Chemosphere 286 (2022) 131684

Contents lists available at ScienceDirect

Chemosphere

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

The cadmium decontamination and disposal of the harvested cadmium accumulator *Amaranthus hypochondriacus* L.

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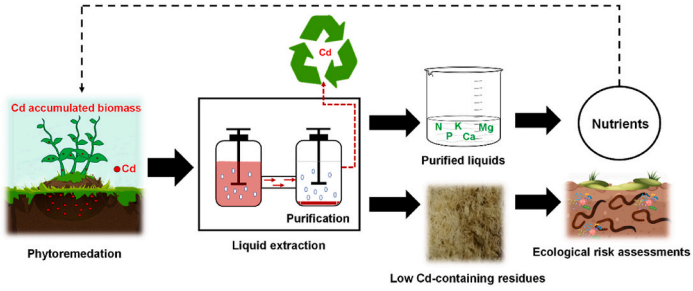
HIGHLIGHTS

- Liquid extraction for Cd removal of *A. hypochondriacus* L. biomass was evaluated.
- 62.1–77.8% of Cd in biomass were removed by 0.25 M HCl after 24 h.
- K_2CO_3, KOH, and 4 Å molecular sieve were effective to remove Cd from waste liquids.
- Extracted liquid after purification using K_2CO_3 was potential to use as a fertilizer.
- Earthworms reduced detriments of Cd-containing biomass for soil microbes.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Lena Q. Ma

Keywords:
Amaranthus hypochondriacus L.
Cadmium
Liquid extraction
Phytoremediation
Risk assessment

ABSTRACT

The heavy metal accumulated biomass after phytoremediation needs to be decontaminated before disposal. Liquid extraction is commonly used to remove and recycle toxic heavy metals from contaminated biomass. In this study, we examined the cadmium (Cd) removal efficiency using different chemical reagents (hydrochloric acid, nitric acid, sulfuric acid, and ethylenediaminetetraacetic acid disodium) of the post-harvest *Amaranthus hypochondriacus* L. biomass. The purifications for the extracted liquids and ecological risk assessments for the extracted residues were also investigated. We have found that 77.8% of Cd in stems and 62.1% of Cd in leaves were removed by 0.25 M HCl after 24 h. In addition, K_2CO_3, KOH, and 4 Å molecular sieve could remove ≥89.0% of Cd in the extracted liquids. Finally, after we returned the extracted residues to the earthworm-incubated soil, the extracted biomass negatively affected the growth (weight loss ≥ 11.0%) and survival (mortality ≥ 33.3%) of *Eisenia fetida*. It should be noted that earthworms decreased soil available Cd concentrations from 0.14–0.05 mg kg^{-1} to 0.11–0.04 mg kg^{-1} and offset the negative effects of the Cd-contaminated biomass on soil microbes. Overall, given the cost of reagents, the Cd removal efficiency, and the ecological risks of the extracted biomass, using 0.25 M HCl for liquid extraction and K_2CO_3 for purification should be recommended. This work highlights

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https://doi.org/10.1016/j.chemosphere.2021.131684
Received 10 May 2021; Received in revised form 22 July 2021; Accepted 25 July 2021
Available online 26 July 2021

the potential of liquid extraction for immediately and directly removing the Cd from fresh contaminated accumulator biomass and the resource cycling potential of the extracted liquids and biomass after purification.

1. Introduction

The over-application of chemical fertilizers and pesticides and the indiscriminate disposal of industrial waste in many areas have resulted in widespread contamination of Cd in agricultural farmland (Qin et al., 2021). It has been estimated that over 126 tons of Cd were released into the environment with sewage irrigation in China after 1975 (Shi et al., 2019). Furthermore, a survey conducted in 2014 showed that 7% of the investigated farmland contained excessive levels of Cd in China (Ministry of Environmental Protection of China, 2014). Soil Cd contamination is of particular concern when Cd accumulates in crops through food chain transfer, being negative to the ecosystem health, and even posing a risk to human health (Gupta et al., 2019; Pecina et al., 2021). Therefore, there is an urgent need to remediate soil Cd contamination, and several technologies have been applied for this aspect.

Phytoremediation is one of the most promising technology for soil heavy metal remediation, which is low-cost, highly feasible, and environmentally friendly (Antoniadis et al., 2017; Ashraf et al., 2019). Heavy metal can be assimilated by hyperaccumulator or high biomass accumulator plant, and removed after plant harvesting (Suman et al., 2018). *A. hypochondriacus* L. is a fast-growing and easily cultivated Cd accumulator plant (Li et al., 2020), which can accumulate over 100 mg kg^{-1} of shoot Cd in soil contaminated with 5 mg kg^{-1} of Cd (Li et al., 2012). In addition, Li et al. (2016) reported that *A. hypochondriacus* L. could yield a total biomass of 71.4 and 46.2 t hm^{-1}, and reduced the soil Cd from 4.5 to 1.0 mg kg^{-1} through a 3.2- year cultivation of double harvesting or a 5.7-year cultivation of single harvesting, respectively. However, several tons of metal-accumulated biomass will be produced when the plants were applied to remediate soil heavy metal contamination. If these large amounts of contaminated biomass are inappropriately disposed, the heavy metal in plant biomass will be discharged into the environment again (Abhilash and Yunus, 2011; Ghosh and Maiti, 2020). However, seldom attention has been focused on the post-treatment of *A. hypochondriacus* L. after harvest. In this case, we should explore an efficient way to decontaminate the harvested accumulator biomass and carry out a risk assessment for the residue before disposal (Sas-Nowosielska et al., 2004; Ghosh and Singh, 2005).

Many researchers have proposed and explored a series of treatment technologies, such as incineration, pyrolysis, ashing, gasification, composting, compaction, and direct disposal (Vocciante et al., 2019). Nevertheless, the final products of these treatments still contain a large amount of heavy metal, posing certain risks for environmental safety, and need to be appropriately pre-treated or post-treated (Cui et al., 2020). Liquid extraction is another pre-treated technology for contaminated biomass. It can extract a high percentage of heavy metals from the contaminated biomass, depending on the metal speciation in plants and the chemical properties of the extracting agents (Kovacs et al., 2013). The common extractants are acid reagents [e. g., hydrochloric acid (HCl), nitric acid (HNO_3), sulfuric acid (H_2SO_4)] or powerful chelators [e. g., ethylenediaminetetraacetic acid (EDTA), citric acid (CA), and calcium chloride ($CaCl_2$)] (Kim et al., 2020; Ma et al., 2020; Wang et al., 2020). The main mechanism for removing the metals using liquid extraction is the destruction of the cell wall structure or chelation between metals and chelators. Wu et al. (2020) announced that 12% H_2SO_4 could remove about 99% of Cd in contaminated rapeseed stalks by changing the chemical components of lignocellulose of plant biomass. Hetland et al. (2001) have reported that EDTA could also remove over 98.5% of lead from the contaminated biomass.

After liquid extraction and is considered non-hazardous material, the plant residues can be recycled for subsequent utilization (Sas-Nowosielska et al., 2004). In addition, the extracted metal in the solution can be recovered by different technologies (Jin and Zhang, 2020; Vocciante et al., 2019). The decontaminated biomass is rich in lignocellulose, which is an ideal material for biofuel production. Using a Cd-accumulator rapeseed for fermentation and pre-treating it with 10% H_2SO_4, Wu et al. (2020) yielded over 10% of the dry matter of ethanol. In addition, the decontaminated biomass can be subsequently used as a nutrient additive. For example, Cd-enriched tobacco leaves could be used as a forage after Cd removal using 0.5% HCl combined with 70% ethanol (Yang et al., 2019). Furthermore, we can recover the extracted metal from the extracted liquid by chemical precipitation or electrodeposition (Delil et al., 2020). Currently, the research of liquid extraction mainly focuses on the treatments of oven-dried and air-dried biomass, which require high energy and high time consumption (Yang et al., 2019; Yuan et al., 2019). Rapid processing of fresh heavy metal accumulator biomass is a means of preventing secondary contamination. Moreover, many studies have attempted to compare the removal efficiency of different extractants and select the most suitable liquid extraction condition, whereas few were conducted to explore the safety treatment and resource utilization of the extracted waste. The ecological risk assessment of the extracted products after liquid extraction is necessary.

The goal of this research was to appraise the viability of whole disposal process consisting of liquid extraction, resource utilization, and ecological risk assessment. In this study, we used different extractants (e. g., HCl, HNO_3, H_2SO_4, CA, $CaCl_2$, and EDTA) to remove Cd from the fresh biomass of *A. hypochondriacus* L., followed by purifying the liquid after extraction with different methods. We carefully compared their extraction efficiency and purification efficiency. Finally, we conducted an ecological risk assessment of the leaching residues after direct disposal using earthworms (*E. fetida*) and soil microbial phospholipid fatty acids (PLFAs) as indicators. Our hypothesis is that (i) the strong acid with relative low concentration can extract most of the Cd in the fresh biomass of *A. hypochondriacus* L., (ii) the extracted liquids may be a useable nutrients or fertilizer for plant after safely purification, and (iii) the low Cd-containing residues after liquid extraction may have a low risk for soil organism. Therefore, our study aims to: (i) select the most suitable conditions for liquid extraction of *A. hypochondriacus* L. biomass (i.e., extractants, concentrations, and time), (ii) compare the purification efficiency of the solution after extraction using precipitation and adsorption methods, then decide the best option, (iii) and conduct the ecological risk assessment for the safely-treated leaching residues using *E. fetida* and PLFAs. This research will provide a practical way to immediately deal with the fresh contaminated biomass and prevent secondary pollution after phytoremediation.

2. Materials and methods

2.1. Soil and plant biomass preparation

The typical southern sandy loam soil with pH 5.29 was collected from the surface farmland soil layer (0–20 cm) in Maba in Shaoguan city, Guangdong province, China (24° 38′ 32″ N, 113° 35′ 55″ E). The total Cd and available Cd concentrations in the soil sample were 2.50 mg kg^{-1} and 1.54 mg kg^{-1}, respectively. Soil basic properties are as follows: 26.8 g kg^{-1} for soil organic matter (SOM), 1.57 g kg^{-1} for total N, 3.09 g kg^{-1} for total P, and 7.48 cmol kg^{-1} for cation exchange capacity (CEC). After being air-dried, crushed, and sieved through 1 cm sieves, the soils were applied to conduct pot experiments.

Plastic pots (10 kg capacity) filled with 8 kg of the tested soil were used for plant culture. 3.02 g urea, 1.94 g ammonium dihydric phosphate [$(NH_4)H_2PO_4$], and 2.97 g potassium sulfate [K_2SO_4] were added

into the pot as basal fertilizer. The seeds of high biomass producing plant, *A. hypochondriacus* L. were purchased from Shuonong agriculture Co., Ltd. in Xinyu city, Jiangxi province, China. Three seedlings of *A. hypochondriacus* L. were planted in each pot at South China Botanical Garden (Guangzhou, China). The water content of all pots was maintained at 70% of soil water-holding capacity. After 60 d of growth, all aboveground plants were harvested. The harvested plant was separated into stems and leaves, and rinsed with deionized water. One part of the samples was weighed and oven-dried at 70 °C for 72 h to constant weight, and the rest of them was crushed with stainless steel grinder (BJ-150, 25000 rpm, 500 W, China) for 3–5 min. The crushed fresh biomass was stored in a sealed bag and stored at 4 °C for later treatment. The water content of stems and leaves of *A. hypochondriacus* L. was 79.3% and 81.0%, and the Cd concentrations were 3.86 mg kg^{-1} and 7.34 mg kg^{-1} (fresh weight), respectively.

2.2. Liquid extraction procedure

Hydrochloric acid (HCl; 0.01 M, 0.05 M, 0.10 M, 0.25 M), nitric acid (HNO_3; 0.25 M), sulfuric acid (H_2SO_4; 0.25 M), Citric Acid (CA; 0.10 M), calcium chloride ($CaCl_2$; 0.10 M), and ethylenediaminetetraacetic acid disodium (EDTA-2Na; 0.10 M) were used as extractants in this experiment, with each of three replicates. The concentration of 0.10 M used in this study was referred to the concentrations of these extractants mostly applied at soil washing or ash leaching (Huang et al., 2011; Xiao et al., 2019). Nevertheless, the concentration of 0.25 M adopted here was aimed to compare the extraction efficiency of different powerful acids (e.g., HCl, HNO_3, H_2SO_4) under a relatively high concentration. The deionized water was used as control. According to the ratio of extraction of 1:20 (dry biomass: extractant, w:v) [equivalent to fresh biomass: extractant = 1:4.1 (stems) and 1:3.8 (leaves)], which is extensively recommended for chemical extraction of heavy metal contained sewage, ash and soil (Xiao et al., 2019; Yuan et al., 2019). All liquid extraction was performed by mixing 4.83 g fresh stems (total Cd amount was 18.6 μg) or 5.25 g fresh leaves (total Cd amount was 38.5 μg) of *A. hypochondriacus* L. with 20 mL of extractants in 50 mL centrifuge tubes. Next, the centrifuge tube was put into a reciprocal shaker under an ambient temperature of 25–30 °C and a vibration speed of 220 rpm for 24 h, which is widely employed in the leaching experiment for heavy metal contaminated soil and sewage (Ke et al., 2020; Prabhakar et al., 2021). After liquid extraction, samples were centrifuged at 4700 rpm for 10 min, then filtered with 0.45 μm micro-filtration membrane. The filtrate was stored at 4 °C for subsequent analysis. The leaching residues after filtration were washed twice with 20 mL ultrapure water, and oven-dried at 70 °C to constant weight for the determination of Cd.

Given the high extraction efficiency, 0.25 M HCl and 0.10 M EDTA were mainly investigated in the subsequent study. In order to evaluate the effects of the extraction time, a series of extractive duration (i.e., 1 d, 2 d, 3 d, and 5 d) was arranged to explore the suitable liquid extraction time for the extractants of 0.10 M HCl, 0.25 M HCl, and 0.10 M EDTA. The liquid extraction process was performed as aforementioned.

2.3. Leachate purification and analysis

Next, we tried to remove the Cd in the liquids after liquid extraction. Two main wastewater treatment techniques, chemical precipitation (using KOH and K_2CO_3) and physical adsorption (using 4 Å molecular sieve), were applied to purify the leachate of liquid extraction. The hydroxide precipitation method was conducted by adding 1 M potassium hydroxide (KOH) into the leachate until the liquid pH attained 11. 0.3% polyaluminium chloride (PAC, w:w) was subsequently added to the mixture to fully construct the metal hydroxide flocculation (Patterson et al., 1977). The carbonate precipitation method was performed by adding 1 M potassium carbonate (K_2CO_3) into the filtrate until the solution pH reached 10. Similarly, 0.3% PAC was added to the solution to form flocculent precipitate (Patterson et al., 1977). For the adsorption methods, 1 M NaOH or 1 M HCl was added to the solution firstly to adjust filtrate pH to 6.5. Then 2.5 g L^{-1} dose of 4 Å molecular sieve was added to the leachate to adsorb Cd. The mixture was put into a reciprocal oscillation machine and shook at a speed of 220 rpm under an ambient temperature of 30 ± 1 °C for 90 min (Rao et al., 2006). For all these three methods, after the reaction was fully completed, the leachate was centrifuged at a speed of 4700 rpm for 10 min and filtered with 0.22 μm micro-filters. The supernatant was stored at 4 °C for subsequent analysis. All trials were replicated three times.

2.4. The risk assessment of the extracted residues using E. fetida

The safety of the disposal of the residues after liquid extraction was examined using *E. fetida* as a bioindicator. The untreated biomass (NT), the deionized water extracted biomass (CK), the 0.25 M HCl extracted biomass, and the 0.10 M EDTA extracted biomass were added to the soil respectively and incubated with *E. fetida.* The tested leaching residues were the aboveground biomass of *A. hypochondriacus* L., which were the mixtures of the equal weight of stems and leaves. The Cd concentrations of the untreated, deionized water extracted, 0.25 M HCl extracted, and 0.10 M EDTA extracted fresh biomass were 5.72, 5.0, 1.59, and 2.41 mg kg^{-1}, respectively. The clean soils were collected from the surface layer (0–20 cm) of a cropland in South China Botanical Garden (23° 10′ 34″ N, 113° 21′ 7″ E). The collected soils were air-dried and sieved to 1 mm, and the maximum water holding capacity was analyzed with OECED (2016). The main physicochemical properties of soils were as follows: silty loam soil, pH (4.58), maximum water holding capacity (47.2%), and SOM (67.8 g kg^{-1}). The model earthworm species utilized in this experiment was *E. fetida,* which was obtained from an earthworm farm (Haolun Ecological Agriculture Co., Jiangmen, China). The adult earthworms were acclimated in the silty loam soil at 20 ± 1 °C for 15 d. Before the tests, earthworms were rinsed with ultrapure water to clear the surface soil and evacuated their gut content on a wet filter paper for 24 h in the dark (Maity et al., 2018). We then picked mature earthworms with obvious clitellum for ecotoxicological tests.

The ecological risk assessment of the residues was conducted by simulating straw returning to the field. According to the ratio of biomass to soil (1%), 3 g aboveground biomass of *A. hypochondriacus* L. was added into 300 g clean soils in a 2 L polypropylene jar. Six gut-cleaned adult earthworms with individual weights from 300 to 400 mg were assigned to each jar. The preservative film with little holes to supply oxygen and rubber band were applied to prevent earthworms from escaping away the jar. The jar was cultured in an artificial climate incubator (LRG-300Y, China) at 20 ± 1 °C, a relative humidity of 80%, and a controlled light-dark cycle of 16 h light/8 h dark (Zhu et al., 2019). The soil moisture was adjusted to 60% of the maximum water holding capacity and kept by adding ultrapure water regularly (Xiao et al., 2021). Tests were performed with three replicates. The dead body of the earthworm was picked out from the jar immediately every day. After 28 d, earthworms were hand collected, counted for mortality, and weighted after surface washing and 24 h of gut voiding. All the recorded earthworms were killed with liquid nitrogen and frozen at – 80 °C for subsequent analysis (Zhang et al., 2019).

2.5. Soil microbial community measurement

The soil microbial community composition and biomass of the tested soil were detected by phospholipid fatty acids (PLFAs) analysis with the method described by Bossio and Scow (1998). The soil samples were freeze-dried and extracted with a mixture: chloroform/methanol/phosphate buffer solvent (1:2:0.8 by volume) for microbial lipids. The lipids were finally detected by an Agilent 6890 gas chromatography coupled with a flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). The concentration of individual PLFAs was reported as nmol g^{-1} of PLFAs to dry soil. Internal standard with a known concentration of Methylnonadecanoate (19:0) was added

into the samples for calculation of the PLFAs concentration of each soil sample (Wu et al., 2020a). The biomarkers of the common bacteria were saturated PLFAs 14:0, 15:0, 16:0, 17:0, and 18:0; the gram-negative was represented by monounsaturated and cyclopropane PLFAs 14:1w5c, 15:1w6c, 16:1w7c, 16:1w7t, 16:1w5c, 18:1w9c, 18:1w7c, 18:1w7t, cy17:0, and cy19:0; a/I branched PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, and a17:0 were for the gram-positive; the biomarkers indicative of the actinomycetes was mythy-branched PLFAs 10Me18:0, 10Me16:0, and 10Me17:0; fungi were considered to be indicated by the PLFAs biomarkers of 18:2w6c, 18:3w6c, and 18:3w3c (Wang et al., 2021; Zheng et al., 2021). General soil microbial composition was defined by fungi: bacteria biomass ratios.

2.6. Soil, plant, and earthworm samples analyses

The soil pH was determined using a glass electrode with a soil to water ratio of 1:2.5 (w:v). Cation exchange capacity was measured with a method described by Huang et al. (2020). Total organic carbon was measured with a modified Walkley-Black method (Meersmans et al., 2009). Concentrated sulfuric acid with a mixture catalyst of potassium sulfate and copper sulfate (3.5 mL H_2SO_4, 1.48 g K_2SO_4, and 0.12 g $CuSO_4$) were utilized to digest soil samples (0.10 g soil) (Taylor, 2000), then the total nitrogen and phosphorous of soil were determined by indophenol blue colorimetric method and molybdate colorimetric method, respectively (Wu et al., 2020b). The maximum water holding capacity of the soil was measured with OECED (2016). The soil particle size distribution was analyzed with a laser diffraction method using an LA-960A particle size analyzer (Partica, HORIBA Ltd., Japan). Based on the international standard system of soil texture classification, the soil particle size was categorized as sand (20–2000 μm), silt (2–20 μm), and clay (<2 μm) (Qiu et al., 2021). The total Cd of soil was determined after digestion with a mixture of concentrated acid (high-purity HNO_3, HCl, HF, 6:3:2, by volume) (Mao et al., 2019), and soil available Cd was extracted with 0.10 M $CaCl_2$ (Huang et al., 2020). The certified reference material (GBW (E) 100357) was used as quality control. Samples Cd was detected by a flame atomic absorption spectrophotometer (AAS, contrAA800, Analytikjena, Germany). The concentrations of total and available Cd of earthworm incubated soil were measured by the same method.

Plant moisture was measured by oven-drying plant samples to constant weight and calculated with the weight change of plant samples. The total Cd of the plant, leaching residues, and earthworms were determined after digestion with high purity concentrated nitric acid using an AAS. The Cd chemical forms analysis of stems and leaves of *A. hypochondriacus* L. were processed according to the chemical sequential extraction procedure proposed by Xin et al. (2017), including ethanol extracted-, deionized water extracted-, NaCl extracted-, acetic acid extracted-, and HCl extracted-, and residual form. The leachate before and after purification was digested with high purity concentrated nitric acid and perchloric acid (HNO_3:$HClO_4$ = 3:1, v:v), and analyzed for the concentrations of Cd, K, Ca, and Mg (Bora et al., 2020). The concentrations of N and P of the leachate were analyzed using indophenol blue colorimetric method and molybdate colorimetric method. The pH of the leachate was directly determined using a glass electrode.

2.7. Statistical analysis

All of the experimental data are presented as mean ± standard error (SE) of triplicates. One-way ANOVA was applied to test the differences between the different extraction treatments. Two-way ANOVA was used to examine the effects of different extraction treatments and earthworms on soil microbial community composition. The significant difference between treatments was analyzed with Tukey's honest significance test ($P < 0.05$). All statistical analyses were performed using SPSS 26.0 software.

3. Results

3.1. Liquid extraction of Cd contaminated A. hypochondriacus L. biomass

Different extractants have different Cd removal performance of *A. hypochondriacus* L. biomass after 24 h of leaching. As Fig. 1 (a) shows, 0.10 M HCl, 0.25 M HCl, 0.25 M HNO_3, 0.25 M H_2SO_4, and 0.10 M EDTA effectively removed > 77% of Cd from stems, with the highest extraction rate being 78.0%. The extraction rates of the rest of the extractants were less than 70%, with the extraction rate of the control (deionized water) being the lowest. Alternatively, Fig. 1 (b) depicted the same trends of the extraction efficiency for Cd of *A. hypochondriacus* L. leaves. 0.25 M HCl, 0.25 M HNO_3, 0.25 M H_2SO_4, and 0.10 M EDTA had the best performance on removing Cd. The highest Cd extraction rate was 63.3%. The removal rates of the other extractants were lower than 40%.

The effects of reaction time on the removal efficiency of Cd in *A. hypochondriacus* L. stems and leaves were shown in Fig. S1. Given the cost and extraction efficiency of extractants, 0.10 M HCl, 0.25 M HCl, and 0.10 M EDTA were investigated using a longer extraction time. After

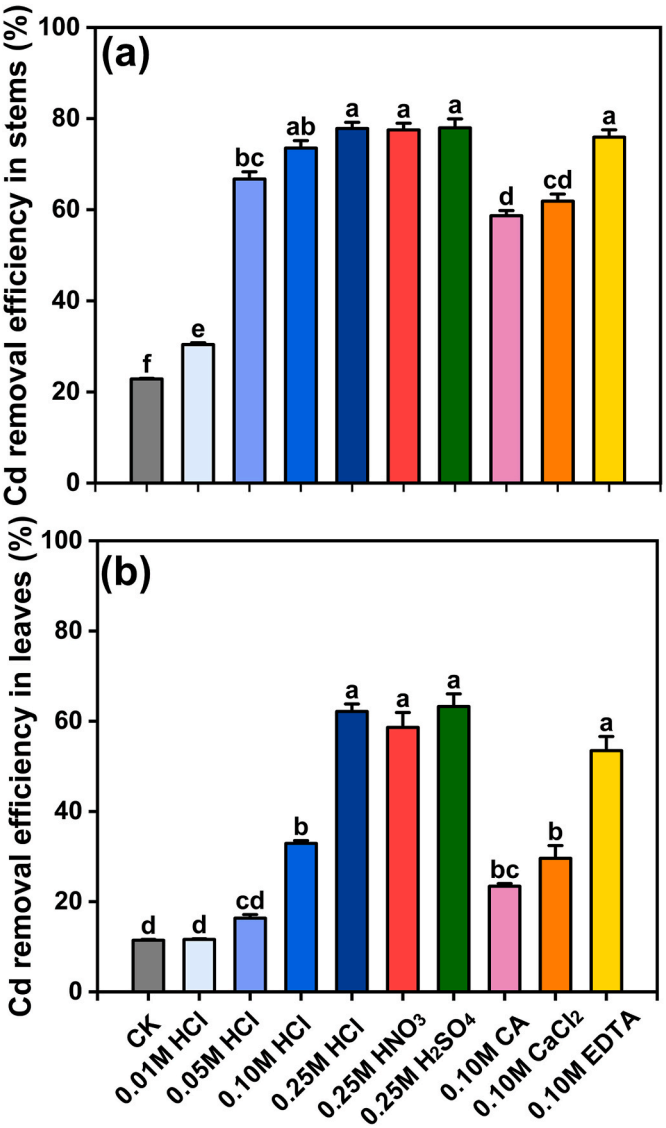

Fig. 1. The removal efficiency of Cd from the stems (a) and leaves (b) of *A. hypochondriacus* L. with different extractants for 24 h. CA, citric acid; EDTA, ethylenediamine tetraacetic acid disodium salt. Data are presented as mean ± standard error (n = 3). Different letters on the column indicate significant differences among the different treatments ($P < 0.05$).

1 d, > 65% of Cd in stems and >40% of Cd in leaves were extracted by the three selected extractants, with the highest extraction efficiency being found for 0.25 M HCl (Fig. S1). However, the extraction efficiency did not increase or even slightly decrease with a longer extraction time.

3.2. Effects of extractants on Cd chemical forms

Cd concentrations of different fractions in stems and leaves of *A. hypochondriacus* L. were remarkably influenced by different extractants (Fig. 2). It can be seen from the figure that the main Cd fractions in stems and leaves of *A. hypochondriacus* L. were NaCl-extracted Cd. All three treatments significantly removed the ethanol-Cd and the deionized water-Cd in the leaves ($P < 0.05$). However, only 0.25 M HCl and 0.10 M EDTA prominently removed the NaCl-Cd, the acetic acid-Cd, the HCl-Cd, and the residual-Cd in stems ($P < 0.05$). Unlike the Cd fractions in stems, the effects of different treatments on ethanol-Cd and deionized water-Cd removal in leaves were different. 0.25 M HCl largely removed both of them, while deionized water only removed the latter one. Interestingly, 0.10 M EDTA failed to remove these two Cd fractions from the leaves, but it substantially increased the concentrations of these two Cd fractions. Similar to the Cd removal in stems, deionized water was hard to remove the rest of Cd fractions in leaves except for the residue Cd. Similarly, the removal efficiency of the rest Cd fractions using 0.25 M HCl and 0.10 M EDTA were same as those in stems, and the latter was better than the former one.

3.3. Purification for liquids after extraction

For Cd removal in stems, the Cd concentrations of liquids extracted with 20 mL of the four extractants (0.25 M HCl, 0.25 M HNO_3, 0.25 M H_2SO_4, and 0.10 M EDTA) were 0.708–0.727 mg L^{-1}. Correspondingly, the Cd concentrations of the leaching residues treated by 0.25 M HCl, 0.25 M HNO_3, 0.25 M H_2SO_4, and 0.10 M EDTA were 0.16–0.95 mg kg^{-1} (Table S1). As for extracting the Cd in leaves with different extractants, the Cd concentrations of leaching liquids treated by 20 mL of 0.25 M HCl, 0.25 M HNO_3, 0.25 M H_2SO_4, and 0.10 M EDTA were 1.03–1.22 mg L^{-1}. Accordingly, the Cd concentrations of the leaching residues were 2.76–3.80 mg kg^{-1}. The recovery rates of Cd in different treatments were 80%–117%, which indicated that extracting the Cd in the shoots of *A. hypochondriacus* L. with the liquid extraction method was feasible.

We then used K_2CO_3, KOH, and 4 Å molecular sieve to purify the Cd from the leaching liquids (Table 1). In our study, we combined the leaching liquids of stems and leaves of *A. hypochondriacus* L. as the ratio of 1:1 for subsequent purification investigation. For the deionized water treatment (CK), the purification efficiencies of the chemical precipitation (using K_2CO_3 or KOH) and the physical adsorption (using 4 Å molecular sieve) methods for removing Cd from liquids were >71%, with the order being 4 Å molecular sieve (96.5%) > KOH (84.3%) > K_2CO_3 (71.8%). The Cd removal proportions of the three methods had no significant differences between them. After being treated with these three methods, the liquid acidity adjusted from low acidic to neutral or weakly alkaline. The pH of the three liquids changed from 4.29 to 9.35 for K_2CO_3 treatment, 8.01 for KOH treatment, and 8.56 for 4 Å molecular sieve treatment. With reference to the extraction using 0.25 M HCl, the three purification treatments prominently removed the Cd in liquids, and all of the liquids satisfied the sewage discharge standard for Cd

Table 1
The removal proportions of Cd from the extraction liquids of *A. hypochondriacus* L. with different treatments.

Liquids	Treatments	Cd (mg•L^{-1})	Removal proportion	pH
CK	NT	0.113 ± 0.013a	–	4.29
	K_2CO_3	0.032 ± 0.0015b	71.8%	9.35
	KOH	0.018 ± 0.0042b	84.3%	8.01
	4 Å molecular sieve	0.004 ± 0.0003b	96.5%	8.56
0.25 M HCl	NT	0.821 ± 0.0098a	–	0.72
	K_2CO_3	0.085 ± 0.011b	89.6%	9.35
	KOH	0.091 ± 0.012b	89.0%	8.05
	4 Å molecular sieve	0.027 ± 0.001c	96.8%	8.17
0.10 M EDTA	NT	0.71 ± 0.007a	–	4.20
	K_2CO_3	0.59 ± 0.0053c	17.5%	9.97
	KOH	0.62 ± 0.0035b	12.4%	10.6
	4 Å molecular sieve	0.73 ± 0.0055a	–	7.16

Note: NT represent no treatment for different initial liquids. – denotes undetectable values. Date are exhibited as mean ± standard error (n = 3). Different letters in the column indicate significant differences among different treatments ($P < 0.05$).

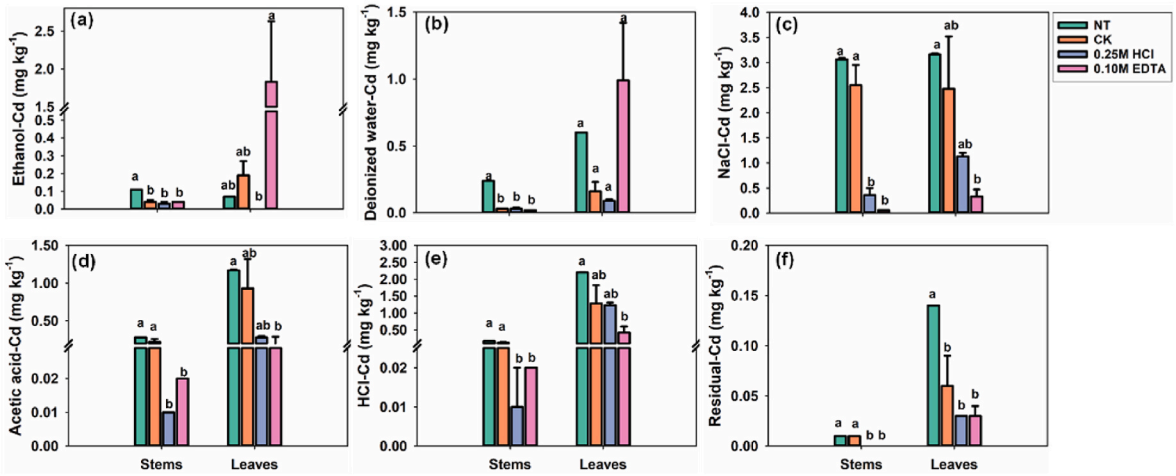

Fig. 2. The chemical forms of Cd in the stems and leaves of *A. hydrochondriacus* L. after different extractants of 24 h extraction. NT indicates the biomass of no liquid extraction treatment; CK indicates deionized water extracted biomass; 0.25 M HCl indicates 0.25 M HCl extracted biomass; 0.10 M EDTA indicates 0.10 M EDTA extracted biomass. Ethanol-Cd, inorganic Cd including nitrate/nitrite, chloride, and aminophenol Cd; Deionized water-Cd, water-soluble Cd including organic acid complexes and $Cd(H_2PO_4)_2$; NaCl-Cd, Cd integrated with pectate and protein; Acetic acid-Cd, insoluble Cd-phosphate including $CdHPO_4$, $Cd_3(PO_4)_2$ and other Cd-phosphate complexes; HCl-Cd, Cd oxalate. Residual-Cd, residue Cd. The data in the column are presented as mean ± standard error (n = 3). The different letters on the column indicate significant difference among different treatments ($P < 0.05$).

(GB8978-1996, ≤0.10 mg L^{-1}) in China. The purification efficiencies were as follows: 4 Å molecular sieve (96.8%) > K_2CO_3 (89.6%) > KOH (89.0%). It should be noted that the acidity of liquids altered from strongly acidic to alkaline. The pH of the three liquids was adjusted from 0.72 to 9.35 for K_2CO_3 treatment, 8.05 for KOH treatment, and 8.17 for 4 Å molecular sieve treatment. However, these three methods could not effectively remove Cd from the liquids after extraction using 0.10 M EDTA.

3.4. Nutrients of the leachate after Cd purification

The nutrient (N, P, K, Ca, and Mg) concentrations of the leaching liquids after purification are listed in Table 2. The nutrient concentrations of the control (extracted with deionized water) were 363 mg L^{-1} for N, 163 mg L^{-1} for P, 2102 mg L^{-1} for K, 5.01 mg L^{-1} for Ca, and 84 mg L^{-1} for Mg. After purification using K_2CO_3, KOH, and 4 Å molecular sieve, the N concentrations in the leaching liquids slightly decreased to 307 mg L^{-1} in K_2CO_3 treatment ($P > 0.05$), and significantly decreased to 276 mg L^{-1} in KOH treatment ($P < 0.05$) and to 296 mg L^{-1} in 4 Å molecular sieve treatment ($P < 0.05$). The P concentrations significantly reduced from 163 mg L^{-1} to 8.11–81.8 mg L^{-1} in the aforementioned three purification treatments. For K concentrations, the K_2CO_3- and KOH-treated liquids were high in K concentrations, reaching 7127 mg L^{-1} in the K_2CO_3 treatment. However, 4 Å molecular sieve reduced K concentrations to 969 mg L^{-1}. The Ca concentrations were significantly reduced to 0.69 mg L^{-1} using K_2CO_3 and to 0.11 mg L^{-1} using 4 Å molecular sieve. As for the Mg concentrations, they were significantly reduced to 27.5 mg L^{-1} using K_2CO_3 and to 53.6 mg L^{-1} using KOH ($P < 0.05$).

Concerning the leaching liquids of 0.25 M HCl, the N concentrations did not change significantly ($P > 0.05$) in the three purification treatments, while the P concentrations were significantly reduced in three purification treatments ($P < 0.05$). Similarly, K_2CO_3 and KOH greatly increased the K concentrations, with the highest K concentrations being 17246 mg L^{-1} in K_2CO_3 treatment. In the same way, 4 Å molecular sieve removed most of the K contents. All three purification treatments significantly reduced the Ca and Mg concentrations ($P < 0.05$). With respect to the leaching liquids of 0.10 M EDTA, the concentrations of N, P, Ca, and Mg were all remarkably decreased after purification.

3.5. Toxicity to earthworms after disposal of the residues in soil

After liquid extraction, the biomass residues were mixed with the agricultural soil and incubated with *E. fetida* for 28 d. No earthworms survived in the soil mixed with 0.10 M EDTA-extracted biomass. 44.4% of earthworms died in the 0.25 M HCl treatments, which was not statistically different from the control (Table 3). A decrease in earthworm fresh weight was observed in all treatments. Except for EDTA treatments, the highest weight loss rate was found in 0.25 M HCl treatments, while the lowest Cd concentration of earthworms was also found in that treatment, which was 1.14 mg kg^{-1}.

Table 3
Fresh biomass, weight loss, mortality, and the concentrations of Cd of earthworms, following 28 d of incubation.

Treatment	Initial biomass (g)	Final biomass (g)	Weight loss (%)	Mortality (%)	Cd (mg•kg^{-1})
NT	0.37 ± 0.01a	0.28 ± 0.02a	24.1 ± 9.13c	27.8 ± 5.55b	4.12 ± 0.38a
CK	0.38 ± 0.03a	0.34 ± 0.03a	11.0 ± 0.50bc	44.4 ± 5.56b	3.47 ± 0.20a
0.25 M HCl	0.41 ± 0.02a	0.26 ± 0.01a	34.7 ± 3.28b	33.3 ± 16.7b	1.14 ± 0.08b
0.10 M EDTA	0.37 ± 0.01a	–	100 ± 0.00a	100 ± 0.00a	–

Note: NT represents no treatment for different initial plant biomass; CK indicates the deionized water extracted biomass; 0.25 M HCl indicates the 0.25 M HCl extracted biomass; 0.10 M EDTA indicates the 0.10 M EDTA extracted biomass. – denotes undetectable values. Date are showed as mean ± standard error (n = 3). Different letters in the column indicate significant differences among different treatments ($P < 0.05$).

3.6. Cadmium concentrations in soils after disposal of the residues

As can be seen from Fig. 3, the concentrations of soil total Cd and available Cd were positively correlated with the Cd concentrations of the tested biomass. Higher soil total Cd concentrations were found in the soils mixed with the untreated biomass and deionized water extracted biomass than those in 0.25 M HCl and 0.10 M EDTA treatments. The lowest soil total Cd was 0.080 mg kg^{-1} (without earthworms) and 0.083 mg kg^{-1} (with earthworms) in soils mixed with the 0.25 M HCl extracted biomass. As regards to the soil available Cd concentrations, the untreated biomass and deionized water extracted biomass increased the soil available Cd concentrations to a higher extent compared to the 0.25 M HCl and 0.10 M EDTA treatments. The lowest soil available Cd concentrations were 0.049 mg kg^{-1} (without earthworms) and 0.044 mg kg^{-1} (with earthworms) in 0.25 M HCl extracted biomass treatments. Overall, earthworms had no effects on soil total Cd concentration while they significantly reduced available Cd concentrations compared to the counterpart treatments ($P < 0.05$).

3.7. Soil microbial community structure

The soil microbial community composition after disposal of the residues is categorized according to the PLFAs concentrations (Fig. 4). Earthworms significantly increased bacterial, fungal, and actinomycete PLFAs concentrations based on a two-way ANOVA test ($P < 0.05$). For the total bacteria PLFAs, in the absence of earthworms, HCl extracted

Table 2
The nutrients (N, P, K, Ca, and Mg) concentrations of the extraction liquids after purification.

Solution	Treatments	N (mg•L^{-1})	P (mg•L^{-1})	K (mg•L^{-1})	Ca (mg•L^{-1})	Mg (mg•L^{-1})
CK	NT	363 ± 17.7a	163 ± 10.9a	2102 ± 37.5c	5.01 ± 0.10a	84.0 ± 1.51a
	K_2CO_3	307 ± 7.68 ab	22.1 ± 2.12c	7127 ± 208a	0.69 ± 0.08b	27.5 ± 0.50c
	KOH	276 ± 16.4b	8.11 ± 1.28c	3743 ± 49.3b	4.78 ± 1.32a	53.6 ± 6.40b
	4 Å molecular sieve	296 ± 5.28b	81.8 ± 2.19b	969 ± 11.2d	0.11 ± 0.03b	102 ± 7.01a
0.25 M HCl	NT	334 ± 20.1a	165 ± 2.37a	2158 ± 30.3c	106 ± 0.31a	216 ± 2.60a
	K_2CO_3	339 ± 10.6a	68.4 ± 8.57b	17246 ± 116a	4.00 ± 0.53bc	87.3 ± 9.82b
	KOH	298 ± 47.1a	8.00 ± 0.64c	8732 ± 129b	5.66 ± 0.80b	75.1 ± 2.38b
	4 Å molecular sieve	301 ± 35.3a	77.4 ± 2.20b	1296 ± 13.7d	2.34 ± 0.07c	64.4 ± 1.11b
0.10 M EDTA	NT	1400 ± 57.0a	166 ± 3.14a	2114 ± 19.4c	70.6 ± 0.53a	8.86 ± 0.01a
	K_2CO_3	339 ± 29.3b	60.9 ± 3.60b	17508 ± 29.5a	1.11 ± 0.18c	0.94 ± 0.10c
	KOH	304 ± 11.3b	54.8 ± 0.31b	7444 ± 143b	1.84 ± 0.07c	1.05 ± 0.05c
	4 Å molecular sieve	275 ± 57.0b	66.3 ± 2.57b	1406 ± 20.7d	6.00 ± 0.45b	2.73 ± 0.26b

Note: NT represent no treatment for different initial liquids. Date are showed as mean ± standard error (n = 3). Different letters in the column indicate significant differences among different treatments ($P < 0.05$).

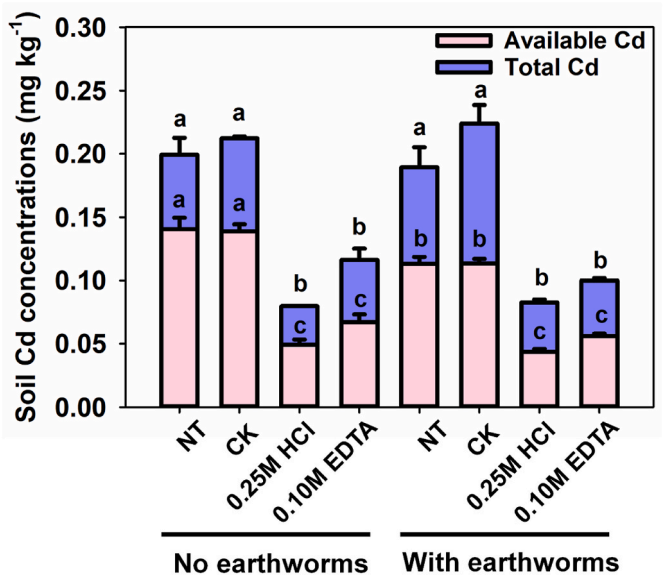

Fig. 3. The soil total Cd and available Cd concentrations after disposal of *A. hypochondriacus* L. residues. NT indicates the biomass of no liquid extraction treatment; CK indicates deionized water extracted biomass; 0.25 M HCl indicates 0.25 M HCl extracted biomass; 0.10 M EDTA indicates 0.10 M EDTA extracted biomass. The data in the column are presented as mean ± standard error (n = 3). The different letters on the column indicate significant difference among different treatments ($P < 0.05$).

biomass significantly increased soil total bacteria PLFAs concentrations compared to those in the untreated biomass and deionized water extracted biomass treatments ($P < 0.05$). In the presence of earthworms, there were no significant differences between the four treatments. With regard to the gram-positive and gram-negative bacteria PLFAs concentrations, similar trends as total bacterial PLFAs were found among different treatments. In the absence of earthworms, HCl extracted and EDTA extracted biomass slightly increased gram-positive and gram-negative bacteria PLFAs concentrations. As for the fungal and actinomycete PLFAs concentrations, in the absence of earthworms, HCl extracted and EDTA extracted biomass significantly increased fungal and actinomycete biomass ($P < 0.05$). However, earthworms offset this effect. In addition, regardless of the presence or absence of earthworms, the adding of HCl extracted biomass slightly and the adding of EDTA extracted biomass significantly decreased the ratios of bacteria PLFAs concentrations to fungal PLFAs concentrations. It can be known that the bacteria played a leading role in the relative abundance of soil microbial community composition in the present study.

4. Discussion

4.1. Hydrochloric acid was effective to remove Cd from biomass after 24 h

In order to immediately dispose the large amounts of accumulator biomass after phytoremediation, we investigated the liquid extraction for fresh biomass of *A. hypochonddriacus* L. Inorganic acid (e.g., H_2SO_4, HNO_3, and HCl), organic acid (e.g., citric acid, oxalic acid, and tartaric acid), and chelating agents (e.g., EDTA, NTA, EDDS) were commonly applied to clean out heavy metal from contaminated soil or sewage (Wang et al., 2019a; Kim et al., 2020). In our study, we designed nine different extractants (Fig. 1) to extract the Cd from stems (18.6 mg kg^{-1}, dry weight) and leaves (38.6 mg kg^{-1}, dry weight) of *A. hypochondriacus* L. The removal efficiency of the nine extractants varied as follows: 0.25 M HCl ≈ 0.25 M HNO_3 ≈ 0.25 M H_2SO_4 > 0.10 M EDTA > 0.10 M HCl > 0.10 M $CaCl_2$ > 0.10 M CA > 0.05 M HCl > 0.01 M HCl. Among them, 0.25 M HCl had the highest removal efficiency. These results were consistent with the research of Yuan et al. (2019) that the HCl was effective in extracting Cd from contaminated biomass and the extraction efficiency increased with HCl concentrations.

A. hypochondriacus L. is an emerging high biomass Cd accumulator

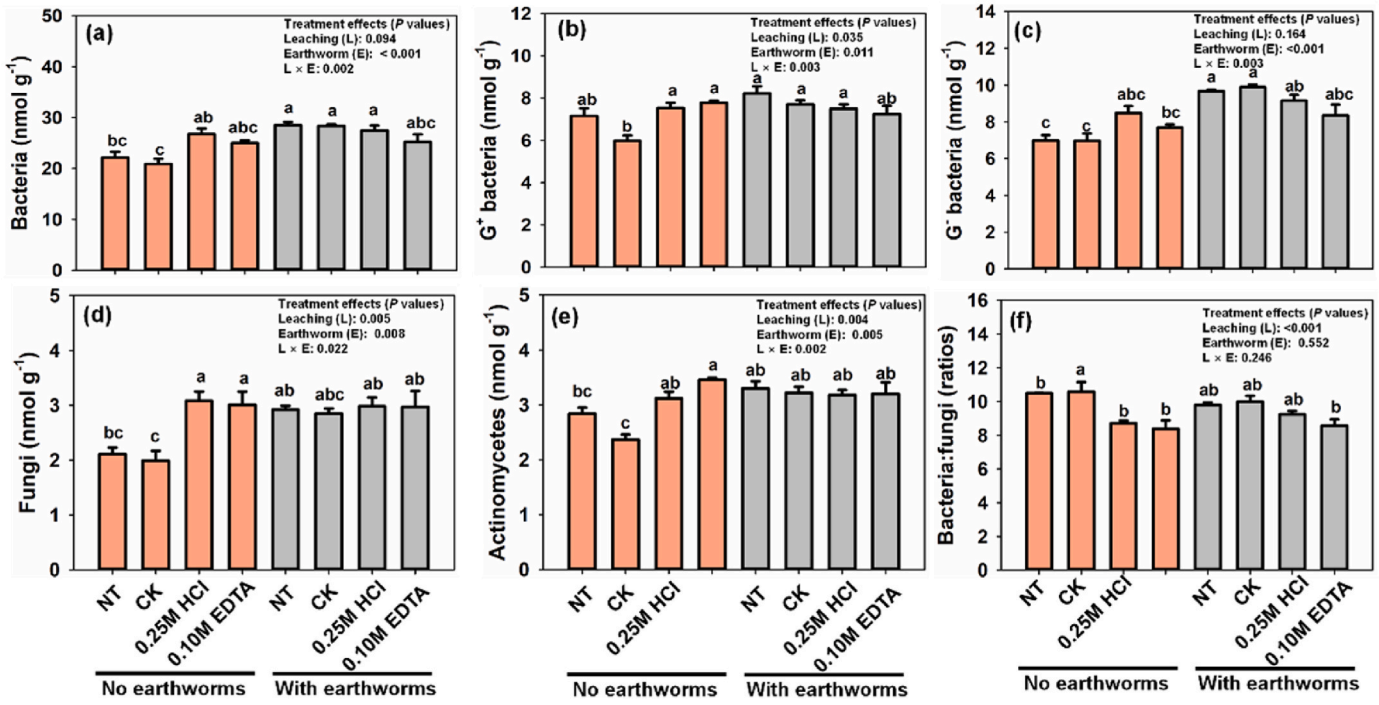

Fig. 4. The soil microbial composition after disposal of *A. hypochondriacus* L. residues. NT, the initial without treated biomass; CK, the deionized water extracted biomass; 0.25 M HCl, the 0.25 M HCl extracted biomass; 0.10 M EDTA, the 0.10 M EDTA extracted biomass. Treatment effects of leaching (L), earthworm (E), and interaction of leaching and earthworm are shown using two-way ANOVA. Data are mean ± standard error (n = 3). The letters on the column indicate significant differences among different treatments ($P < 0.05$).

(Wang et al., 2019b), which can enrich more than 100 mg kg^{-1} of Cd in aboveground biomass in Cd-contaminated soil (Li et al., 2010; Yu et al., 2020). Lu et al. (2017) discovered that the Cd mainly distributed in the cell wall of *A. hypochondriacus* L., and soluble fraction and pectate/protein-integrated Cd were the major chemical forms, which were in line with our results (Fig. 2(c)). The cell wall is the first barrier for preventing heavy metal from entering into the plant tissue, which is mainly constituted by cellulose, hemicellulose, pectate, and protein (Lu et al., 2020). In the current study, three different types of inorganic acid extractants (HCl, HNO_3, and H_2SO_4) had similar effects on the Cd removal under the concentration of 0.25 M, which was consistent with the results observed by Zhu et al. (2013), due to the similar pH of them (0.22, 0.23, and 0.19 respectively). Yang et al. (2020) discovered that the heavy metals removal efficiency of different acids was mainly determined by the movement of proton at low pH. Normally, high acid concentration destroyed cell wall structure, released Cd^{2+} from protein and polysaccharide, and replaced H^+ to Cd^{2+} (Wu et al., 2020). This result was verified by the data from Fig. 2. In addition, the vacuole compartment is another important mechanism for plants to tolerate and detoxify Cd (Wang et al., 2021). A previous study observed that the soluble cellular fraction of Cd in plants mainly distributes in the vacuole (Zhang et al., 2019). Thus, the high acid concentration may change the osmotic pressure of the cell, which results in the leakage of vacuolar content and subsequent Cd removal.

Our results show that 0.10 M EDTA also effectively removed Cd from plant biomass. Previous studies have reported that EDTA was of higher efficiency to remove Cd from the soil compared to HCl at the same concentrations (Li et al., 2019). This may be due to the different mechanisms of them to extract Cd from biomass–as HCl tends to destroy the cell wall structure to release Cd while EDTA prefers to mobile Cd by chelation and complexation. (Li et al., 2019).

The reaction time is another critical factor controlling extraction efficiency. In the present study, 24 h is the most suitable extraction time and extraction efficiency did not increase with a longer extraction time (Fig. S1). In fact, the acid concentrations and temperature (i.e., 0.25 M HCl; ambient temperature) we used were relatively low, compared to those commonly used for lignocellulose pretreatment (i.e., 12% H_2SO_4 combined with 121 °C in autoclave). This limited the destruction of plant cell wall polymers and influenced the extraction efficiency of different extractants with the longer reaction time (Wu et al., 2020). Microwave-assisted liquid extraction seems a proper option to improve the extraction efficiency, and this is the subsequent investigation we should work on.

4.2. Effects of different extractants on the Cd chemical forms in *A. hypochondriacus L*

The Cd chemical forms in plants determined the migration and toxicity of Cd, and the activity of the different Cd fractions gradually weakened from the ethanol extracted-Cd to the residue-Cd (Xin et al., 2017). In the present work, the NaCl extracted-Cd was the main Cd fraction in the stems and leaves of *A. hypochondriacus* L. (Fig. 2), which were consistent with the previous observations (Lu et al., 2017). The three different extractants were variably effective in the removal of different Cd fractions in stems and leaves (Fig. 2). The significant differences among the three treatments were that the deionized water had little effect on extracting the different Cd fractions except for the ethanol extracted- and the deionized water extracted-Cd. 0.25 M HCl could substantially remove all of the six Cd fractions in stems and leaves, especially for the NaCl extracted-Cd (pectates and proteins-integrated Cd) compared to the deionized water. A possible explanation for this result may be large amounts of chlorine ions with a powerful metal chelating ability than the chelates in cells can form a more stable substance (Song et al., 2015). In addition, the ethanol extracted- and deionized water extracted-Cd were the water-soluble Cd fractions in plants, and had the highest translocation capacity (Guan et al., 2018). This is powerful evidence for the Cd removal capacity of HCl. However, 0.10 M EDTA remarkably increased the concentrations of ethanol and deionized water extracted-Cd in leaves. It has been reported that EDTA could dissolve the inertia Cd fractions in soil and change them into labile fractions (Li and Shuman, 1996), as found in the 0.10 M EDTA extracted biomass.

4.3. The potential to utilize the extraction liquid as fertilizer after Cd purification

After liquid extraction, heavy metal wastewater will be generated. Thus, proper purification for extracted wastewater is necessary. In the present work, the purification efficiency of the three different treatments varied in different extracts (Table 2). For the extraction liquid of 0.25 M HCl, 4 Å molecular sieve outperformed the other two treatments, which indicated that the physical adsorption was better than the chemical precipitation for Cd purification. When adopting chemical precipitation, the purification efficiency of K_2CO_3 surpassed that of KOH, denoting carbonate precipitation was better than hydroxide precipitation in removing Cd from waste extracts. With respect to the extraction liquid of 0.10 M EDTA, surprisingly, all three treatments had no remarkable removal effects. Interestingly, EDTA is always considered as the interference substance during metal precipitation (Pohl, 2020). The low Cd removal efficiency of K_2CO_3 and KOH in 0.10 M EDTA was possibly due to the formation of EDTA-Cd chelates in the leachate, which preventing itself from precipitation or adsorption.

Chemical precipitation is the most universally and widely-used method for heavy metal-containing wastewater treatment, which is low cost and convenient. Lime, calcium hydroxide, and sodium hydroxide were always employed to purify the heavy metal-containing wastewater (Vikrant et al., 2019). It should be noted that the solubility of metal compounds normally follows the order of sulfides < carbonates < hydroxides (Pohl, 2020; Wang et al., 2005). In this study, the Cd removal efficiency of K_2CO_3 (after adjusting pH to 10) was higher than that of KOH (after adjusting pH to 11). Patterson et al. (1977) reported that when applying K_2CO_3 for wastewater purification, the yield of Cd precipitates under the pH value of 8.4 was equal to that of KOH treatment under the pH value of 10.4. This is due to that $CdCO_3$ is of lower solubility than $Cd(OH)_2$, and $Cd(OH)_2$ can be dissolved at higher pH (Malik et al., 2019).

Adsorption is another method to purify heavy metal-containing wastewater. The conventional purification techniques (e.g., chemical precipitation, coagulation, and flocculation) produce a high volume of chemical waste, which requires an extra cost for secondary disposal. Therefore, applying recyclable adsorbents such as activated carbon, zeolite, molecular sieve, and bentonite to absorb and remove heavy metal ions in sewage is more cost-effective (Burakov et al., 2018). 4 Å molecular sieve had a strong ability to adsorb Cd in wastewater (Rao et al., 2006). The main mechanism of 4 Å molecular sieve removing the Cd^{2+} in wastewater is ion-exchange adsorption (Xie et al., 2018). In the present work, the 4 Å molecular sieve had an extraordinary performance in removing Cd in leachate with low and high Cd concentrations. 4 Å molecular sieve also has surface precipitation functions for removing the metals (Xie et al., 2018). It changed the pH of leachate to partial alkalinity. Moreover, 4 Å molecular sieve can be regenerated by the way of chemical washing using NaCl and Na_2EDTA after adsorption (Shen et al., 2017). This can avoid the secondary disposal of an abundance of chemical waste during the chemical precipitation, and make the 4 Å molecular sieve more applicable and economical. However, in the extraction liquid of 0.10 M EDTA, Cd mainly existed as EDTA-Cd, preventing itself from adsorption. The ligand of EDTA can create an interference between Cd^{2+} and the ions in surface of 4 Å molecular sieve, stopping the exchange between ions.

The extracts of plant biomass after liquid extraction always contain large amounts of nutrients (e.g., N, K, P, Ca, and Mg) (Table 2). Some previous works showed that the liquid leaching from varies of plants

could be employed to assist phytoremediation by adding them to soil as nutrient additives (Han et al., 2019, 2020). Therefore, the extracted liquids after liquid extraction and safe treatment may be recycled as a useful resource for plant cultivation, hence the waste convert to the precious source. It can be seen from the results that the liquid extraction using 0.25 M HCl and 0.10 M EDTA not only had high Cd removal efficiency, but also released more nutrients in leachates. After Cd purification, three different purification techniques all significantly decreased the concentrations of total N and P in different extracts except for the total N in the extracts of 0.25 M HCl. In previous works, the flocculant of polyaluminium chloride and adsorbent of 4 Å molecular sieve were widely utilized to remove N and P in the aqueous solution for eutrophication management (Heiderscheidt et al., 2020; Zhang et al., 2021). In our study, both of the floccule after adding PAC or 4 Å molecular sieve can adsorb N. However, in the extracts of 0.25 M HCl, the adsorption sites of the floccule or 4 Å molecular sieve were occupied by the high concentration of Cd^{2+}, which limited their absorption for N. With respect to the K concentrations, the addition of KOH and K_2CO_3 reasonable introduced K into the solutions, while 4 Å molecular sieve could remove the K in solutions by ion exchange-adsorption (Cardoso et al., 2015). Concerning the Ca and Mg concentrations, the treatments of KOH and K_2CO_3 remarkably reduced Ca and Mg concentrations by forming carbonate- and hydroxide-precipitates. Similarly, 4 Å molecular sieve had substantially removed Ca and Mg in solution by ion exchange-adsorption (Chang et al., 2017). In general, nutrient concentrations of the solutions after purification with K_2CO_3 and 4 Å molecular sieve were higher than that with KOH.

Over all, the best option using 0.25 M HCl for liquid extraction meet the sewage discharge standard (GB 8978–1996, Cd $\leq$ 0.10 mg L^{-1}) and fertilizers standard (GB 38400–2019, Cd $\leq$ 3 mg kg^{-1}) of Cd in China after purifications. The cost of whole process was shown in Table S2. Using 0.25 M HCl for liquid extraction, the average total reagent cost for using potassium carbonate, potassium hydroxide, and 4 Å molecular sieve for liquid purification were 318 US$•$t^{-1}$, 104 US$•t^{-1}, and 32.5–32.7 US$•$t^{-1}$, respectively – we assume the biomass yield were 150–225 t hm^{-1}•y^{-1}, as reported by Li et al. (2013). Though the cost of using 0.25 M HCl for extraction combining with K_2CO_3 for purification was a little higher than the other two treatments, but this mean that the potassium resource is precious to recycle. This may be an additional way for reducing the waste production and restoration cost during phytoremediation and thus making the process more recyclable.

4.4. Soil toxicity of the leaching residues of A. hypochondriacus L

To investigate the possibility of direct and convenient disposal of the extracted residues after liquid extraction, our study appraised the viability and safety of direct returning the extracted residues into soil. However, soil ecological process which is complex and various may be severely disturbed by Cd-containing residues returning even at low soil total Cd concentrations (Wu et al., 2018). Therefore, our research carefully explored the influence of the extracted residues returning on soil chemical and biological components.

Earthworms are key and major organism components of soil community; they play a crucial role in soil physicochemical and biological functions. Earthworms have been widely used as a sensitive bioindicator of ecological toxicity of heavy metals in the terrestrial environments (Bai et al., 2020; Li et al., 2020). *E. fetida* has been widely used as a representative model species to assess the ecotoxicity of soil pollutants, which is also the standard species favored by the OECED for ecotoxicology test (Chai et al., 2020). Growth indexes had been recommended as sensitive biomarkers of heavy metal exposure (Sinkakarimi et al., 2020). As the results described herein, returning the biomass after Cd extraction to the soil influenced the growth of *E. fetida* (Table 3). The weight-loss rate of earthworms treated with the 0.25 M HCl extracted-biomass was higher than the control. As for the mortality of the tested earthworms, the inhibitory and lethal effects of EDTA on earthworms in our study was consistent with the previous work that EDTA significantly restrained the growth and survival of *E. fetida*. Duo et al. (2019) reported that 77% of earthworms were dead when exposed to 15 mmol kg^{-1} EDTA after 35 d, and Jones et al. (2007) recorded that only 56% of earthworms survived in an acidic sediment added with EDTA. The EDTA remained in the plant biomass and the acidity of the tested soil (acidic soil) led to the high earthworm mortality. Many researchers reported that earthworms contacted with a high dose of chemical contaminants had symptoms of toxicity and even death (Ye et al., 2016). In addition, the significant growth inhibitory effect of earthworms treated with the biomass after 0.25 M HCl extraction may be due to the nutrient loss from the biomass and the lower acidity compared to control (Table 2). Also, the chloride ion remaining in the plant biomass may be toxic to the growth and survival of earthworms. Owojori et al. (2008) reported that soil salinity (NaCl) negatively affected the activity of *E. fetida*. With reference to the Cd concentrations of earthworms, they generally reduced along with the decrease of Cd concentrations of the tested biomass. This was in line with the trends that the Cd concentration of *E. fetida* was higher than that of the control under higher Cd exposure in previous work (Sinkakarimi et al., 2020).

Biomass has been extensively applied to agricultural soil by direct disposal (Zhang et al., 2020). That biomass of metal accumulators may become a new source of metal contamination. Generally, the soil total Cd concentrations were positively related to the Cd amounts of the treated biomass. Earthworms did not influence the soil total Cd concentrations in all treatment because the burrowing and casting activities of them were not active, making the low accumulation of Cd in their body (1.1–4.1 mg kg^{-1}). However, earthworms decreased the soil available Cd concentrations, in agreement with the work reported by Wu et al. (2016) that earthworms reduced the availability and mobility of Cd by incorporating it into large aggregates. Li et al. (2019) also confirmed that the burrowing and ingesting activity of earthworms promoted the mixture of soil organic matter and the formation of organo-metal complexes. Meanwhile, the total Cd and available Cd concentrations of the soils treated with 0.25 M HCl-extracted biomass were all lower than those of the soil treated with 0.10 M EDTA-extracted biomass, which was attributed to the lower Cd concentrations and higher earthworm activity in the former treatments.

Microbial population is extremely sensitive to environmental disturbance (Frey et al., 2001; Stefanowicz et al., 2020). Thus, soil microorganisms are favorable indicators for monitoring the soil contamination (Wu et al., 2020a). Phospholipid fatty acids serve as biomarkers for determining certain microbial community composition and relative abundance of microorganisms, which has been employed to determine the heavy metals' toxic effects on soil microbes (Wu et al., 2020c). In the current investigation, earthworm addition had a positive effect on the abundance and biomass of total bacteria, gram-positive bacteria, gram-negative bacteria, fungi, actinomycetes, and the ratios of bacteria to fungi after returning the extracted biomass to the soil (Fig. 4). This finding is consistent with the results of Ren et al. (2021) that earthworm introduction could favor the activity of aerobic organism. Hussain et al. (2018) demonstrated the positive priming effect of earthworms on soil microbial diversity by improving soil structure and enhancing nutrient availability. In the absence of earthworm, the biomass of microbes was determined by the Cd concentrations of the biomass. The biomass and diversity of soil microbes normally decreased with higher concentrations of soil Cd (Wang et al., 2018). Meanwhile, in the presence of earthworms, no significant differences were found between all treatments, indicating the detoxification functions of earthworms to the contaminated biomass, and to maintain soil health and microbial activity. Thus, earthworm introduction is beneficial to the soil quality when the extracted biomass is returned to the field soil, which provides a potential way to the agricultural application for contaminated biomass disposal after liquid extraction.

5. Conclusions

Different extractants were evaluated for their potential of removing the Cd in the stems and leaves of *A. hypochondriacus* L. In addition, the purification treatments for Cd removal in the leachates and the possible impacts of directly returning the extracted biomass into soil were assessed. Firstly, we used HCl, HNO_3, H_2SO_4, and 0.10 M EDTA for liquid extraction, and found that 0.25 M HCl for 24 h was the best option. Secondly, with respect to the subsequent purification of Cd containing leachates, K_2CO_3 was better than KOH, and 4 Å molecular sieve was of the highest efficiency. Considering the secondary disposal of chemical sludge produced in chemical precipitation, utilizing the 4 Å molecular sieve to purify the extracted waste was more suitable and cost-effective. Finally, we examined the toxicity of the extracted biomass to *E. fetida* and microbes after disposal in soil. Although the concentrations of soil total Cd were all lower than the soil Cd contamination screening standard (GB15618-2018, pH $\leq$ 5.5, 0.3 mg kg^{-1}) in China, HCl- or EDTA-extracted biomass inhibited the growth of earthworms, probably due to the runoff of nutrients and the residual chemicals in the biomass. It should be noted that earthworms could offset the negative effects of the Cd-contaminated biomass on soil microbes, and earthworm introduction is recommended when the extracted biomass is returned to the field soil. These results demonstrated that 0.25 M HCl is the best option for removing the Cd in the *A. hypochondriacus* L., the 4 Å molecular sieve is the most economical and efficient purification reagent for the extracted liquids, and the extracted residues possess a certain of risk for soil organism but earthworms have a powerful ability to offset this limitation.

Further efforts of improving the liquid extraction efficiency and building a more practical and recyclable mode to use the extracted products are essential. To improve the liquid extraction efficiency for heavy metal decontamination of *A. hypochondriacus* L., the combined disposal technologies like alkali hydrolysis- and enzyme hydrolysis-pretreatment will be conducted.

Credit author statement

Long Lei: Investigation, Writing – original draft. Xiaoying Cui: Data curation, Investigation. Cui Li: Data curation, Investigation. Meiliang Dong: Data curation, Investigation. Rong Huang: Data curation, Investigation. Yingwen Li: Data curation, Investigation. Yongxing Li: Data curation, Investigation. Zhian Li: Supervision, Methodology, Writing – review & editing. Jingtao Wu: Supervision, Methodology, Writing – review & editing.

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.

Acknowledgements

The study were financially supported by the Program of Bureau of Science and Information Technology of Guangzhou Municipality (201903010022); the R & D program of Guangdong Provincial Department of Science and Technology (2018B030324003); the Key Special Project for Introduced Talents Team of Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou) (GML2019ZD0408); the National natural Science Foundation of China (31670513).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.131684.

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