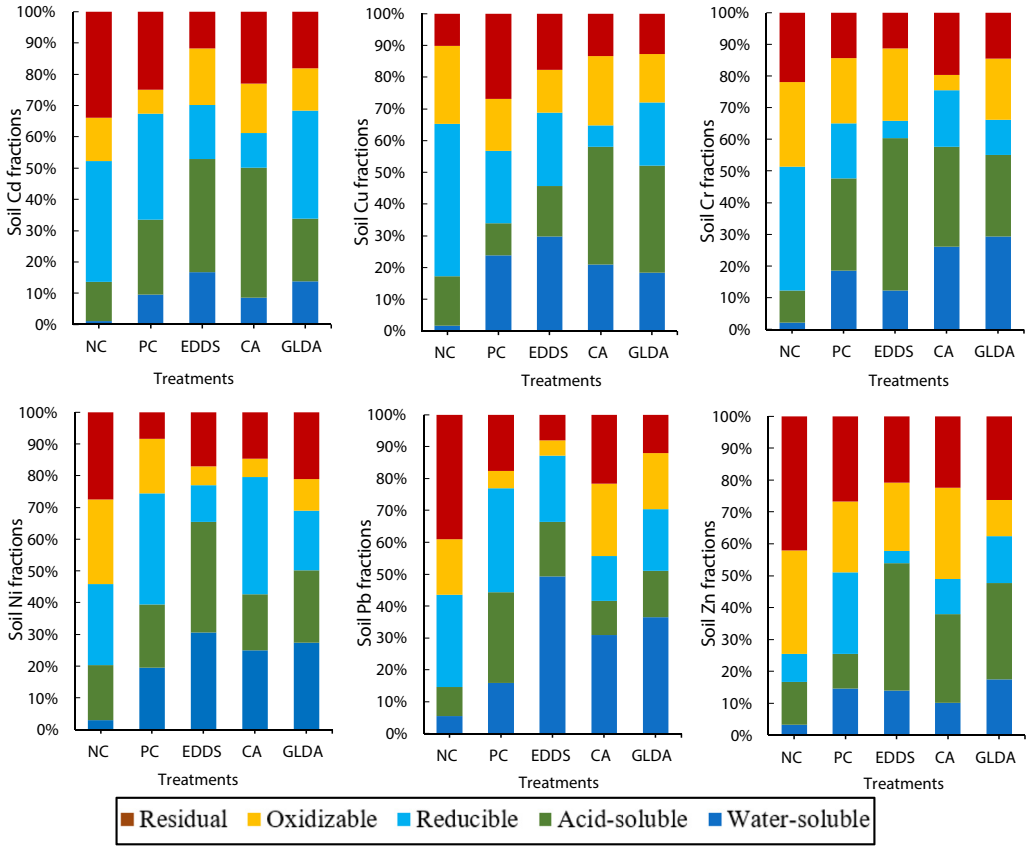


Fig. 3. Heavy metal fractions in the experimental soil following BCR sequential extraction.

plant processes. It is widely reported that Cr phytotoxicity occurs in plants even in minute concentrations (Ghani et al., 2017). Significant Cr extraction occurred in both *B. juncea* and *B. rapa* in HM−spiked soil (51.3 and 54.2 mg kg^{-1}, respectively), (Fig. 4A). *B. juncea* recorded the highest average Cr in tissues with a total of 154 mg kg^{-1} followed by *B. rapa* with 137.5 mg kg^{-1}. However, the translocation of this HM was poor in both plants, with twice as much Cr accumulated in plant roots compared to shoots. *B. juncea* accumulated an average of 42.2 mg kg^{-1} of Cr in shoots, whereas *B. rapa* had 37.4 mg kg^{-1} in shoots. Cr does not have any specific transporter for its uptake by plants and primarily enters the plants through specific and non−specific channels of essential ions and is therefore accumulated predominantly in plant root tissues with very limited translocation to shoots (Shahid et al., 2017). While chelator application significantly increased Cr accumulation in both plants, there was a statistical difference between all treatments except between EDDS and GLDA ($P = 0.237$). CA induced the highest average accumulation of Cr in plants (215.3 mg kg^{-1}), while GLDA and EDDS induced Cr uptake of 201.6 and 197.7 mg kg^{-1}, respectively. While some studies on the *Brassica* family have reported that CA application did not induce any significant increase in Cr uptake (Quartacci et al., 2006), others have reported similar trends to the present study (Afshan et al., 2015).

3.3.2. Zn uptake

Zn accumulation in *B. juncea* and *B. rapa* are shown in Fig. 4B. While relatively lower Zn accumulation occurred in the unspiked treatments, significant uptake was recorded in the spiked treatments ($P < 0.05$) indicating that uptake increased with soil bioavailability period. Both plants performed almost identically in terms of Zn accumulation with shoot and root Zn concentrations of 77.7 and 170 mg kg^{-1} in *B. juncea* and 77.4 and 173 mg kg^{-1} in *B. rapa*, respectively. It is well established that plants in the *Brassicaceae* family are very tolerant of Zn (Feigl et al., 2016); however, Zn accumulation in *B. juncea* was far below the values earlier reported (do Nascimento et al., 2006; Purakayastha et al., 2008). Numerous studies have reported higher accumulation of Zn in root structures when compared to aboveground biomass (Feigl et al., 2016; Murtaza et al., 2017). While all chelating agents increased Zn accumulation, significant differences in uptake pattern between plant, plant parts and chelators were observed. In *B. juncea*, EDDS and CA performed marginally better compared to GLDA. Average plant Zn concentrations induced by CA and EDDS was 166.2 and 172.0 mg kg^{-1}, respectively, largely accumulated in the plant roots. In *B. rapa*, however, GLDA and EDDS treatments induced relatively higher root and shoot Zn concentrations (168.4 mg kg^{-1}) compared to CA (153.0 mg kg^{-1}). This is likely as a result of the stress suffered by plants under EDDS and CA treatments, which impacted plant biomass and thus, reduced HM uptake. This observation suggests an antagonism and competition between Zn and other HMs not at the entry point in roots, but probably later during xylem loading/unloading, restricting the translocation of Zn to aerial parts. Zn is known to interact with several soil properties such as pH, as well as with soil micro and macronutrient supply (Loneragan and Webb, 1993). These interactions have likely contributed to the depression of Zn absorption by roots and translocation to shoots.

3.3.3. Cd uptake

As represented in Fig. 4C, average Cd uptake was particularly low in unspiked soil (1.2 mg kg^{-1}) due to low Cd bioavailability, however this significantly increased to 42.74 mg kg^{-1} after HM spiking. The average tissue concentrations were 55.1 and

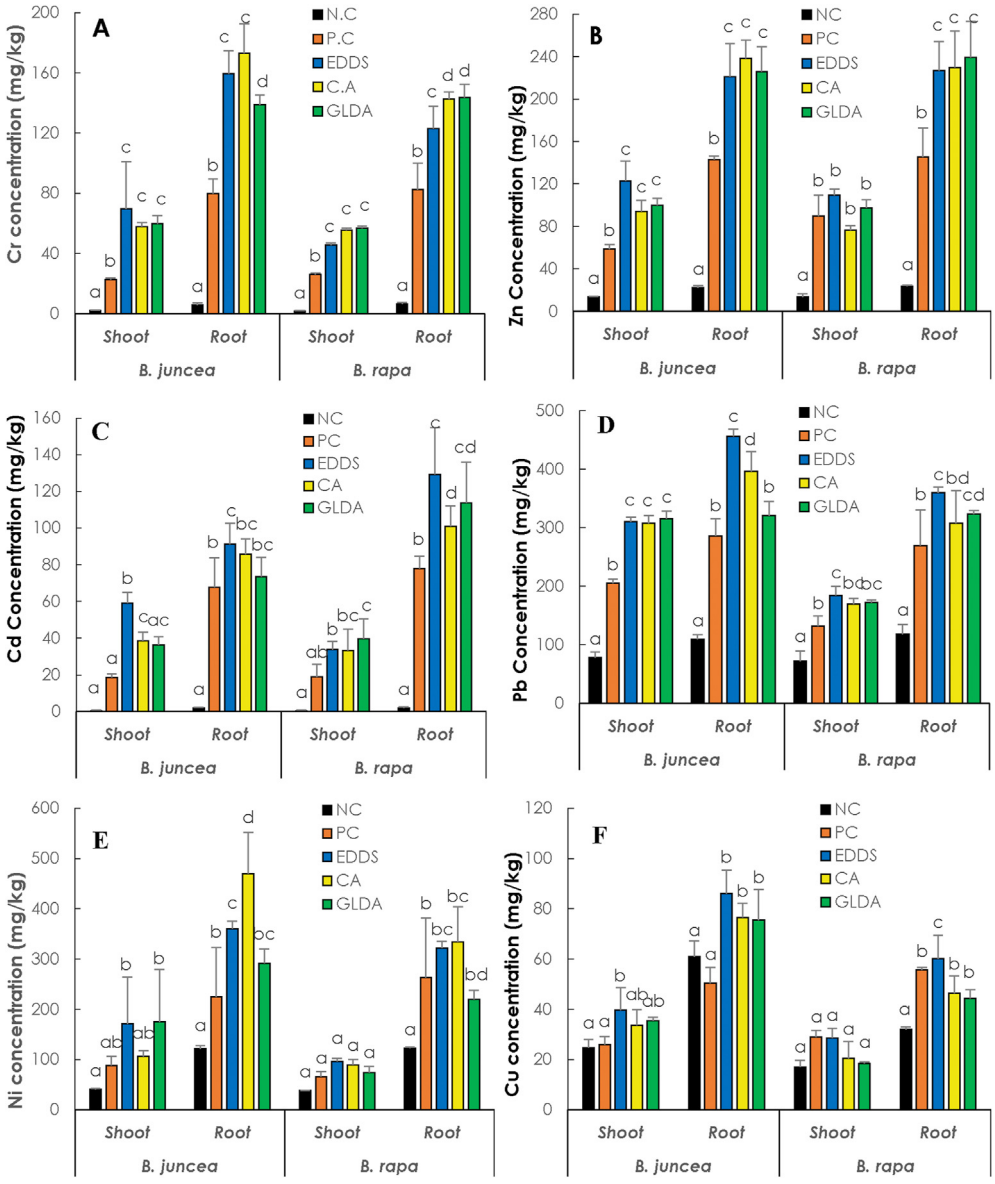


Fig. 4. Mean metal concentrations (mg kg^{-1}) in the shoot and root of *B. juncea* and *B. rapa* at harvest. Error bars represent standard deviation (n = 3). Different letters denote significant difference between treatments ($P < 0.05$).

47.3 mg kg^{-1} for *B. rapa* and *B. juncea*, respectively. Most of the Cd uptake occurred in plant roots with less than 50% translocation in both plants (Table 5). Shoot concentration was limited to only 25.3 mg kg^{-1}. Cd was highest in *B. juncea* shoots, with an average concentration of 30.6 mg kg^{-1}, exceeding *B. rapa*. This is corroborated in a hydroponic study by Nouairi et al. (2006) which observed that *B. juncea* accumulated more Cd in root structures compared to the shoot. Although observations on chelating agents was inconsistent, it was clear that EDDS induced the highest uptake in both *Brassica* spp. The total Cd uptake in *B. juncea* was 150.99 mg kg^{-1} and 162 mg kg^{-1} in *B. rapa*. CA was second only to EDDS with Cd accumulation increasing by 45.1% and 36.4% in *B. juncea* and *B. rapa* plants, respectively. Contrary to results published by Wang et al. (2019), GLDA showed the lowest affinity for Cd among all three chelating agents. In *B. juncea*, GLDA did not significantly increase Cd concentrations, when compared to the control. However, in *B. rapa* plants, GLDA was second only to EDDS, inducing an uptake of 152.1 mg kg^{-1}. Several reports suggest that *B. juncea* is capable of accumulating high levels of Cd within its shoots from soil or hydroponic solution (Salt et al., 1995). In *B. juncea*, Cd has been found to accumulate preferentially in the trichomes of younger leaf surfaces. The storage of Cd in trichomes may represent a detoxification mechanism, since trichomes represent an external tissue to the leaf (Nouairi et al., 2006).

3.3.4. Pb uptake

Pb accumulation in plants increased from 94.4 mg kg^{-1} to 222.8 mg kg^{-1}, representing an increase of 136%. While Pb was well accumulated by both plant species, *B. rapa* translocated significantly less Pb than *B. juncea* ($P < 0.05$). Pb is highly immobile in soil since it readily forms a precipitate because of its low aqueous solubility within the soil matrix. In addition, many plants retain Pb in their roots via sorption and precipitation with only minimal transport to the above ground harvestable plant portions (Jiang et al., 2000; Liu et al., 2000). Pb availability is also affected by the presence of other HMs and has been shown to decline in the

Table 5
Pearson correlation coefficient for selected parameters in *Brassica juncea* and *Brassica rapa* (n = 15).

B. juncea	$Shoot_{DM}$	$Root_{DM}$	$Shoot_{Cr}$	$Root_{Cr}$	$Shoot_{Zn}$	$Root_{Zn}$	$Shoot_{Cd}$	$Root_{Cd}$	$Shoot_{Pb}$	$Root_{Pb}$	$Shoot_{Ni}$	$Root_{Ni}$	$Shoot_{Cu}$	$Root_{Cu}$
$Shoot_{DM}$	1.00													
$Root_{DM}$	.594[b]	1.00												
$Shoot_{Cr}$	-.491[a]	-.06	1.00											
$Root_{Cr}$	-.612[b]	.01	.918[b]	1.00										
$Shoot_{Zn}$	-.614[b]	-.08	.898[b]	.935[b]	1.00									
$Root_{Zn}$	-.585[a]	.05	.884[b]	.974[b]	.943[b]	1.00								
$Shoot_{Cd}$	-.622[b]	-.11	.875[b]	.888[b]	.966[b]	.860[b]	1.00							
$Root_{Cd}$	-.665[b]	.00	.831[b]	.944[b]	.915[b]	.943[b]	.862[b]	1.00						
$Shoot_{Pb}$	-.596[b]	-.04	.911[b]	.967[b]	.949[b]	.984[b]	.879[b]	.938[b]	1.00					
$Root_{Pb}$	-.667[b]	-.03	.856[b]	.918[b]	.928[b]	.903[b]	.916[b]	.943[b]	.909[b]	1.00				
$Shoot_{Ni}$	-.474[a]	-.21	.723[b]	.638[b]	.727[b]	.695[b]	.687[b]	.572[a]	.686[b]	.588[a]	1.00			
$Root_{Ni}$	-.537[a]	-.07	.734[b]	.852[b]	.741[b]	.772[b]	.687[b]	.780[b]	.757[b]	.743[b]	.41	1.00		
$Shoot_{Cu}$	-.580[a]	-.24	.627[b]	.652[b]	.690[b]	.653[b]	.710[b]	.579[a]	.666[b]	.654[b]	.504[a]	.30	1.00	
$Root_{Cu}$	-.44	-.23	.774[b]	.688[b]	.715[b]	.627[b]	.752[b]	.513[a]	.678[b]	.682[b]	.542[a]	.621[b]	.647[b]	1.00

B. rapa	$Shoot_{DM}$	$Root_{DM}$	$Shoot_{Cr}$	$Root_{Cr}$	$Shoot_{Zn}$	$Root_{Zn}$	$Shoot_{Cd}$	$Root_{Cd}$	$Shoot_{Pb}$	$Root_{Pb}$	$Shoot_{Ni}$	$Root_{Ni}$	$Shoot_{Cu}$	$Root_{Cu}$
$Shoot_{DM}$	1.00													
$Root_{DM}$	-.026	1.00												
$Shoot_{Cr}$	-.464[a]	.315	1.00											
$Root_{Cr}$	-.508[a]	.399	.981[b]	1.00										
$Shoot_{Zn}$	-.511[a]	.599[b]	.739[b]	.821[b]	1.00									
$Root_{Zn}$	-.503[a]	.346	.943[b]	.923[b]	.790[b]	1.00								
$Shoot_{Cd}$	-.393	.394	.899[b]	.915[b]	.816[b]	.903[b]	1.00							
$Root_{Cd}$	-.535[a]	.376	.882[b]	.893[b]	.879[b]	.883[b]	.846[b]	1.00						
$Shoot_{Pb}$	-.487[a]	.276	.899[b]	.902[b]	.860[b]	.922[b]	.868[b]	.912[b]	1.00					
$Root_{Pb}$	-.545[a]	.404	.833[b]	.887[b]	.936[b]	.820[b]	.804[b]	.899[b]	.916[b]	1.00				
$Shoot_{Ni}$	-.640[b]	.074	.841[b]	.835[b]	.747[b]	.891[b]	.766[b]	.871[b]	.905[b]	.831[b]	1.00			
$Root_{Ni}$	-.706[b]	.272	.649[b]	.744[b]	.755[b]	.676[b]	.580[a]	.708[b]	.695[b]	.781[b]	.801[b]	1.00		
$Shoot_{Cu}$	-.193	-.019	-.406	-.338	.125	-.206	-.288	-.119	-.043	-.005	.027	.125	1.00	
$Root_{Cu}$	.230	-.36	-.707[b]	-.692[b]	-.330	-.541[a]	-.545[a]	-.485[a]	-.394	-.397	-.310	-.333	.776[b]	1.00

$Shoot_{DM}$ = Shoot dry matter; $Root_{DM}$ = Root dry matter; $Shoot_{Cr}$ = Cr concentration in shoot; $Root_{Cr}$ = Cr concentration in root; $Shoot_{Zn}$ = Zn concentration in shoot; $Root_{Zn}$ = Zn concentration in root; $Shoot_{Cd}$ = Cd concentration in shoot; $Root_{Cd}$ = Cd concentration in root; $Shoot_{Pb}$ = Pb concentration in shoot; $Root_{Pb}$ = Pb concentration in root; $Shoot_{Ni}$ = Ni concentration in shoot; $Root_{Ni}$ = Ni concentration in root; $Shoot_{Cu}$ = Cu concentration in shoot; $Root_{Cu}$ = Cu concentration in root.
[a] Correlation is significant at the 0.05 level.
[b] Correlation is significant at the 0.01 level.

presence of other HMs (Cd, Zn, Cr, Cu, and Ni), however, in ternary combination (with Zn and Cu), its availability increases due to the antagonistic interaction between Cu and Zn (Orroño et al., 2012). Although the application of CA and GLDA both induced higher roots to shoot Pb translocation, EDDS induced the highest Pb uptake in both plants, with average tissue concentrations of 245.3 and 200.3 mg kg^{-1}. Pb uptake in *B. juncea* and *B. rapa* shoots, although significantly exceeded the control, did not significantly differ in the CA and GLDA treatments. The CA treatment also displayed significant Pb uptake with a 26.1% increase over the control. CA decreases soil pH, which increases the mobile form of Pb by mainly mobilizing the acid–soluble fraction, which corresponds to the Pb bound to carbonates. Similarly, GLDA induced high Pb accumulation with an average of 663.11 mg kg^{-1} in plants. In *B. juncea*, GLDA increased shoot Pb concentration by 52.2% and root concentration by 11.4% while in *B. rapa*, the increase was 28.0% and 20.3% for shoots and roots, respectively. The high Pb accumulation particularly in shoots can be attributed to the fact that chelating agents at threshold concentrations overcome the physiological barrier(s) in roots that normally function to control the uptake and the translocation of solutes.

3.3.5. Ni uptake

The concentration of Ni in the roots and shoots of the harvested plants is provided in Fig. 4E. The average uptake in the unspiked soil was about 80.65 mg kg^{-1}, while in the spiked treatment, the average concentration reached 160.40 mg kg^{-1}, an increase of 99%. The mean Ni concentrations in shoots and roots were 116.2 and 293.5 mg kg^{-1} for *B. juncea* and 72.6 and 252.2 mg kg^{-1} for *B. rapa*, respectively. The This is in contrast to some studies that have reported *B. rapa* as a Ni hyperaccumulator with concentrations >1000 mg kg^{-1} in both roots and shoots (Delil, 2017). *B. rapa* accumulated 71% more Ni in roots compared to shoots, whereas *B. juncea* exhibited a higher root concentration of up to 39%. In a similar study with *Brassica* spp., Panwar et al. (2002) established that although *B. juncea* was tolerant to Ni, root to shoot translocation seemed to be restricted both by high Ni concentration and similarly by the application of chelating agents. We observed that chelate application generally increased Ni uptake in both the roots and shoots of all plants. CA was the most effective ligand for Ni mobilization and accumulation with an average of 249.8 mg kg^{-1} followed closely by EDDS at 237.3 mg kg^{-1}. Ni accumulation was relatively poor under GLDA treatment with an average value of 190.0 mg kg^{-1}. It is worth mentioning that the average uptake of Ni in *B. rapa* declined by 12% due to GLDA application. It has been reported that GLDA possesses excellent protonation and complexation characteristics for Ni (Begum et al., 2012), however, our observations showed that it did not effectively increase Ni uptake in *Brassica* plants.

3.3.6. Cu uptake

The uptake of Cu by *B. juncea* and *B. rapa* plants is shown in Fig. 4F. Cu uptake was relatively high for all plants in the unspiked soil treatment, considering that the bioavailable soil Cu fraction was 72.58 mg kg^{-1}. The average plant Cu concentration in the unspiked treatment was 67.3 mg kg^{-1}, while in the HM–spiked treatment, this value increased to 80.5 mg kg^{-1}. In *B. juncea* plants, HM uptake declined between the unspiked and HM–spiked treatments. This contradicts the findings of previous studies including (Ultra et al., 2005; Turan and Esringü, 2018), which have all reported an

increase in Cu accumulation with increasing soil concentration. However, a decline in plant biomass and HM phytotoxicity are both known to affect Cu uptake (Ebbs and Kochian, 1997; Cook et al., 1998). Amongst all the HMs in this study, Cu has the strongest covalent bonds with oxygen atoms on any particular mineral (McBride et al., 1994), and thus, difficult to desorb into the soil solution. This may have restricted the bioavailability of Cu which had the lowest BCF values among all HMs in this study. Additionally, Murtaza et al. (2017) found that at higher Cd loads, the uptake and accumulation of Zn and Cu were inhibited. Therefore, the decline in Cu uptake may be caused by the synergistic effects of the spiked HMs on the plant. As observed by Kutrowska et al. (2017), the presence of HMs altered the distribution of micronutrients in plants by lowering Cu concentration in plants and increasing Zn uptake by several folds. Although Cu uptake was generally increased by chelating agents, great variability was observed between plants, plant parts (shoots and roots), and chelating agents. It appears that all chelating agents alleviated the stress which affected *B. juncea* plants in the HM−spiked treatments. However, the current results showed that the chelating ligands did not significantly increase Cu accumulation in both plants.

3.4. Phytoextraction efficiency of metals by B. juncea and B. rapa

The bioconcentration factors (BCF) and translocation factors (TF) for *B. juncea* and *B. rapa* are presented in Table 4. BCF has been classified into three categories by Baker (1981) as follows: Plants with a BCF of less than 1 are considered excluders, between 1 and 10 are accumulators and those with values above 10 are called hyperaccumulators. Plant micronutrients including Cu, Fe, Ni and Zn play essential roles in plant biochemical processes and as such, are well tolerated and accumulated in plant. Therefore, the levels of accumulated HMs significantly differ for essential and non−essential metals in control plants, with the latter occurring at very low levels. The average HM BCF values in decreasing order were Ni > Cd > Zn > Pb > Cu > Cr for both plants. The average BCF in *B. juncea* plants was above 1 for all HMs except Cr, whereas in *B. rapa*, only the average BCF values for both Cr and Cu were below 1. The low bioaccumulation of Cr is not surprising as Cr is considered an immobile element in soils, particularly when abundant trivalent Cr exists in chromite and Fe oxides (Ertani et al., 2017). Similarly, in the HM−spiked treatment, the BCF values for Cr, Zn, and Cd increased, while the values for Pb, Ni, and Cu decreased in both plants. We observed a decline in Ni and Cu bioconcentration in treatments with higher HM concentrations. Amongst the HMs, BCF values for Ni and Cd were higher with average values of 3.4 and 2.3 in *B. juncea*, and 2.8 and 2.6 in *B. rapa*, respectively. The application of chelating agents led to a significant increase in BCF values for all HMs, except Cu. The effect of chelators on enhancing accumulation capacity was in the following order: EDDS > CA > GLDA. Among the six HMs in this study, four (Zn, Cd, Pb, and Cu) had the highest BCF values in EDDS treatment, while two (Cr and Ni) were the highest in the CA treatment. This is highly consistent with the study by Zhao et al. (2015) which reported that 5 mmol kg^{-1} EDDS treatment induced the highest BCF values for Cu (13.26), Cd (3.31), Pb (1.64), and Zn (6.1) compared to EDTA. BCF values in GLDA treatment, although generally lower than in the EDDS and CA treatments, were generally above 1. In fact, in *B. rapa* plants, GLDA treatment had the highest BCF values for Cr and Zn.

The root to shoot mobility (translocation) of all studied HMs was generally < 50%, except for Pb and Zn (Table 4). The average TF values for both plants in decreasing order were; Pb > Zn > Cu > Cr > Ni > Cd. Both plants showed poor translocation of Cd and Ni to plant aboveground structures. The HM−spiked treatments showed no significant difference in TF values compared to the unspiked treatments. The effect of chelators on increasing HM translocation was negligible. However, among the three chelators, GLDA was found to possess the most potential for HM translocation. While EDDS and GLDA minutely increased HM translocation in *B. juncea* plants, there was no discernible increase in *B. rapa* plants. Soluble chemical elements can enter roots either via cell−wall free space (apoplastic pathway) or by transport, across the plasma membrane of root cells and movement through the cytoplasm (symplastic pathway) (Goswami and Das, 2015). Numerous studies have established that roots act as a barrier for HM translocation and protect stem and other aerial plant parts from HM contamination to reduce oxidative stress (Panwar et al., 2002; Liu et al., 2019). The differences in the HM concentrations of the plant parts suggest different cellular mechanisms of bioaccumulation that may manage and control their translocation and partitioning in plant systems (Sinha et al., 2007; Sharma and Dietz, 2009). In addition, HM uptake and translocation to shoots is intrinsically linked to the element speciation, soil pH and SOM (Kabata-Pendias, 2010).

The observed HM concentrations in both *Brassica* spp. in this study were below the values reported by previous studies (Blaylock et al., 1997; Gisbert et al., 2006; Delil, 2017). This can be attributed to both the stress suffered by plants under several HM which produce both antagonistic and synergistic effects, as well as the moderate levels of HMs applied in this study. Perhaps most consequential, the neutral soil pH and relatively high SOM content of the present experiment did not favour soil HM solubilisation and may have limited plant accumulation. It is well established that at high pH, HMs tend to form insoluble metal mineral phosphates and carbonates, whereas at low pH they tend to be found as free ionic species or as soluble organometals and are more bioavailable (Olaniran et al., 2013).

3.5. Comparison of heavy metal uptake by chelating agents

Chelating agents can enhance absorption, translocation, and accumulation of HMs in plants through enhanced desorption of HMs from the soil matrix to the soil solution, increasing bioavailability and mobility, facilitating HM transport into the xylem and hence, increasing HM translocation from roots to shoots (Song et al., 2005; Tandy et al., 2006; Evangelou et al., 2007). In the present study, the application of chelating agents generally increased the bioavailability and hence, accumulation of HMs by plants. However, chelating agents displayed variability in the type of HM extracted and the level of extraction. It is important to note the application rate in the present study; CA was applied at the highest concentration of 10 mmol kg^{-1} of soil, followed by EDDS and GLDA at 5 mmol kg^{-1} and 3 mmol kg^{-1} of soil, respectively. EDDS applied at 5 mmol kg^{-1} proved to be the most effective chelating agent, inducing the highest accumulation of all but one HM in this study (Cr). Similar investigations have reported that EDDS resulted in an increase in the uptake of Pb (Cheng et al., 2012), Cu (Ultra et al., 2005), Zn (Fässler et al., 2010), and Cd (Wang et al., 2019). In fact, for all HMs except Cu and Cr in both plants, the average BCF in EDDS treatment was >2.5. Although CA was applied at the highest concentration of all chelating agents (10 mmol kg^{-1}), it displayed high BCF for Cr alone. Even though CA was second to EDDS in terms of Pb, Zn, Ni, Cd, and Cu, BCF values were still high enough for CA to be considered efficient. CA is advantageous because of its organic nature, ensuring it biodegrades fast in the environment. Additionally, CA has a moderate impact on growth parameters while still inducing significant HM uptake in hyperaccumulators. HM phytoextraction with GLDA revealed that this ligand was highly suited for Cr but not effective for the other HMs

under the current conditions. However, it must be noted that GLDA was applied at the lowest concentration of all three chelators (3 mmol kg^{-1}) and may perform equally or better at similar concentrations to EDDS and CA. Nonetheless, phytoextraction studies using GLDA have shown that concentrations of 2.5–3.0 mmol kg^{-1} provided optimum results in terms of HM accumulation and phytotoxicity (Yuan et al., 2016; Wang et al., 2019). Low concentrations of chelators can mitigate HM toxicity in plants, however; high levels can significantly increase HM ions in the soil solution, which in turn induces severe stress in plants (do Nascimento et al., 2006; Epelde et al., 2008). According to Wei et al. (2015), low concentrations (2.5 mmol kg^{-1}) GLDA promoted biomass production by *S. alfredii*, whereas high concentrations (10 mmol kg^{-1}) inhibited growth. Despite the slightly improved the mobility of selected HMs in plants, translocation remained low and most HMs were predominantly accumulated in the roots of the plants. The average increase in HM concentration of shoots, although concurrent with previous studies exploring chelate-induced phytoextraction (Meers et al., 2005a; Zeremski-Škorić et al., 2010; Bouquet et al., 2017), is insufficient to be considered effective in realistic phytoremediation programmes.

3.6. Pearson correlation analysis

The correlation coefficients (r) among plant variables, including shoot DM, root DM, shoot and root HM contents for both *B. juncea* and *B. rapa* are provided in Table 5. In *B. juncea* plants, shoot DM was positively correlated ($P < 0.01$) with root DM, revealing that both parameters decreased in the presence of HMs and chelating agents, whereas in *B. rapa*, there was no clear correlation between these parameters ($r = -0.026$). Additionally, shoot DM in both plants showed a significant negative correlation with HM concentrations, except for Cu. Several studies have also reported an inverse relationship between DM and HM concentrations (Sridhar et al., 2005; Guo et al., 2019). However, no significant relationship existed between root DM and HM uptake in both plants, except for *B. rapa* root DM, which showed a strong positive correlation with shoot Zn uptake. Excluding a few exceptions, root and shoot HM concentrations in both plant species showed strong positive correlations. The major exception was Cu concentration in *B. rapa*, which showed significant negative correlations with other HM concentrations. This is corroborated by Kutrowska et al. (2017) which reported that Cu interacts and sometimes competes with Cd, Pb and Zn both at uptake and during translocation to root. Such HM interactions are limiting factors which can affect the tolerance, bioconcentration and translocation of HMs by plants. Consistent with the findings of previous research (Tandy et al., 2004; Kutrowska et al., 2017), these correlations suggest possible competition and synergies between the HMs.

4. Conclusion

The findings of the present study validate that the application of biodegradable chelating agents increases heavy metal (HM) phytoextraction in *Brassica* plants. The addition of HMs (Cd, Cr, Cu, Ni, Pb, and Zn) significantly declined growth parameters including leaf length, shoot length, photosynthetic pigments, and dry matter. EDDS at 5 mmol kg^{-1} had a significant effect on the growth of all plants, while the effect of CA at 10 mmol kg^{-1} although significant, was milder. In contrast, GLDA at 3 mmol kg^{-1} enhanced plant growth and biomass production. Chelating agents increased the bioavailable soil HM fractions in the order EDDS > GLDA > CA. Among the plant species, *B. juncea* showed a higher affinity for a wider range of HMs compared to *B. rapa* and displayed relatively high bioconcentration of several HMs even in the absence of chelating agents. Additionally, HM concentrations in the roots were found to always exceed those in the shoots, with a lower translocation from roots to shoots (<1), suggesting a strategy of these plants to compartmentalize the potentially toxic elements in physiologically fewer active parts in order to preserve younger tissues. The results of HM extraction with chelating agents revealed that EDDS had the strongest potential for HM extraction, inducing the highest uptake of Pb, Zn, Ni, Cd, and Cu. In comparison, CA and GLDA induced the highest accumulation of Cr. The results showed that EDDS provided the best phytoextraction potential especially in a multi–metal soil setting. However, it is yet to be established whether EDDS and GLDA could be used to improve HM accumulation *in situ*. In addition, the degradation of GLDA and its effects on soil microbial community is worth exploring.

Credit author statement

Ivan Diarra, Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - original draft, preparation, Visualization, Project administration. Krishna Kotra, Supervision, Writing - review & editing. Surendra Prasad, Supervision, Resources, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are grateful to The University of the South Pacific for funding this research through the research office (Vote code: FUND F3216 ORG FST12).

References

Afshan, S., Ali, S., Bharwana, S.A., Rizwan, M., Farid, M., Abbas, F., Ibrahim, M., Mehmood, M.A., Abbasi, G.H., 2015. Citric acid enhances the phytoextraction of chromium, plant growth, and photosynthesis by alleviating the oxidative damages in Brassica napus L. Environ. Sci. Pollut. Res. Int. 22, 11679–11689. https://doi.org/10.1007/s11356-015-4396-8.

Álvarez, E.A., Mochón, M.C., Sánchez, J.C.J., Rodrıguez, M.T., 2002. Heavy metal extractable forms in sludge from wastewater treatment plants. Chemosphere 47, 765–775. https://doi.org/10.1016/S0045-6535(02)00021-8.

Anjum, N.A., Ahmad, I., Pereira, M.E., Duarte, A.C., Umar, S., Khan, N.A. (Eds.), 2012. The Plant Family Brassicaceae: Contribution towards Phytoremediation. Springer Netherlands.

Arikibe, J.E., Prasad, S., 2020. Determination and comparison of selected heavy metal concentrations in seawater and sediment samples in the coastal area of Suva. Fiji. Mar. Pollut. Bull. 157 (111157) https://doi.org/10.1016/j.marpolbul.2020.111157.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in beta vulgaris. Plant Physiol. 24, 1–15. https://doi.org/10.1104/pp.24.1.1.

Baker, A.J.M., 1981. Accumulators and excluders -strategies in the response of plants to heavy metals. J. Plant Nutr. 3, 643–654. https://doi.org/10.1080/01904168109362867.

Barona, A., Aranguiz, I., Elías, A., 2001. Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. Environ. Pollut. 113, 79–85. https://doi.org/10.1016/s0269-7491(00)00158-5.

Begum, Z.A., Rahman, I.M.M., Tate, Y., Egawa, Y., Maki, T., Hasegawa, H., 2012. Formation and stability of binary complexes of divalent ecotoxic ions (Ni, Cu, Zn, Cd, Pb) with biodegradable aminopolycarboxylate chelants (dl-2-(2-Carboxymethyl)Nitrilotriacetic acid, GLDA, and 3-hydroxy-2,2′-iminodisuccinic acid, HIDS) in aqueous solutions. J. Solut. Chem. 41, 1713–1728. https://doi.org/10.1007/s10953-012-9901-9.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, B.D., Raskin, I., 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol. 31, 860–865. https://doi.org/10.1021/es960552a.

Bouquet, D., Braud, A., Lebeau, T., 2017. Brassica juncea tested on urban soils

moderately contaminated by lead: origin of contamination and effect of chelates. Int. J. Phytoremediation 19, 425–430. https://doi.org/10.1080/15226514.2016.1244160.
Brynhildsen, L., Rosswall, T., 1997. Effects of metals on the microbial mineralization of organic acids. Water Air Soil Pollut. 94, 45–57. https://doi.org/10.1007/BF02407092.
Chandra, S., Gangaiya, P., Togamana, C., Prasad, S., 2015. First screening study of metal content in soil from a mixed waste receptacle. S. Pac. J. Nat. Appl. Sci. 33, 7–12.
Cheng, G.L., Ma, X.F., Sun, X.B., Zhao, S.Q., 2012. Effects of EDTA, EDDS and citric acid on growth of maize and uptake of lead by maize in contaminated soil. Adv. Mater. Res. 534, 277–280. https://doi.org/10.4028/www.scientific.net/AMR.534.277.
Chibuike, G.U., Obiora, S.C., 2014. Heavy metal polluted soils: effect on plants and bioremediation methods. Appl. Environ. Soil Sci. 2014 (12) https://doi.org/10.1155/2014/752708.
Cook, C.M., Kostidou, A., Vardaka, E., Lanaras, T., 1998. Effects of copper on the growth, photosynthesis and nutrient concentrations of Phaseolus plants. Photosynthetica 34, 179–193. https://doi.org/10.1023/A:1006832321946.
Corley, M., Mutiti, S., 2017. The effects of lead species and growth time on accumulation of lead in Chinese cabbage. Glob. Chall 1 (1600020). https://doi.org/10.1002/gch2.201600020. http://onlinelibrary.wiley.com/doi/10.1002/gch2.201600020/abstract.
del Mundo Dacera, D., Babel, S., 2006. Use of citric acid for heavy metals extraction from contaminated sewage sludge for land application. Water Sci. Technol. 54, 129–135. https://doi.org/10.2166/wst.2006.764.
Delil, A.D., 2017. Chelate-induced phytoextraction potential of Brassica rapa for soil contaminated with nickel. Eur. J. Eng. Nat. Sci. 2, 194–203.
Dewailly, E., Suhas, E., Mou, Y., Dallaire, R., Chateau-Degat, L., Chansin, R., 2008. High fish consumption in French Polynesia and prenatal exposure to metals and nutrients. Asia Pac. J. Clin. Nutr. 17, 461–470.
Diarra, I., Prasad, S., 2020. The current state of heavy metal pollution in Pacific Island Countries: a review. Appl. Spectrosc. Rev. 1–25. https://doi.org/10.1080/05704928.2020.1719130.
Ding, Y.Z., Song, Z.G., Feng, R.W., Guo, J.K., 2014. Interaction of organic acids and pH on multi-heavy metal extraction from alkaline and acid mine soils. Int. J. Environ. Sci. Technol. 11, 33–42. https://doi.org/10.1007/s13762-013-0433-7.
do Nascimento, C.W.A., Amarasiriwardena, D., Xing, B., 2006. Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil. Environ. Pollut. 140, 114–123. https://doi.org/10.1016/j.envpol.2005.06.017.
Ebbs, S., Kochian, L., 1997. Toxicity of zinc and copper to brassica species: implications for phytoremediation. J. Environ. Qual. 26, 776–781. https://doi.org/10.2134/jeq1997.00472425002600030026x.
Epelde, L., Hernández-Allica, J., Becerril, J.M., Blanco, F., Garbisu, C., 2008. Effects of chelates on plants and soil microbial community: comparison of EDTA and EDDS for lead phytoextraction. Sci. Total Environ. 401, 21–28. https://doi.org/10.1016/j.scitotenv.2008.03.024.
Ertani, A., Mietto, A., Borin, M., Nardi, S., 2017. Chromium in agricultural soils and crops: a review. Water Air Soil Pollut. 228 (190) https://doi.org/10.1007/s11270-017-3356-y.
Evangelou, M.W.H., Ebel, M., Schaeffer, A., 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68, 989–1003. https://doi.org/10.1016/j.chemosphere.2007.01.062.
Fässler, E., Evangelou, M.W., Robinson, B.H., Schulin, R., 2010. Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS). Chemosphere 80, 901–907. https://doi.org/10.1016/j.chemosphere.2010.04.077.
Feigl, G., Kolbert, Z., Lehotai, N., Molnár, Á., Ördög, A., Bordé, Á., Laskay, G., Erdei, L., 2016. Different zinc sensitivity of Brassica organs is accompanied by distinct responses in protein nitration level and pattern. Ecotoxicol. Environ. Saf. 125, 141–152. https://doi.org/10.1016/j.ecoenv.2015.12.006.
Förstner, U., 1995. Land contamination by metals: global scope and magnitude of problem. In: Metal Speciation and Contamination of Soil. Lewis Publishers, Germany, pp. 1–33.
Ghani, A., Hussain, M., Ikram, M., Yaqoob, M., Shaukat, R., Munawar, A., Ullah, R., Imtiaz, A., 2017. Effect of chromium toxicity on the growth and mineral composition of brown mustard (Brassica juncea l.). J. Multidiscip. Res. Dev. 3, 36–38.
Gisbert, C., Clemente, R., Navarro-Aviñó, J., Baixauli, C., Ginér, A., Serrano, R., Walker, D.J., Bernal, M.P., 2006. Tolerance and accumulation of heavy metals by Brassicaceae species grown in contaminated soils from Mediterranean regions of Spain. Environ. Exp. Bot. 56, 19–27. https://doi.org/10.1016/j.envexpbot.2004.12.002.
Goswami, S., Das, S., 2015. A study on cadmium phytoremediation potential of indian mustard, Brassica juncea. Int. J. Phytoremediation 17, 583–588. https://doi.org/10.1080/15226514.2014.935289.
Guo, D., Ali, A., Ren, C., Du, J., Li, R., Lahori, A.H., Xiao, R., Zhang, Ziyang, Zhang, Zengqiang, 2019. EDTA and organic acids assisted phytoextraction of Cd and Zn from a smelter contaminated soil by potherb mustard (Brassica juncea, Coss) and evaluation of its bioindicators. Ecotoxicol. Environ. Saf. 167, 396–403. https://doi.org/10.1016/j.ecoenv.2018.10.038.
Hart, J.R., 2011. Ethylenediaminetetraacetic acid and related chelating agents. In: Ullmann's Encyclopedia of Industrial Chemistry. American Cancer Society. https://doi.org/10.1002/14356007.a10_095.pub2.
Imo, T.S., Latu, F., Vaurasi, V., Yoshida, J., Amosa, P., Sheikh, M.A., 2014. Distribution of heavy metals in sediments at the commercial and fishing ports in Samoa. Int. J. Environ. Sustain Dev. 5, 517–521. https://doi.org/10.7763/ijesd.2014.v5.537, v5.537.
Jean, L., Bordas, F., Gautier-Moussard, C., Vernay, P., Hitmi, A., Bollinger, J.-C., 2008. Effect of citric acid and EDTA on chromium and nickel uptake and translocation by Datura innoxia. Environ. Pollut. 153, 555–563.
Jiang, W., Liu, D., Hou, W., 2000. Hyperaccumulation of lead by roots, hypocotyls, and shoots of Brassica juncea. Biol. Plant. (Prague) 43, 603–606. https://doi.org/10.1023/A:1002804100215.
Kabata-Pendias, A., 2010. Trace Elements in Soils and Plants. CRC Press. https://doi.org/10.1201/b10158.
Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I., Dumat, C., 2016. A comparison of technologies for remediation of heavy metal contaminated soils. J. Geochem. Explor. 182, 247–268. https://doi.org/10.1016/j.gexplo.2016.11.021.
Khan, A.H.A., Butt, T.A., Mirza, C.R., Yousaf, S., Nawaz, I., Iqbal, M., 2019. Combined application of selected heavy metals and EDTA reduced the growth of Petunia hybrida L. Sci. Rep. 9 (4138) https://doi.org/10.1038/s41598-019-40540-7. https://www.nature.com/articles/s41598-019-40540_7.
Kim, S.-H., Lee, I.-S., 2010. Comparison of the ability of organic acids and EDTA to enhance the phytoextraction of metals from a multi-metal contaminated soil. Bull. Environ. Contam. Toxicol 84, 255–259. https://doi.org/10.1007/s00128-009-9888-0.
Kołodyńska, D., 2013. Application of a new generation of complexing agents in removal of heavy metal ions from different wastes. Environ. Sci. Pollut. Res. Int. 20, 5939–5949. https://doi.org/10.1007/s11356-013-1576-2.
Kumar, A., Prasad, M.N.V., Sytar, O., 2012. Lead toxicity, defense strategies and associated indicative biomarkers in Talinum triangulare grown hydroponically. Chemosphere 89, 1056–1065. https://doi.org/10.1016/j.chemosphere.2012.05.070.
Kutrowska, A., Małecka, A., Piechalak, A., Masiakowski, W., Hanć, A., Barałkiewicz, D., Andrzejewska, B., Zbierska, J., Tomaszewska, B., 2017. Effects of binary metal combinations on zinc, copper, cadmium and lead uptake and distribution in Brassica juncea. J. Trace Elem. Med. Biol. 44, 32–39. https://doi.org/10.1016/j.jtemb.2017.05.007.
Lanigan, R.S., Yamarik, T.A., 2002. Final report on the safety assessment of EDTA, calcium disodium EDTA, diammonium EDTA, dipotassium EDTA, disodium EDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium EDTA, HEDTA, and trisodium HEDTA. Int. J. Toxicol. 21, 95–142. https://doi.org/10.1080/10915810290096522.
Lesage, E., Meers, E., Vervaeke, P., Lamsal, S., Hopgood, M., Tack, F.M.G., Verloo, M.G., 2005. Enhanced phytoextraction: ii. effect of EDTA and citric acid on heavy metal uptake by Helianthus annuus from a calcareous soil. Int. J. Phytoremediation 7, 143–152. https://doi.org/10.1080/16226510590950432.
Lewis, K.M., Greene, C.L., Sattler, S.A., Youn, B., Xun, L., Kang, C., 2020. The structural basis of the binding of various aminopolycarboxylates by the periplasmic EDTA-binding protein EppA from chelativorans sp. BNC1. Int. J. Mol. Sci. 21 https://doi.org/10.3390/ijms21113940.
Liu, B., Wang, J., Xu, M., Zhao, L., Wang, Z., 2019. Spatial distribution, source apportionment and ecological risk assessment of heavy metals in the sediments of Haizhou Bay national ocean park, China. Mar. Pollut. Bull. 149 https://doi.org/10.1016/j.marpolbul.2019.110651.
Liu, D., Jiang, W., Liu, C., Xin, C., Hou, W., 2000. Uptake and accumulation of lead by roots, hypocotyls and shoots of Indian mustard [Brassica juncea (L.)]. Bioresour. Technol. 71, 273–277. https://doi.org/10.1016/S0960-8524(99)00082-6.
Loneragan, J.F., Webb, M.J., 1993. Interactions between Zinc and Other Nutrients Affecting the Growth of Plants. In: Robson, A.D. (Ed.), Zinc in Soils and Plants. Springer Netherlands, Dordrecht, pp. 119–134. https://doi.org/10.1007/978-94-011-0878-2_9.
Maeaba, W., Prasad, S., Chandra, S., 2019. First assessment of metals contamination in road dust and roadside soil of Suva city, Fiji. Arch. Environ. Contam. Toxicol 77, 249–262. https://doi.org/10.1007/s00244-019-00635-8.
Mahmud, J.A., Hasanuzzaman, M., Nahar, K., Bhuyan, M.H.M.B., Fujita, M., 2018. Insights into citric acid-induced cadmium tolerance and phytoremediation in Brassica juncea L.: coordinated functions of metal chelation, antioxidant defense and glyoxalase systems. Ecotoxicol. Environ. Saf. 147, 990–1001. https://doi.org/10.1016/j.ecoenv.2017.09.045.
Mai, X., Luo, D., Wei, L., Liu, Y., Huang, X., Wu, Q., Yao, G., Liu, G., Liu, L., 2019. Evaluation method for the measuring comprehensive suitability of chelating agents: a study of the temporal dynamics of heavy metal activation. Int. J. Phytoremediation 21, 1415–1422. https://doi.org/10.1080/15226514.2019.1633262.
McBride, M.B., Mcbride, M.B.A., McBride, P.D., 1994. Environmental Chemistry of Soils. Oxford University Press.
Meers, E., Ruttens, A., Hopgood, M., Lesage, E., Tack, F.M.G., 2005a. Potential of Brassica rapa, Cannabis sativa, Helianthus annuus and Zea mays for phytoextraction of heavy metals from calcareous dredged sediment derived soils. Chemosphere 61, 561–572. https://doi.org/10.1016/j.chemosphere.2005.02.026.
Meers, E., Ruttens, A., Hopgood, M.J., Samson, D., Tack, F.M.G., 2005b. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere 58, 1011–1022. https://doi.org/10.1016/j.chemosphere.2004.09.047.
Morkunas, I., Woźniak, A., Mai, V.C., Rucińska-Sobkowiak, R., Jeandet, P., 2018. The role of heavy metals in plant response to biotic stress. Molecules 23 (2320).

https://doi.org/10.3390/molecules23092320.
Morrison, R.J., Munro, A.J., 1999. Waste management in the small island developing states of the south pacific: an overview. Aust. J. Environ. Manag. 6, 232–246. https://doi.org/10.1080/14486563.1999.10648474.
Mourato, M.P., Moreira, I.N., Leitão, I., Pinto, F.R., Sales, J.R., Louro Martins, L., 2015. Effect of heavy metals in plants of the genus Brassica. Int. J. Mol. Sci. 16, 17975–17998. https://doi.org/10.3390/ijms160817975.
Murtaza, G., Javed, W., Hussain, A., Qadir, M., Aslam, M., 2017. Soil-applied zinc and copper suppress cadmium uptake and improve the performance of cereals and legumes. Int. J. Phytoremediation 19, 199–206. https://doi.org/10.1080/15226514.2016.1207605.
Naidu, S.D., Morrison, R.J., 1994. Contamination of Suva harbour. Fiji. Mar. Pollut. Bull. 29, 126–130. https://doi.org/10.1016/0025-326X(94)90436-7.
Ning, Y., Liu, N., Song, Y., Luo, J., Li, T., 2019. Enhancement of phytoextraction of Pb by compounded activation agent derived from fruit residue. Int. J. Phytoremediation 21, 1449–1456. https://doi.org/10.1080/15226514.2019.1633266.
Nouairi, I., Ammar, W.B., Youssef, N.B., Daoud, D.B.M., Ghorbal, M.H., Zarrouk, M., 2006. Comparative study of cadmium effects on membrane lipid composition of Brassica juncea and Brassica napus leaves. Plant Sci. 170, 511–519. https://doi.org/10.1016/j.plantsci.2005.10.003.
Olaniran, A.O., Balgobind, A., Pillay, B., 2013. Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. Int. J. Mol. Sci. 14, 10197–10228. https://doi.org/10.3390/ijms140510197.
Orroño, D.I., Schindler, V., Lavado, R.S., 2012. Heavy metal availability in Pelargonium hortorum rhizosphere: interactions, uptake and plant accumulation. J. Plant Nutr 35, 1374–1386. https://doi.org/10.1080/01904167.2012.684129.
Oviedo, C., Rodríguez, J., 2003. EDTA: the chelating agent under environmental scrutiny. Quím. Nova 26, 901–905. https://doi.org/10.1590/S0100-40422003000600020.
Panwar, B.S., Ahmed, K.S., Mittal, S.B., 2002. Phytoremediation of nickel-contaminated soils by Brassica species. Environ. Dev. Sustain. 4, 1–6. https://doi.org/10.1023/A:1016337132370.
Park, E.-K., Wilson, D., Choi, H.-J., Wilson, C.T., Ueno, S., 2013. Hazardous metal pollution in the republic of Fiji and the need to elicit human exposure. Environ. Health Toxicol 28. https://doi.org/10.5620/eht.2013.28.e2013017 https://dx.doi.org/10.5620%2Feht.
Purakayastha, T.J., Viswanath, T., Bhadraray, S., Chhonkar, P.K., Adhikari, P.P., Suribabu, K., 2008. Phytoextraction of zinc, copper, nickel and lead from a contaminated soil by different species of Brassica. Int. J. Phytoremediation 10, 61–72. https://doi.org/10.1080/15226510701827077.
Quartacci, M.F., Argilla, A., Baker, A.J.M., Navari-Izzo, F., 2006. Phytoextraction of metals from a multiply contaminated soil by Indian mustard. Chemosphere 63, 918–925. https://doi.org/10.1016/j.chemosphere.2005.09.051.
Quartacci, M.F., Irtelli, B., Gonnelli, C., Gabbrielli, R., Navari-Izzo, F., 2009. Naturally-assisted metal phytoextraction by Brassica carinata: role of root exudates. Environ. Pollut. 157, 2697–2703. https://doi.org/10.1016/j.envpol.2009.04.035.
Rascio, N., Navari-Izzo, F., 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci. 180, 169–181. https://doi.org/10.1016/j.plantsci.2010.08.016.
Rauret, G., 1998. Extraction procedures for the determination of heavy metals in contaminated soil and sediment. Talanta 46, 449–455. https://doi.org/10.1016/S0039-9140(97)00406-2.
Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, P., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit. 1, 57–61. https://doi.org/10.1039/A807854H.
RoyChowdhury, A., Datta, R., Sarkar, D., Török, B., Dransfield, T., 2018. Heavy metal pollution and remediation. In: Green Chemistry. Elsevier, pp. 359–373. https://doi.org/10.1016/B978-0-12-809270-5.00015-7.
Salt, D.E., Prince, R.C., Pickering, I.J., Raskin, I., 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol. 109, 1427–1433. https://doi.org/10.1104/pp.109.4.1427.
Schmidt, C.K., Brauch, H.-J., 2006. Aminopolycarboxylate complexing agents, in: organic pollutants in the water cycle. John Wiley & Sons, Ltd 155–180. https://doi.org/10.1002/352760877X.ch7.
Schowanek, D., Feijtel, T.C.J., Perkins, C.M., Hartman, F.A., Federle, T.W., Larson, R.J., 1997. Biodegradation of [S,S], [R,R] and mixed stereoisomers of ethylene diamine disuccinic acid (EDDS), a transition metal chelator. Chemosphere 34, 2375–2391. https://doi.org/10.1016/S0045-6535(97)00082-9.
Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N.K., Dumat, C., Rashid, M.I., 2017. Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review. Chemosphere 178, 513–533. https://doi.org/10.1016/j.chemosphere.2017.03.074.
Sharma, S.S., Dietz, K.-J., 2009. The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci. 14, 43–50. https://doi.org/10.1016/j.tplants.2008.10.007.
She, W., Cui, G., Jie, Y., Bai, Y., Cao, Y., Xiao, C., 2014. Comparative effects of chelants on plant growth, cadmium uptake and accumulation in nine cultivars of Ramie (Boehmeria nivea). Acta Agric. Scand 64, 71–78. https://doi.org/10.1080/09064710.2014.885077.
Sillanpää, M., 1997. Environmental fate of EDTA and DTPA. Rev. Environ. Contam. Toxicol 152, 85–111. https://doi.org/10.1007/978-1-4612-1964-4_3.
Sinha, S., Gupta, A.K., Bhatt, K., 2007. Uptake and translocation of metals in fenugreek grown on soil amended with tannery sludge: involvement of antioxidants. Ecotoxicol. Environ. Saf. 67, 267–277. https://doi.org/10.1016/j.ecoenv.2006.07.005.
Song, J., Luo, Y.M., Wu, L.H., 2005. Chelate-enhanced phytoremediation of heavy metal contaminated soil. In: Biogeochemistry of Chelating Agents, ACS Symposium Series. American Chemical Society, pp. 366–382. https://doi.org/10.1021/bk-2005-0910.ch022.
Sridhar, B.B.M., Diehl, S.V., Han, F.X., Monts, D.L., Su, Y., 2005. Anatomical changes due to uptake and accumulation of Zn and Cd in Indian mustard (Brassica juncea). Environ. Exp. Bot. 54, 131–141. https://doi.org/10.1016/j.envexpbot.2004.06.011.
Szczygłowska, M., Piekarska, A., Konieczka, P., Namieśnik, J., 2011. Use of Brassica plants in the phytoremediation and biofumigation processes. Int. J. Mol. Sci. 12, 7760–7771. https://doi.org/10.3390/ijms12117760.
Takahashi, R., Fujimoto, N., Suzuki, M., Endo, T., 1997. Biodegradabilities of ethylenediamine-N,N'-disuccinic acid (EDDS) and other chelating agents. Biosci. Biotechnol. Biochem. 61, 1957–1959. https://doi.org/10.1271/bbb.61.1957.
Tandy, S., Bossart, K., Mueller, R., Ritschel, J., Hauser, L., Schulin, R., Nowack, B., 2004. Extraction of heavy metals from soils using biodegradable chelating agents. Environ. Sci. Technol. 38, 937–944. https://doi.org/10.1021/es0348750.
Tandy, S., Schulin, R., Nowack, B., 2006. Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration. Environ. Sci. Technol. 40, 2753–2758. https://doi.org/10.1021/es052141c.
Turan, M., Esringü, A., 2018. Phytoremediation based on canola (Brassica napus L.) and Indian mustard (Brassica juncea L.) planted on spiked soil by aliquot amount of Cd, Cu, Pb, and Zn. Plant Soil Environ. 53, 7–15. https://doi.org/10.17221/3188-pse.
Ultra, V.U., Yano, A., Iwasaki, K., Tanaka, S., Kang, Y., Sakurai, K., 2005. Influence of chelating agent addition on copper distribution and microbial activity in soil and copper uptake by brown mustard (Brassica juncea). Soil Sci. Plant Nutr. 51, 193–202. https://doi.org/10.1111/j.1747-0765.2005.tb00023.x.
USEPA, 1996. Acid digestion of sediments, sludges and soils. Environ. Prot. Agency (Washington, DC, USA). https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf.
Vandevivere, P.C., Saveyn, H., Verstraete, W., Feijtel, T.C., Schowanek, D.R., 2001. Biodegradation of metal-[S,S]-EDDS complexes. Environ. Sci. Technol. 35, 1765–1770. https://doi.org/10.1021/es0001153.
Verbruggen, N., Hermans, C., Schat, H., 2009. Molecular mechanisms of metal hyperaccumulation in plants. New Phytol. 181, 759–776. https://doi.org/10.1111/j.1469-8137.2008.02748.x.
Wang, K., Liu, Y., Song, Z., Wang, D., Qiu, W., 2019. Chelator complexes enhanced Amaranthus hypochondriacus L. phytoremediation efficiency in Cd-contaminated soils. Chemosphere 237, 124–480. https://doi.org/10.1016/j.chemosphere.2019.124480.
Wang, X., Wang, Y., Mahmood, Q., Islam, E., Jin, X., Li, T., Yang, X., Liu, D., 2009. The effect of EDDS addition on the phytoextraction efficiency from Pb contaminated soil by Sedum alfredii Hance. J. Hazard Mater. 168, 530–535. https://doi.org/10.1016/j.jhazmat.2009.02.074.
Wei, Z., Chen, X., Wu, Q., Tan, M., 2015. Enhanced phytoextraction of heavy metals from contaminated soils using sedum alfredii hance with biodegradable chelate GLDA. Huan Jing Ke Xue Huanjing Kexue 36, 1864–1869.
Wilkins, D.A., 1978. The measurement of tolerance to edaphic factors by means of root growth. New Phytol. 80, 623–633. https://doi.org/10.1111/j.1469-8137.1978.tb01595.x.
Wuana, R.A., Okieimen, F.E., Imborvungu, J.A., 2010. Removal of heavy metals from a contaminated soil using organic chelating acids. Int. J. Environ. Sci. Technol. 7, 485–496. https://doi.org/10.1007/BF03326158.
Yang, L., Luo, C., Liu, Y., Quan, L., Chen, Y., Shen, Z., 2013. Residual effects of EDDS leachates on plants during EDDS-assisted phytoremediation of copper contaminated soil. Sci. Total Environ. 444, 263–270. https://doi.org/10.1016/j.scitotenv.2012.11.085.
Yeh, T.Y., Pan, C.T., 2012. Effect of chelating agents on copper, zinc, and lead uptake by Sunflower, Chinese cabbage, Cattail, and Reed for different organic contents of soils. J. Environ. Anal. Toxicol. 2, 1–5. https://doi.org/10.4172/2161-0525.1000145.
Yuan, J., Li, Y., Xu, J.-C., Yang, H.-G., 2016. Enhanced phytoremediation of heavy metal polluted soil from contaminated soils using plant by biodegradable chelate GLDA in combination with plant hormones. J. Wuhan Univ. Technol. 38, 82–86. https://doi.org/10.3963/j.issn.1671-4431.2016.02.015.
Zeremski-Škorić, T., Sekulić, P., Maksimović, I., Šeremešić, S., Ninkov, J., Milić, S., Vasin, J., 2010. Chelate-assisted phytoextraction: effect of EDTA and EDDS on copper uptake by Brassica napus L. J. Serbian Chem. Soc. 75, 1279–1289. https://doi.org/10.2298/jsc091207086z.
Zhao, Y., Mao, G., Hongxia, H., Gao, L., 2015. Effects of EDTA and EDDS on heavy metal activation and accumulation of metals by soybean in alkaline soil. Soil Sediment Contam. 24, 353–367. https://doi.org/10.1080/15320383.2015.962125.