The Comparative Potential of Four Compositae Plants for Phytoremediation of Karst Lead/Zinc Mine Tailings Contaminated Soil

Guangxu Zhu,^{a,*} Jingjing Zhao,^{b,#} Qing Chen,^a Qingjun Guo,^{c,*} Dandan Cheng,^a Ghatri Chhetri Dhruba Bijaya,^a and Wangjun Li^d

A tailings concentration gradient experiment was done to demonstrate the comparative evaluation of phytoremediation for Cd, Pb, and Zn in karst lead/zinc mine tailings contaminated soil by four Compositae plants (Crassocephalum crepidioides, Bidens pilosa, Ageratum conyzoides, and Cosmos bipinnatus). The results indicated that the four species grown in the dose-gradient mine tailings were tolerant to these metals to varying degrees. C. crepidioides and B. pilosa were more tolerant to heavy metals, while C. bipinnatus was the most sensitive species. Despite the high concentrations of Pb and Zn in the culture substrate, there was only a small increase in the concentration of these elements in the plant organs compared to the control. However, all species were observed to be shoot accumulators for Cd. C. crepidioides accumulated a maximum of 132.1 and 159.1 mg kg⁻¹ of Cd in leaves and stems, respectively, and these results were higher than those in the roots (67.2 mg kg⁻¹) and soil (75.8 mg kg⁻¹). C. crepidioides can be regarded as a Cd-hyperaccumulator, with a Cd removal of 4.56% to 9.97% from the soil polluted by lead/zinc mine tailings after single season cultivation. The comprehensive analysis result indicated that C. crepidioides exhibited the highest tolerance, biomass production, and removal of heavy metals, indicating its ability in the phytoremediation of contaminated soil in Pb/Zn tailings affected area.

DOI: 10.15376/biores.17.2.2997-3013

Keywords: Phytoremediation; Pb/Zn tailing; Compositae plants; Cd-hyperaccumulator; Southwest China

Contact information: a: College of Biology and Environment Engineering, Guiyang University, Guiyang, 550005, Guizhou, China; b: School of Resource & Chemical Engineering, Sanming University, Sanming, 365004, Fujian, China; c: Center for Environmental Remediation, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, 100101, China; d: The School of Environmental Science and Engineering, Suzhou University of Science and Technology, Suzhou, 215009, Jiangsu, China; *Corresponding authors: zhugx.10b@igsnrra.c.cn; guoqj@igsnrr.ac.cn; #The author contributed equally to this work.

INTRODUCTION

Tailings, in context to mining, are defined as the waste or noneconomic by-product generated during mineral exploitation and utilization, and other materials, which are generally deposited in open-air tailing ponds without any treatment (Adiansyah *et al.* 2015). The annual discharge of mine tailings in the world exceeds 10 billion tons (Adiansyah *et al.* 2015). In China, the major industrial solid waste comprehensive utilization of the 12^{th} Five-Year Plan of China indicates that mining activities generated 1.21×10^8 tons of mine tailings, with less than 14% comprehensive utilization rate every year since 2010 (Huang *et al.* 2018). As the materials remaining after the extraction and

beneficiation of ores, tailings often contain an elevated level of heavy metals, such as As, Cd, Cu, Pb, and Zn (Mendez and Maier 2008). Most of the heavy metal ions exhibit carcinogenic, teratogenic, mutagenic, and bioaccumulative amplification effects, as well as being non-biodegradable (Kong *et al.* 2021). These toxic elements can spread to surrounding areas through water erosion and wind erosion, which pose a great threat to the ecological environment and human health (Xiao *et al.* 2017; Ghazaryan *et al.* 2019). Therefore, tailings and ecological environmental issues are giving rise to growing concern from the community.

Conventional technologies for the remediation of heavy metal contaminated soils, such as soil excavation, fixation, landfilling, or soil leaching, mainly involve physical and chemical methods (Sun et al. 2018). However, due to the large quantity of soils in tailings surrounding area, decontamination by such conventional methods appears to be unfeasible due to the following: high cost; an additional pollution involved and adverse effects on the biological activity; structure; and fertility of soils (Wang et al. 2017; Cui et al. 2019). In comparison to traditional techniques, phytoremediation has been proposed as an environmentally friendly and low-cost remediation strategy, because it is a technology that uses plants to mitigate environmental problems without excavating the contaminating material and disposing of it elsewhere. Thus, phytoremediation reduces exposure risks for cleanup personnel and secondary contamination in transport (Idaszkin et al. 2017; Strachel et al. 2018). Phytoremediation mainly includes the methods of phytoextraction, stabilization, and -volatilization, among which, phytoextraction seems to be the most attractive environmental remediation strategy due to its versatility in usage. Phytoextraction mainly refers to the process of using naturally growing accumulator or hyperaccumulator to absorb, transfer, and enrich soil heavy metals to the aboveground part of plants, and then to harvest, incinerate, and recycle the heavy metals (Krämer et al. 1996; Ali et al. 2013). The key effect of this remediation technique depends on how to increase the aboveground biomass and its heavy metal content, that is, to find or select more plants with fast growth rate, good adaptability, large biomass, and the ability to accumulate high levels of the metal in its harvestable parts (Marrugo-Negrete et al. 2016; Aishah et al. 2019; Xu et al. 2019). However, relatively few plants possess all these attributes. The areas surrounding metal mines usually suffer from a wide range of chemical and physical limitations to plant growth including extreme pH, poor soil structure, high metal toxicities, low water availability, and a lack of organic matter and basic plant nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Nirola et al. 2016). Furthermore, in Guizhou, Guangxi, and other Southwest karst areas of China, tailings provide a more unfavorable and extreme substrate for plant growth (Li and Yang 2008). Therefore, screening for accumulator plants is of enormous importance for the future development of efficient plants for phytoremediation purposes in karst metal mining areas.

The Compositae (Asteraceae) family is one of the largest flowering plant families worldwide. Compositae are important primarily because of their many beautiful ornamentals, which are widely used as landscape greening and horticultural ornamentation (Liu *et al.* 2019). Compositae plants are excellent candidates for phytoremediation purposes because of their rapid growth, high biomass, strong breeding ability, ability to grow in soils polluted with heavy metals, and low impact on the food chain and human health (Wan *et al.* 2017; Yongpisanphop *et al.* 2017). In addition, Compositae plants are widely planted throughout China. Few studies have evaluated the utility of Compositae plants for phytoremediation of mine tailings or contaminated soil (Chen *et al.* 2005; Liu *et*

al. 2019). The authors performed a field investigation on the content of heavy metals in soils and 17 types of dominant plants from wasteland downstream of a lead/zinc (Pb/Zn) mine in the Northwest Guangxi Zhuang Autonomous Region. The authors compared the absorption and accumulation characteristics of heavy metals among different plants and demonstrated that the vegetation in the slag had formed a natural community dominated by herbaceous plants, in which Compositae plants were the dominant species. The authors' previous results also showed that Compositae plants, especially Crassocephalum crepidioides, Bidens pilosa, and Ageratum conyzoides, were more capable of enriching heavy metals in their tissues from contaminated soil than other studied plants (Zhu et al. 2018). The adaptability and remediation ability of these plants to heavy metal contaminated soils with different degrees need to be further studied. Cosmos bipinnatus, a popular floricultural plant, is widely distributed in China. It has been widely used as landscape greening and horticultural ornamentation with many advantages (Huang et al. 2017). Therefore, C. bipinnatus may be an important plant species for beautifying the environment in mine areas and remediating soils contaminated with heavy metals. However, little information is available on the accumulation potential of heavy metals by C. bipinnatus.

In the present study, four types of widely distributing Compositae plants, namely, *A. conyzoides*, *B. pilosa*, *C. crepidioides*, and *C. bipinnatus*, were tested as candidates for phytoremediation by growing seedlings in different grades of Pb/Zn mine tailings. The growth, tolerance index, and metal accumulation of seedlings were assessed. The objectives of this study were to elucidate the adaptive capabilities of the selected Compositae species for growth on mine tailings and the safe possible use of these plants for the phytoremediation of soils contaminated by tailings in the karst region of China.

EXPERIMENTAL

Experimental Materials

Mine tailings were randomly collected from the top 20 cm of a Pb/Zn tailings pond in Bijie City, Guizhou Province, China (26°46′ N, 104°23′ E). The area is characterized by a typical subtropical plateau monsoon humid climate with a mean annual temperature of 11.1 °C and a mean annual precipitation of approximately 1100 mm. The mine tailings pond, measuring 15 m in height, covering an area of around 1.0×10^4 m², has been stored for more than 30 years. Uncontaminated soil for the amendment was collected from the top 20 cm of a forest site in Guiyang suburb (26°33 N, 106°45′ E), which was 300 m away from roads and residential areas. The soil type is typical mountain yellow soil, which is consistent with that in the area of tailings pond. Seeds of plants under test were all collected from a flower market in Guiyang City.

Pot Experimental Design

A pot experiment was conducted under glasshouse conditions at Guiyang University. The experiment was arranged in a randomized block design with four treatments in quadruplicate, totaling 64 pots. The four treatments included 100% soil (CK), 20% mine tailings + 80% soil (w/w, T1), 80% mine tailings + 20% soil (w/w, T2), and 100% mine tailings (T3).

After mine tailings and soils were air-dried, passed through a 2-mm mesh sieve, and mixed thoroughly, 4 kg of each were transferred to cylindrical plastic pots (diameter

19 cm \times height 23 cm). The seeds of the four species were sown directly into the pots and covered with a layer of nutrient soil (1 to 1.5 cm) to ensure a high germination rate.

Before sowing, seeds were disinfected with 2.5% NaClO for 10 min and thoroughly washed with distilled water. After germination, four uniform seedlings were cultured in each pot. During the growth period, deionized water was supplied every other day to maintain soil moisture between 50% and 60% of water retention capacity. The plants were cultivated under greenhouse conditions with a natural photoperiod of 12 to 13 h, day/night temperature of 20 to 30/15 to 22 °C, and relative humidity of 70/85%. Metal-related phytotoxicity symptoms including chlorosis, reddish veins, and petioles, or curled leaves under any of the treatments were recorded during the experiment. The plants were harvested for measurements after 110 days of germination.

Sampling and Analysis

The soil physicochemical characteristics for each treatment were determined using standard procedures (Shi *et al.* 2020) and are listed in Table 1. During the harvesting stage, plants and rhizosphere soil samples were collected, and the plant height and root length were measured. The plant samples were separated into different parts (*e.g.*, leaf, stem, and root) and washed thoroughly with tap water followed by distilled water to remove adhering soil particles. The dry weights of the samples were recorded after treating with high-temperature desiccation under 105 °C for 30 min and drying at 65 °C for 3 days to gain a constant weight. Subsequently, each part was milled to a fine powder prior for chemical analysis. The soil samples were air-dried and passed through a 2-mm mesh sieve.

The plant samples were digested with a 4:1 (v/v) ratio of concentrated nitric acid (HNO₃) to perchloric acid (HClO₄) for the analysis of total heavy metals. The soil samples were digested using a concentrated mixture of HNO₃, HClO₄, and hydrofluoric acid (HF) with a ratio of 6:2:2 (v/v). The sample digestion process is as follows: 0.1 g of dry plant sample (0.5 g for plant samples) was placed in a Teflon crucible with 15 mL mixed acid The solution was then heated successively to 190 °C in a heating plate until it was nearly dry. The procedure was repeated with 2 mL mixed acid in order to totally remove silica and organic matter from the sample. After evaporating the digestion liquids to near dryness, the residuals were re-dissolved by HNO₃ (2%) and diluted with distilled water. The available metals were extracted with a 0.05 mol·L⁻¹ diethylene triamine pentaacetic acid (DTPA) solution, a 0.01 mol·L⁻¹ calcium chloride solution, and a 0.1 mol·L⁻¹ triethanolamine solution.

Water used for dilution and dissolution was purified using a Millipore deionizing system (Merck Millipore Co., Billerica, MA, USA) at 18.2 M Ω . The utilized HNO₃, HF, and HClO₄ were super pure reagents (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). The total metal concentrations in digestive solutions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 5300DV, PerkinElmer, Waltham, MA, USA) at the Center for Environmental Remediation, Institute of Geographic Sciences and Natural Resources Research (IGSNRR), Chinese Academy of Sciences. Certified reference materials (GSS- and GSV-) obtained from the Center of National Standard Reference Materials of China, as well as blank samples, were included in each batch of analyses for quality assurance procedures. Good agreement was obtained between the authors' method and certified values. All samples were analyzed in duplicate and the analytical precision was accepted when the relative standard deviation was within 5%.

Statistical Analysis

Statistical analyses in this study were performed using Microsoft Excel (Ver. 2013, Microsoft Corp., Redmond, WA, USA) and SPSS (Ver. 22.0, SPSS Inc., Chicago, IL, USA) software. Data were subjected to a one-way analysis of variance (ANOVA) and least significant difference (LSD) tests for mean comparisons. Differences at p < 0.05 were considered significant.

RESULTS AND DISCUSSION

Heavy Metal Concentrations in Test Soils

The total and available concentrations of individual heavy metals (Cd, Pb, and Zn) in the four different treated soils are summarized in Table 1. The content of heavy metals in the investigated soils varied among different cultivation patterns, and many of the examined metals were found at levels that greatly exceeded the regional background values. The contents of Pb and Zn in the CK soil exceeded the background values in Guizhou (35.2 mg·kg⁻¹ and 99.5 mg·kg⁻¹ for Pb and Zn, respectively) and the Cd content was close to the background value of 0.659 mg kg^{-1} (Zhu *et al.* 2014). In addition, the contents of the three elements in the pure tailing (T3 treatment) were much higher than the background values. The contents of Cd, Pb, and Zn were 115.1, 669.1, and 247.7 times higher than their corresponding contextual or original values, respectively. Among the metals, Zn is an essential minor nutrient for plant growth and is associated with many enzyme systems and certain other proteins. Thus, it is critical for a number of plant functions and health; however, excessive amounts of this element can limit plant growth, causing general chlorosis, and inhibiting photosynthesis, decreasing ATP synthesis and generating oxidative damage (Disante et al. 2010). Cd and Pb are toxic metals for plants and are usually adversely affect the growth, metabolism, and water status of plants by disturbing metabolic functions, increasing reactive oxygen species in cells and by interacting with functional groups in proteins and nucleic acids (Küpper and Andresen 2016; Hariharan et al. 2020). Regarding the effective coefficient of heavy metals (the proportion of the available state content in the total), the effective coefficient of Cd in the tailings was up to 57%, followed by Zn (28%), and Pb (15%).

Growth and Biomass Measurements

To select a candidate plant for phytoremediation, it is important to maintain good growth in the contaminated matrix (Alvarenga *et al.* 2008; Han *et al.* 2018). The growth parameters of plants including the average shoot height, root length, and dry weights of leaves, stems, roots, and the whole plant in each treatment are shown in Figs. 1 through 3. In the present study, the four species responded differently to heavy metal levels contained in the substrates. Generally, plant roots are the first organ to withstand metal toxicity in metal media. Therefore, root systems are more easily influenced by metals than aerial organs (Liu *et al.* 2018). In the dose-gradient experiments, the root lengths of *C. crepidioides* and *B. pilosa* showed no differences under various doses of metal stress (P > 0.05). However, under T3 treatment, the root lengths of *B. pilosa* and *C. bipinnatus* were reduced 27% and 34%, compared with the values for the control.

Treatment	pН	Alkali Hydrolysis	Available P	Available K	Organic	Tota	al Content (m	ng∙kg⁻¹)	Available Content (mg·kg ⁻¹)			
	-	N (mg kg ⁻¹)	(mg∙kg⁻¹)	(mg⋅kg⁻¹)	C (%)	Cd	Pb	Zn	Cd	Pb	Zn	
CK	6.52	193.5	14.6	95.3	0.85	0.62	153.9	209.1	0.27	5.44	35.3	
T1	6.84	155.8	12.4	90.4	0.72	15.4	3534	4806	10.4	661.3	2090	
T2	7.69	76.4	8.9	81.4	0.51	45.2	11,966	14,650	21.0	1647	2970	
Т3	8.03	38.6	7.2	76.3	0.37	75.8	23,550	24,640	43.2	3291	4874	

Table 1. Characteristics of the Mixtures in the Treatments

Table 2. Contents of Cd, Pb, and Zn in the Studied Plant Species (mg·kg⁻¹)

Tractmont	Orgon	C. crepidioides			A. conyzoides			B. pilosa			C. bipinnatus		
meannenn	Organ	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
CK		0.81	12.2	45.2	0.34	5.76	21.6	0.47	7.55	58.5	3.28	4.67	98.4
T1	Loof	35.7	19.6	248.6	26.8	21.5	232.1	18.6	9.32	186.3	7.55	29.0	195.1
T2	Leai	58.1	24.2	273.2	57.6	29.7	291.5	34.9	13.4	258.7	18.2	296.0	843.9
T3		132.1	31.3	568.3	99.7	74.1	683.0	47.3	19.9	376.6	34.8	998.8	1708
CK		0.76	13.2	43.2	0.38	3.84	24.6	0.31	18.6	39.8	7.27	2.38	57.4
T1	Stom	28.5	19.1	194.2	8.95	20.9	86.9	14.4	22.0	79.4	21.3	10.2	300.8
T2	Stern	44.1	25.2	266.0	28.2	33.1	130.6	23.5	26.0	149.4	41.1	40.3	569.6
Т3		159.1	86.5	550.4	58.4	81.1	287.6	42.0	64.6	259.8	53.1	80.5	775.7
CK		0.57	25.8	31.9	0.46	11.8	24.6	0.27	46.9	48.2	5.8	5.16	58.0
T1	Poot	19.0	69.2	130.2	6.9	59.8	112.8	11.7	71.3	94.7	13.3	33.1	335.1
T2	RUUL	35.6	169.1	283.1	22.9	121.4	176.5	25.1	142.1	167.9	30.0	169.1	482.7
Т3		67.2	257.2	452.7	25.8	158.2	253.5	30.8	175.8	275.0	60.2	680.9	1547.9

The data pertaining to plant height indicate that all the *C. bipinnatus* grown on contaminated soil (T1~T3) showed reduced heights and shorter roots when compared with CK. In comparison to the CK, the shoot heights of *C. crepidioides* and *B. pilosa* were significantly inhibited only by the T3 treatment, while that of *A. conyzoides* began to decrease significantly under the T2 treatment (P < 0.05).





Fig. 1. Shoot height of studied plant species for different treatments

Fig. 2. Root length of studied plant species for different treatments

With respect to the plant biomass, the dry weight of organs in the four selected species subjected to the tailings followed the order: leaf > stem > root. Among all of the species, *C. crepidioides* produced the highest leaf, stem, root, and total biomass measurements, followed by *B. pilosa*. With increasing doses of metals, there were distinct differences in the plant biomass among the study plants. Relative to the CK, there were no

significant differences in the biomass of all species under the T1 treatment (P > 0.05), except for *C. bipinnatus*. The T2 treatment provided significant biomass inhibition for *B. pilosa* (P < 0.05). After exposure to the T3 treatment, the biomasses of all species were significantly lower (P < 0.05), which is consistent with the decreased shoot height of the plant species.



Fig. 3. Dry biomass of studied plant species for different treatments

The tolerance index (TI) based on biomass was used to assess the tolerance of the four species in the same tailing level. The TI refers to the ratio of the total biomass under various tailing application treatments and the corresponding biomass in the control. A higher value represented a higher tolerance in the plants. Under T2 treatment, TI values for C. crepidioides, A. conyzoides, B. pilosa, and C. bipinnatus were 1.03, 0.94, 0.85, and 0.52, respectively, and those were 0.53, 0.54, 0.49, and 0.25 in the T3 treatment (Fig. 4). According to Audet and Charest (2007), if TI is less than 1, this implies that the plant suffered a stress due to metal pollution. In contrast, if TI is equal to 1, the plant is unaffected by metal pollution. The results shown in Table 4 indicated that C. crepidioides was the most tolerant species to tailings, while C. bipinnatus was the most sensitive one. The different abilities to maintain normal growth in the substrates reflected the difference in resistance or tolerance of plants to metal toxicity. Tolerance index values also demonstrated that a high concentration of complex heavy metals in soil had toxic and stressful effects on plant growth. Growth inhibition causing reductions in biomass production are commonly observed in plants subjected to high metal levels (Shi et al. 2016). Decreased plant biomass may be a result of many factors, such as metal-induced micronutrient deficiencies (Kováčik et al. 2009). In the present experiments, there were no obvious toxicity symptoms for the studied plants. Therefore, these findings at least partly indicate that the studied plants are heavy metal-tolerant plants.



Fig. 4. Tolerance index values of studied plant species for different treatments

Heavy Metal Uptake and Accumulation in Plant Organs

The mean concentrations of Cd, Pb, and Zn in the organs of the studied plants grown in the treatments containing different proportions of mine tailings are depicted in Table 2. Generally, heavy metal concentrations in the test plants exhibited a linear increase in response to an increasing amount of tailings; however, the extent of the increase also differed among the plant species.

Kabata-Pendias and Pendias (2001) proposed that the phytotoxic levels (based on the total contents in plant) for plant growth, should be established at 5 to 30, 30 to 300, and 100 to 400 mg·kg⁻¹ for Cd, Pb, and Zn, respectively. In the present study, most Pb, Zn, and Cd concentrations in the shoot organs after exposure to the high metal dose approached or were higher than the normal or phytotoxic levels. Among the metals, the higher mine tailing levels in treatments T1 to T3 led to even more marked increases in Cd concentrations when compared with other metals. C. bipinnatus showed best accumulation of Pb and Zn, especially under the T2 and T3 treatments. The highest concentrations of Pb and Zn were found in the leaf of C. bipinnatus grown in T3 treatment soil, which were 31.9 and 3.0, 13.5 and 2.5, 50.1 and 4.5 times those of in C. crepidioides, A. conyzoides, and B. pilosa, respectively. However, the biomass of C. bipinnatus subjected to the pure tailings was approximately one-fourth that of the control. In addition, C. crepidioides contained higher Cd concentrations in tissues than those of other plants. Note that the highest Cd concentration was 132.1 mg·kg⁻¹ in the leaf and 159.1 mg·kg⁻¹ in the stem of C. crepidioides grown in T3 treatment soil, and these values exceeded the Cdhyperaccumulator critical threshold values, fixed at 100 mg·kg⁻¹ (Baker and Brooks 1989). Moreover, the content of Cd in the shoot of C. crepidioides was higher than that in the root and soil. C. crepidioides demonstrated the basic characteristics of a Cd-hyperaccumulator (Baker and Brooks 1989; Antoniadis et al. 2017).

Regarding the distribution of heavy metals in plant organs, absorbed Cd and Zn mainly accumulated in the shoots, while Pb was mainly distributed in the root system. The bioaccumulation factor (BCF) and bio-transfer factor (BTF) were calculated to investigate the accumulation and translocation ability of trace metals in the soil-plant system. The BCF refers to the ratio of concentration of metals in the plant and the corresponding available

content of heavy metals in the soil (Monterroso *et al.* 2014). A greater BCF indicates a stronger accumulation ability of heavy metals. BCF value > 1 indicates the plant potential for the remediation of metal-contaminated soils (Branzini *et al.* 2012). The BTF was the ratio of the metal content in aboveground parts to that in roots, which roughly reflects the translocation capability for heavy metals from roots to the aboveground parts in plants (Pandey *et al.* 2016). The bioaccumulation factor and bio-transfer factor are important factors when one considers the potential of phytoextraction for a given species.

Tables 3 and 4 compare the average BCF and BTF values for Cd, Pb, and Zn in the different organs of the selected four species. The BCFs differ between plants species, indicating their different strategies for metal accumulation. As expected, the BCF values in the tested plants mostly decreased with increased tailing application. This decrease may be due to limited root-to-shoot transport. Similarly, a decrease in BCF values was reported by Sidhu et al. (2018) and Zhang et al. (2011) with enhanced metal concentrations in soil. In addition, the accumulation ability for metals decreased in the order of Cd > Zn > Pb, the study plants were favorable for accumulating Cd in their tissues compared to other metals. The most BCF values for Cd of > 1 in tested plants was mainly related to the higher availability coefficient of Cd in each treatment (Table 1). The values of BCFs in most plant organs for Pb did not exceed 0.1, except for the CK treatment, suggesting lower enrichment and movement ability of Pb in plants and soil. The BTF values varied among species and heavy metals in the same substrate. In general, the BTF values for Cd in the four species were higher than 1, except in the leaf of C. bipinnatus. These BTF values of > 1, indicative of Cd accumulated by the studied species were largely found in aerial part of the plant. It is worth noting that both BCF and BTF values for Cd in C. crepidioides were > 1, demonstrating that C. crepidioides may be a candidate for phytoextraction of Cd. For all plant species studied, the BTF values for Pb were less than 1. This is in agreement with the fact that Pb seems to be a metal with low mobility within plants, which accumulates mainly in roots (binding to ion exchange sites and extracellular precipitation; Bidar et al. 2007). The BTF values for Zn varied from 0.50 to 2.69 among the species, with the highest value occurring in A. conyzoides leaves.

Treat-	Organ	C. crepidioides		A. conyzoides			B. pilosa			C. bipinnatus			
ment	Organ	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
CK		3.00	2.25	1.28	1.26	1.08	0.61	1.74	1.41	1.66	12.15	0.86	2.79
T1	Loof	3.42	0.03	0.12	2.57	0.03	0.11	1.79	0.01	0.09	0.72	0.04	0.09
T2	Leai	2.76	0.01	0.09	2.74	0.02	0.10	1.66	0.01	0.09	0.86	0.18	0.28
T3		3.06	0.01	0.12	2.31	0.02	0.14	1.09	0.01	0.08	0.80	0.30	0.35
CK		2.81	2.44	1.22	1.41	0.71	0.70	1.15	3.45	1.13	26.93	0.44	1.63
T1	Stom	2.73	0.03	0.09	0.86	0.03	0.04	1.38	0.03	0.04	2.04	0.02	0.14
T2	Stern	2.10	0.02	0.09	1.34	0.02	0.04	1.12	0.02	0.05	1.95	0.02	0.19
T3		3.68	0.03	0.11	1.35	0.02	0.06	0.97	0.02	0.05	1.23	0.02	0.16
CK		2.11	4.78	0.90	1.70	2.18	0.68	1.00	8.70	1.37	21.59	0.96	1.64
T1	Deet	1.82	0.10	0.06	0.66	0.09	0.05	1.13	0.11	0.05	1.27	0.05	0.16
T2	RUUL	1.69	0.10	0.10	1.09	0.07	0.06	1.19	0.09	0.06	1.43	0.10	0.16
T3		1.55	0.08	0.09	0.60	0.05	0.05	0.71	0.05	0.06	1.39	0.21	0.32

Table 3. BCFs of Cd, Pb, and Zn in the Studied Plant Species

Treat-	Organ C.		C. crepidioides		A. conyzoides			B. pilosa			C. bipinnatus		
ment	Organ	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn	Cd	Pb	Zn
CK		1.42	0.47	1.42	0.74	0.50	0.90	1.74	0.16	1.21	0.56	0.89	1.70
T1	Loof	1.88	0.28	1.91	3.90	0.36	2.06	1.59	0.13	1.97	0.57	0.89	0.58
T2	Lear	1.63	0.14	0.96	2.51	0.24	1.65	1.39	0.09	1.54	0.61	1.75	1.75
T3		1.97	0.12	1.26	3.87	0.47	2.69	1.54	0.11	1.37	0.58	1.47	1.10
CK		1.33	0.51	1.36	0.83	0.32	1.02	1.15	0.40	0.82	1.25	0.45	0.99
T1	Stem	1.50	0.28	1.49	1.30	0.35	0.77	1.23	0.31	0.84	1.60	0.31	0.90
T2		1.24	0.15	0.94	1.23	0.27	0.74	0.93	0.18	0.89	1.37	0.24	1.18
T3		2.37	0.34	1.22	2.27	0.51	1.13	1.37	0.37	0.95	0.88	0.12	0.50

Phytoextraction Potential

By combining the weighted mean concentrations of the four analysed metals and dry weight in different organs, the metal extraction amount and removal rate by the whole plant from the experimental soil were calculated under the tailing application treatments (Table 5). As shown in Table 5, *C. crepidioides* took up relatively higher metals dose compared to the other plant species assayed in the current experiment. The accumulation amount pattern of Cd among the species was in the order of *C. crepidioides* > *B. pilosa* > *A. conyzoides* > *C. bipinnatus*; The Pb extraction amount in the various organs of species were ranked as *C. crepidioides* > *C. bipinnatus* > *B. pilosa* > *A. conyzoides*; except for *C. bipinnatus* under T3 treatment; the relative order of Zn uptake was seen as *C. crepidioides* > *B. pilosa* > *A. conyzoides*. *B. pilosa* > *A. conyzoides*; except for *C. bipinnatus* > *A. conyzoides*. *B. pilosa* > *A. conyzoides*; except for *C. bipinnatus* > *A. conyzoides*. *B. pilosa* > *A. conyzoides*; except for *C. bipinnatus* > *A. conyzoides*. *B. pilosa* > *A. conyzoides*; except for *C. bipinnatus* > *A. conyzoides*. *B. pilosa* > *A. conyzoides*; except for *C. bipinnatus* > *A. conyzoides*. *B. pilosa* has been demonstrated comparable Cd hyperaccumulative characteristics (Sun *et al.* 2009). In the present study, the extraction amount of Cd by *C. crepidioides* was 2.43 to 2.90 times that of *B. pilosa* under the same conditions.

Species	Treatment	Extraction	n Amount	(mg·pot⁻¹)	Removal Percentage (%)			
		Cd	Pb	Zn	Cd	Pb	Zn	
	T1	6.15	5.00	42.53	9.97	0.04	0.22	
C. crepidioides	T2	10.23	8.47	54.91	5.66	0.02	0.09	
	Т3	13.84	7.72	56.85	4.56	0.01	0.06	
A. conyzoides	T1	1.16	1.91	10.94	1.88	0.01	0.06	
	T2	2.57	3.05	13.64	1.42	0.01	0.02	
	Т3	2.52	3.24	16.96	0.83	0.01	0.02	
	T1	2.12	2.92	18.39	3.44	0.02	0.10	
B. pilosa	T2	3.94	4.83	27.93	2.18	0.01	0.05	
	Т3	5.71	7.50	43.21	1.88	0.01	0.04	
	T1	0.79	1.05	14.07	1.28	0.01	0.07	
C. bipinnatus	T2	1.44	6.43	28.59	0.80	0.01	0.05	
	Т3	1.05	10.19	25.89	0.35	0.01	0.03	

Table 5. Extraction Amount and Remo	oval Percentage of Metals by Test Plants
-------------------------------------	--

Concerning the metal removal percentage, there is a gradual decrease in percent removal of heavy metals with increasing concentration of tailings in the growth medium. The removal rates for Cd were the highest in the tested plants, followed by Zn, and Pb. The removal rates of Zn and Pb in the plants were minimal, generally less than 0.1% and less than 0.02%, respectively. With respect to Cd removal, *C. crepidioides* performed surprisingly well and its Cd removal rates reached 4.56% to 9.97%, indicating a strong potential to remediate Cd-contaminated soil.

When planning phytoremediation practices, the selection of suitable plant species is one of the most important factors in the process of phytoremediation. Plants that are used for remediation of tailings must tolerate a high concentration of heavy metals and harsh growth environment. In addition, the selected plants also should grow rapidly and have a high biomass production (Aishah et al. 2019; Xu et al. 2019). C. crepidioides, an annual herb Compositae plant with a fast reproduction rate, occurs widely throughout tropical and subtropical China. As field weeds, they are rarely used currently. While field weeds are a highly evolved plant group produced under the dual pressure of human and natural selection. Compared with crops, weeds have stronger resistance to adversity. After longterm natural evolution and artificial selection, they have extensive adaptability and tenacious vitality, and weeds also have a strong ability to compete for light, water, and fertilizer. The importance of this species is further increased by its luxuriant and fast growth rate and high biomass production that this plant can generate in natural conditions. More importantly, triple cropping would be possible within one year for C. crepidioides, which is an obvious superiority over other previously reported Cd accumulators. The findings of the present study denoted that C. crepidioides has better tolerance to metal toxicity than other plants. The underlying mechanism study, including physiological and biochemical indexes of plants needs to be further investigated. The high biomass production, the greater BTF and BCF values for Cd, and the higher concentrations of accumulated Cd in shoots and roots are characteristics of this species may be employed as a promising candidate to mitigate Cd from contaminated soils. Moreover, C. crepidioides can be used as ornamental plants and possess the double advantages of beautifying the environment and purifying the soil and has a high prospect of engineering application.

Nevertheless, the above research results were observed in a pot experiment only. In general, plants grown in pot experiment usually contain higher concentrations of trace elements than plants grown in the field. Plants grown in the field showed a 20% decrease of remediation efficiency compared to the plants from pot experimental conditions (Schmidt 2003). In spite of this, it is necessary to understand the principles of phytoremediation and the processes that occur under regulated conditions prior to use in contaminated areas. Additional studies are required to investigate the phytoremediation performance of *C. crepidioides* for heavy metals in combination with plant growth promoters and chelating agents to maximize the removal efficiency of heavy metals. Furthermore, long-term field experiments using *C. crepidioides* to remediate soils contaminated with mine tailings should be conducted in the future.

CONCLUSIONS

- 1. Pb/Zn tailings contained elevated concentrations of total Cd, Pb, and Zn, which impose high stress to species planted for revegetation. The four tested Compositae species exhibited distinctly different responses of growth and biomass yield when grown in media with high concentrations of Pb/Zn tailings. In general, *C. bipinnatus* was poorly tolerant to heavy metals and *C. crepidioides* was the most metal-tolerant species among the tested ones.
- 2. Based on the growth traits, BCF and BTF for Cd >1, accumulating Cd above 100 mg·kg⁻¹, which is the threshold value of Cd-hyperaccumulator, *C. crepidioides* can be regarded as a Cd-hyperaccumulator, with the Cd removal rates of 4.56% to 9.97% from the soil polluted by lead/zinc mine tailings after single season cultivation.
- 3. *C. crepidioides* exhibited the highest biomass production and concentration of heavy metals and demonstrated the greatest potential for remediation of those mine tailings rich of Cd in Southwest China.

ACKNOWLEDGMENTS

The authors are grateful for the National Natural Science Foundation of China (No. 41803018; 41625006), Discipline and Master's Site Construction Project of Guiyang University by Guiyang City Financial Support Guiyang University [SH-2020], Youth Science and Technology Talent Growth Project of Education Department of Guizhou Province (KY [2018] 294), and Innovation and Entrepreneurship Program for College Students of Guizhou Province (S202110976019).

REFERENCES CITED

- Adiansyah, J. S., Rosano, M., Vink, S., and Keir, G. (2015). "A framework for a sustainable approach to mine tailings management: Disposal strategies," *Journal of Cleaner Production* 108(Part A), 1050-1062. DOI: 10.1016/j.jclepro.2015.07.139
- Ali, H., Khan, E., and Sajad, M. A. (2013). "Phytoremediation of heavy metals- concepts and applications," *Chemosphere* 91(7), 869-881. DOI: 10.1016/j.chemosphere.2013.01.075
- Aishah, R. M., Shamshuddin, J., Fauziah, C. I., Arifin, A., and Panhwar, Q. A. (2019).
 "Using plant species for phytoremediation of highly weathered soils contaminated with zinc and copper with application of sewage sludge," *BioResources* 14(4), 8701-8727. DOI: 10.15376/biores.14.4.8701-8727
- Alvarenga, P., Gonçalves, A. P., Fernandes, R. M., Varennes, A. D., Vallini, G., Duarte, E., and Cunha-Queda, A. C. (2008). "Evaluation of composts and liming materials in the phytostabilization of a mine soil using perennial ryegrass," *Science of The Total Environment* 406(1-2), 43-56. DOI: 10.1016/j.scitotenv.2008.07.061
- Antoniadis, V., Levizou, E., Shaheen, S. M., Ok, Y. S., Sebastian, A., Baum, C., Prasad, M. N. V., Wenzel, W. W., and Rinklebe, J. (2017). "Trace elements in the soil-plant

interface: Phytoavailability, translocation, and phytoremediation – A review," *Earth-Science Reviews* 171, 621-645. DOI: 10.1016/j.earscirev.2017.06.005

- Audet, P., and Charest, C. (2007). "Heavy metal phytoremediation from a meta-analytical perspective," *Environmental Pollution* 147(1), 231-237. DOI: 10.1016/j.envpol.2006.08.011
- Baker, A. J. M., and Brooks, R. R. (1989). "Terrestrial higher plants which hyperaccumulate metallic elements – A review of their distribution, ecology and phytochemistry," *Biorecovery* 1, 811-826.
- Bidar, G., Garcon, G., Pruvot, C., Dewaele, D., Cazier, F., Douay, F., and Shirali, P. (2007). "Behavior of *Trifolium repens* and *Lolium perenne* growing in a heavy metal contaminated field: Plant metal concentration and phytotoxicity," *Environmental Pollution* 147(3), 546-553. DOI: 10.1016/j.envpol.2006.10.013
- Branzini, A., Gonzalez, R. S., and Zubillaga, M. (2012). "Absorption and translocation of copper, zinc and chromium by Sesbania virgata," Journal of Environmental Management 102, 50-54. DOI: 10.1016/j.jenvman.2012.01.033
- Chen, B. D., Tang, X. Y., Zhu, Y. G., and Peter, C. (2005). "Metal concentrations and mycorrhizal status of plants colonizing copper mine tailings: Potential for revegetation," *Science in China, Series C* 48(1), 156-164. DOI: 10.1007/BF02889814
- Cui, L. Q., Chen, T. M., Yin, C. T., Yan, J. L., Ippolito, J. A., and Hussain, Q. (2019).
 "Mechanism of adsorption of cadmium and lead ions by iron-activated biochar," *BioResources* 14(1), 842-857. DOI: 10.15376/biores.14.1.842-857
- Disante, K. B., Fuentes, D., and Cortina, J. (2010). "Sensitivity to zinc of Mediterranean woody species important for restoration," *Science of The Total Environment* 408(10), 2216-2225. DOI: 10.1016/j.scitotenv.2009.12.045
- Ghazaryan, K., Movsesyan, H., Ghazaryan, N., and Watts, B. A. (2019). "Copper phytoremediation potential of wild plant species growing in the mine polluted areas of Armenia," *Environmental Pollution* 249, 491-501. DOI: 10.1016/j.envpol.2019.03.070
- Han, Z. Y., Guo, Z. H., Zhang, Y., Xiao, X. Y., and Peng, C. (2018). "Potential of pyrolysis for the recovery of heavy metals and bioenergy from contaminated *Broussonetia papyrifera* biomass," *BioResources* 13(2), 2932-2944. DOI: 10.15376/biores.13.2.2932-2944
- Hariharan, A., Harini, V., Sandhya, S., and Rangabhashiyam, S. (2020). "Waste Musa acuminata residue as a potential biosorbent for the removal of hexavalent chromium from synthetic wastewater," Biomass Conversion and Biorefinery 1-14. DOI: 10.1007/s13399-020-01173-3
- Huang, J. J., Yang, Z. B., Li, J. H., Liao, M. A., Lin, L. J., Wang, J., Yang, Y. X., Liang, D., Xia, H., Wang, X., and Ren, W. (2017). "Cadmium accumulation characteristics of floricultural plant, *Cosmos bipinnatus*," *Chemistry and Ecology* 33(9), 807-816. DOI: 10.1080/02757540.2017.1384820
- Huang, L. G., Li, Y. Y., Zhao, M., Chao, Y. Q., Qiu, R. L., Yang, Y. H., and Wang, S. Z. (2018). "Potential of *Cassia alata* L. coupled with biochar for heavy metal stabilization in multi-metal mine tailings," *International Journal of Environmental Research and Public Health* 15(3), 494. DOI: 10.3390/ijerph15030494
- Idaszkin, Y. L., Lancelotti, J. L., Pollicelli, M. P., Marcovecchio, J. E., and Bouza, P. J. (2017). "Comparison of phytoremediation potential capacity of *Spartina densiflora*

and *Sarcocornia perennis* for metal polluted soils," *Marine Pollution Bulletin* 118(1-2), 297-396. DOI: 10.1016/j.marpolbul.2017.03.007

- Kabata-Pendias, A., and Pendias, H. (2001). *Trace Element in Soils and Plants*, 3rd Ed., CRC Press, Boca Raton, FL, USA.
- Kong, Q. P., Shi, X. Q., Ma, W. W., Zhang, F. Z., Yu, T., Zhao, F, Zhao, D. D., and Wei, C. H. (2021). "Strategies to improve the adsorption properties of graphene-based adsorbent towards heavy metal ions and their compound pollutants: A review," *Journal of Hazardous Materials* 415, article no. 125690. DOI: 10.1016/j.jhazmat.2021.125690
- Kováčik, J., Klejdus, B., Hedbavny, J., Štork, F., and Bačkor, M. (2009). "Comparison of cadmium and copper effect on phenolic metabolism, mineral nutrients and stressrelated parameters in *Matricaria chamomilla* plants," *Plant and Soil* 320(1), 231-242. DOI: 10.1007/s11104-009-9889-0
- Krämer, U., Cotter-Howells, J. D., Charnock, J. M., Baker, A. J. M., and Smith, J. A. C. (1996). "Free histidine as a metal chelator in plants that accumulate nickel," *Nature* 379(6566), 635-638. DOI: 10.1038/379635a0
- Küpper, H., and Andresen, E. (2016). "Mechanisms of metal toxicity in plants," *Metallomics* 8(3), 269-285. DOI: 10.1039/c5mt00244c
- Li, M. S., and Yang, S. X. (2008). "Heavy metal contamination in soils and phytoaccumulation in a manganese mine wasteland, South China," *Air, Soil and Water Research* 1(1), 257-273. DOI: 10.4137/ASWR.S2041
- Liu, S. L., Yang, R. J., Tripathi, D. K., Li, X., He, W., Wu, M. X., Ali, S., Ma, M. D., Cheng, Q. S., and Pan, Y. Z. (2018). "The interplay between reactive oxygen and nitrogen species contributes in the regulatory mechanism of the nitro-oxidative stress induced by cadmium in Arabidopsis," *Journal of Hazardous Materials* 344(C), 1007-1024. DOI: 10.1016/j.jhazmat.2017.12.004
- Liu, Z. L., Chen, W., and He, X. Y. (2019). "Evaluation of hyperaccumulation potentials to cadmium (Cd) in six ornamental species (Compositae)," *International Journal of Phytoremediation* 20(2), 1464-1469. DOI: 10.1080/15226514.2018.1501343
- Marrugo-Negrete, J., Marrugo-Madrid, S., Pinedo-Hernández, J., Durango-Hernández, J., and Díez, S. (2016). "Screening of native plant species for phytoremediation potential at a Hg-contaminated mining site," *Science of The Total Environment* 542(A), 809-816. DOI: 10.1016/j.scitotenv.2015.10.117
- Mendez, M. O., and Maier, R. M. (2008). "Phytostabilization of mine tailings in arid and semiarid environments – An emerging remediation technology," *Environmental Health Perspectives* 116(3), 278-283. DOI: 10.2307/40040140
- Monterroso, C., Rodríguez, F., Chaves, R., Diez, J., Becerra-Castro, C., Kidd, P. S., and Macías, F. (2014). "Heavy metal distribution in mine-soils and plants growing in a Pb/Zn mining area in NW Spain," *Applied Geochemistry* 44, 3-11. DOI: 10.1016/j.apgeochem.2013.09.001
- Nirola, R., Megharaj, M., Aryal, R., and Naidu, R. (2016). "Screening of metal uptake by plant colonizers growing on abandoned copper mine in Kapunda, South Australia," *International Journal of Phytoremediation* 18(4), 399-405. DOI: 10.1080/15226514.2015.1109599
- Pandey, S. K., Bhattacharya, T., and Chakraborty, S. (2016). "Metal phytoremediation potential of naturally growing plants on fly ash dumpsite of Patratu thermal power

station, Jharkhand, India," *International Journal of Phytoremediation* 18(4), 87-93. DOI: 10.1080/15226514.2015.1064353

- Schmidt, U. (2003). "Enhancing phytoextraction: The effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals," *Journal of Environmental Quality* 32(6), 1939-1954. DOI: 10.2134/jeq2003.1939
- Shi, P., Zhang, Y., Zhang, Y., Yu, Y., Li, P., Li, Z. B., Xiao, L., Xu, G. C., and Zhu, T. T. (2020). "Land-use types and slope topography affect the soil labile carbon fractions in the Loess hilly-gully area of Shaanxi, China," *Archives of Agronomy and Soil Science* 66(5), 638-650. DOI: 10.1080/03650340.2019.1630824
- Shi, X., Chen, Y. T., Wang, S. F., Pan, H. W., Sun, H. J., Liu, C. X., Liu, J. F., and Jiang, Z. P. (2016). "Phytoremediation potential of transplanted bare-root seedlings of trees for lead/zinc and copper mine tailings," *International Journal of Phytoremediation* 18(11), 1155-1163. DOI: 10.1080/15226514.2016.1189399
- Sidhu, G. P. S., Bali, A. S., Bhardwaj, R., Singh, H. P., Batish, D. R., and Kohli, R. K. (2018). "Bioaccumulation and physiological responses to lead (Pb) in *Chenopodium murale* L.," *Ecotoxicology and Environmental Safety* 151, 83-90. DOI: 10.1016/j.ecoenv.2017.12.068
- Strachel, R., Wyszkowska, J., and Baćmaga, M. (2018). "An evaluation of the effectiveness of sorbents in the remediation of soil contaminated with zinc," *Water Air and Soil Pollution* 229(7), 235. DOI: 10.1007/s11270-018-3882-2
- Sun, W., Ji, B., Khoso, S. A., Tang, H. H., Liu, R. Q., Wang, L., and Hu, Y. H. (2018). "An extensive review on restoration technologies for mining tailings," *Environmental Science and Pollution Research* 25(34), 33911-33925. DOI: 10.1007/s11356-018-3423-y
- Sun, Y., Zhou, Q., Wang, L., and Liu, W. (2009). "Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator," *Journal of Hazardous Materials* 161(2-3), 808-814. DOI: 10.1016/j.jhazmat.2008.04.030
- Wang, L., Ji, B., Hu, Y. G., Liu, R. Q., and Sun, W. (2017). "A review on *in situ* phytoremediation of mine tailings," *Chemosphere* 184(1), 594-600. DOI: 10.1016/j.chemosphere.2017.06.025
- Wan, X. M., Lei, M., and Yang, J. X. (2017). "Two potential multi-metal hyperaccumulators found in four mining sites in Hunan Province, China," *Catena* 148(1), 67-73. DOI: 10.1016/j.catena.2016.02.005
- Xiao, R., Wang, S., Li, R., Wang J. J., and Zhang, Z. (2017). "Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China," *Ecotoxicology and Environmental Safety* 141, 17-24. DOI: 10.1016/j.ecoenv.2017.03.002
- Xu, L., Xing, X., Liang, J., Peng, J., and Zhou, J. (2019). "In situ phytoremediation of copper and cadmium in a co-contaminated soil and its biological and physical effects," RSC Advances 9(2), 993-1003. DOI: 10.1039/C8RA07645F
- Yongpisanphop, J., Babel, S., Kruatrachue, M., and Pokethitiyook, P. (2017).
 "Phytoremediation potential of plants growing on the Pb-contaminated soil at Song Tho Pb mine, Thailand," *Soil and Sediment Contamination* 26(4), 426-437. DOI: 10.1080/15320383.2017.1348336
- Zhang, X. F., Xia, H. P., Li, Z. A., Zhuang, P., and Gao, B. (2011). "Identification of a new potential Cd-hyperaccumulator *Solanum photeinocarpum* by soil seed bank-

metal concentration gradient method," *Journal of Hazardous Materials* 189(1-2), 414-419. DOI: 10.1016/j.jhazmat.2011.02.053

- Zhu, G. X., Xiao, H. Y., Guo, Q. J., Song, B., Zheng, G. D., Zhang, Z. Y., Zhao, J. J., and Okoli, P. C. (2018). "Heavy metal contents and enrichment characteristics of dominant plants in wasteland of the downstream of a lead-zinc mining area in Guangxi, Southwest China," *Ecotoxicology and Environmental Safety* 151, 266-271. DOI: 10.1016/j.ecoenv.2018.01.011
- Zhu, H. L., Liu, H. Y., Long, J. H., and Yan, Z. Y. (2014). "Pollution characteristics of heavy metals in soils in typical contaminated areas, Guizhou Province," *Earth and Environment* 42(4), 505-512. DOI: 10.14050/j.cnki.1672-9250.2014.04.017

Article submitted: February 5, 2021; Peer review completed: April 24, 2021; Revised version received and accepted: February 8, 2022; Published: April 7, 2022 DOI: 10.15376/biores.17.2.2997-3013