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Remediation for trace metals in polluted soils by turfgrass assisted with chemical reagents

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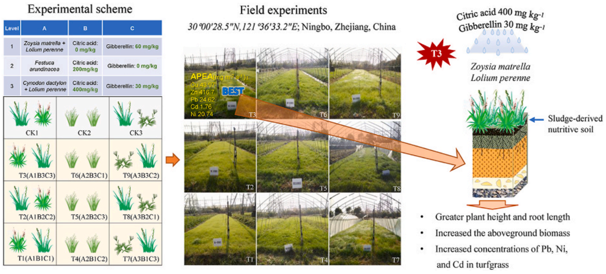
HIGHLIGHTS

- An orthogonal test of turfgrass remediation of trace metals (TMs) was designed.
- Grass species and planting patterns affected the growth factor and TMs enrichment.
- Citric acid increased turfgrass biomass and the bioconcentration factors of TMs.
- Auxin spraying facilitated the bioaccumulation of Cd by turfgrass.
- Intercropping of *Z. matrella* and *L. perenne* showed a potential for TMs remediation.

GRAPHICAL ABSTRACT



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ABSTRACT

Trace metal pollution in soils is one of the universal environmental problems in the world. Phytoremediation is a green, safe, ecological, and economic method to achieve continuous reduction of soil pollutants. Turfgrass is a plant with great landscape value and has considerable biomass when used for remediation of trace metal contaminated soil. However, its remediation ability needs to be improved in future application. The combined application of turfgrass, citric acid (CA) and auxin (gibberellin, GA_3) were applied in the phytoremediation of an artificial nutritive soil derived from sludge, and a field scale orthogonal experiment (L_9) was conducted to understand the interaction effect and obtain the optimum phytoremediation. Experimental results showed that the types and cultural patterns of turfgrass mainly determined plant height, root length and trace metal concentration in turfgrass, however CA treatment was prone to increase the aboveground biomass and the concentrations of most trace metals in turfgrasses, especially the concentration of Ni in turfgrass. GA_3 spraying significantly increased the concentration of Cd in turfgrass. The culture patterns of turfgrass played 42.4% influence on acid-extractable Cd, while CA applying had 53.8% influence on the acid-extractable Ni. The annual phytoextraction amount of trace metals based on five mowing a year were proposed to assess the remediation ability of treatments, which of the combination treatment (T3, intercropping *Zoysia matrella* and *Lolium perenne*, and applying 400 mg kg^{-1} CA and 30 mg kg^{-1} GA_3) were 1.6–2.1 times higher CK group. This research provides technical reference for intercropping turfgrass for remediation of trace metals in sludge-derived nutritive soil.

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1. Introduction

With the rapid development of modern industry and agriculture, these industrial and agricultural activities are easy to release potentially toxic trace metals into soil and water (Oladoye et al., 2022). Seriously, trace metals cannot be biodegraded, resulting the continuous accumulation and migration of trace metals in the environment will pose a great threat to human health and ecological security (Sidhu et al., 2020). Compared with traditional remediation methods, phytoremediation is an innovative method to reduce or detoxify pollutants by using green plants (Sidhu et al., 2020; Li et al., 2021b). Turfgrass has the characteristics of rapid growth, huge root and biomass, strong stress resistance, and more valuably, it can enrich a variety of trace metals from soils such as Cu, Zn, Pb, Cd, and Ni, showing excellent potential on phytoremediation (Desjardins et al., 2018; Zhang and Ji, 2019; Steliga and Kluk, 2020). Even more surprisingly, turfgrasses are highly regenerative and can be mowed multiple times in a year. Each mowing removes the trace metals accumulated in the aerial parts and allows the turfgrass to start new growth and obtain additional biomass (Zhao et al., 2011). Even with all its benefits, turfgrass has one deficiency is that the concentrations of trace metals accumulated in it are much lower than those in hyperaccumulator plants, such as *Sedum alfredii H.* and *Brassica juncea* L. (Cao et al., 2020). Therefore, improving the extraction ability of trace metals in turfgrass is the key to realize the phytoremediation of trace metals by turfgrass.

Improving the low bioavailability of heavy metals in contaminated soil is an effective way to increase phytoremediation efficiency (Ma et al., 2020). Therefore, chelating agents, such as ethylenediaminetetraacetic acid (EDTA), citric acid (CA) are used to assist remediation (Lestan et al., 2008; Gul et al., 2021). Chelating agents can activate metal ions in soil and improve their bioavailability through direct metal chelation (Sarret et al., 2001), thus improving the accumulation and extraction efficiency of heavy metals in plants. (Bulak et al., 2014; Rostami and Azhdarpoor, 2019). For large-scale field applications, biodegradable chelating agents are preferred, such as CA, a natural organic acid owning an appropriate biodegradation rate in the soil (Ehsan et al., 2014).

Another strategy to improve the efficiency of phytoremediation is to increase the biomass of plant. Many plant growth regulators have been used as modifiers for phytoremediation (Bulak et al., 2014), such as gibberellins, cytokinins, and salicylic acid. In addition, these growth regulators had been proved to improve plant extraction efficiency by promoting plant tolerance to trace metals (Gou et al., 2010; Chen et al., 2020). For example, spraying gibberellin acid ($C_{19}H_{22}O_6$, CAS:77-06-5) and indoleacetic acid ($C_{10}H_9NO_2$, CAS:87-51-4) on leaves improved the ability of plants to extract Pb (Hadi et al., 2013). Moreover, the polycultures in intercropping was demonstrated as a promising cultivation to improve phytoremediation system flexibility (Cui et al., 2018; Desjardins et al., 2018) and biomass (Cui et al., 2021). Recently, the combination of growth regulators and chelating agents showed a great potential on increasing metal absorption. The chelating agent can penetrate the membrane and reinforce metal absorption, while the growth regulators can attenuate metal stress through promoting chelation, vacuolar sequestration, and modulation of antioxidant pathways (Mao et al., 2015; Liang et al., 2019; Singh et al., 2021).

Based on the research achievements stated above, we hypothesized that coupling chelating agents and plant growth agents could improve the ability of turfgrass (monocropping or intercropping) to remediate heavy metals in soil. Therefore, a field experiment was designed, including three factors of turfgrass planting pattern, citric acid spraying, and GA_3 spraying. The annual extracted amount was put forward as an evaluation index of phytoextraction efficiency, which presented the amount of trace metals removed from contaminated soil by mowing the turfgrass. The research provides scientific basis and feasibility for the large-scale utilization of turfgrass as a phytoremediation in the later period.

2. Materials and methods

2.1. Experimental site and materials

The experimental field locates at Ruixue Flower Company (30°00′28.5 ″N, 121°36′33.2 ″E) in Zhenhai District, Ningbo City, Zhejiang Province. The area of the tested land is 600 m^2 and rectangular. Before the experiments, the land was divided into 10 blocks of similar size, the size of each block is 3 × 20 m^2, numbered from T1 to T9, and the left block was marked as CK and used as a blank control group.

The artificial nutritive soil derived from sludge, namely SDN soil, used in this study was provided by Ningbo High-tech Zone Chunli Energy-saving Technology Co., Ltd. The SDN soil was dark brown, which was produced by mixing the aerobic compost sludge with lime, rice husk, and clay mineral. According to the physicochemical properties listed in Table 1, SDN soil was slightly alkaline, with high cation exchange capacity (CEC) and soil organic matter (SOM) content, and is more loose than ordinary soil. The concentrations of trace metals of Cu, Zn, Pb, Cd, and Ni in SDN soil were lower than the risk screening values of the risk control standard for soil contamination of development land (GB 36600–2018), but still higher than their respective risk screening values of the risk control standard for agricultural land (GB 15618–2018). Only by reducing the heavy metal concentrations to lower than values in GB 15618, can the potential risk of heavy metals be eliminated.

The turfgrass seeds of *Zoysia matrella*, *Lolium perenne*, *Festuca arundinacea*, and *Cynodon dactylon* were provided by the Ruixue Flowers Company. Citric acid (CA) was purchased from Huayan Chemical Co., Ltd., and gibberellin (GA_3) was obtained from Shangdong Bestway Pesticide Co., Ltd.

2.2. Experimental design and turfgrass harvesting

Orthogonal experimental design is a an efficient, rapid, and economical experimental design method to study multi-factors at multi-levels, and had been widely used in the field of environmental science (Dai et al., 2016). An orthogonal test of L_9 (3 × 3) was designed involving three regulatory factors: the turfgrass species and cropping pattern (Factor A), CA treatment (Factor B), GA_3 treatment (Factor C), as shown in Table 2. In terms of Factor A, there are four kinds of turfgrass species commonly used in local gardens, and monocropping or intercropping were determined according to their growth characteristics. *F. arundinacea* grows upright and has strong stress resistance and vigorous growth, so it can achieve good turfgrass coverage even if

Table 1
Physicochemical properties of SDN soil.

Indicator	Values				
The moisture content (%)	29.9 ± 2.97				
Bulk density (g cm^{-3})	0.93 ± 0.04				
pH	7.6 ± 0.1				
Soil organic matter (g kg^{-1})	205 ± 59				
Cation exchange capacity (cmol kg^{-1})	190 ± 25				
Metal concentration	Total	BCR1	BCR2	BCR3	Residue
Cu (mg kg^{-1})	45.38 ± 1.54	2.9%	11.4%	29.4%	56.3%
Zn (mg kg^{-1})	393.6 ± 24.3	16.7%	28.2%	15.3%	39.7%
Pb (mg kg^{-1})	100.4 ± 3.5	9.6%	9.7%	7.2%	73.5%
Cd (mg kg^{-1})	5.45 ± 0.13	12.6%	26.0%	13.0%	48.5%
Ni (mg kg^{-1})	64.32 ± 0.67	9.3%	10.3%	12.6%	67.8%

Table 2
Design of orthogonal test.

Experiment code	Treatment	Factor A	Factor B	Factor C
		Culturing pattern	CA (mg kg^{-1})	GA_3 (mg kg^{-1})
T1	A1B1C1	A1 (*Zoysia matrella* + *Lolium perenne*)	B1 (0)	C1 (60)
T2	A1B2C2		B2 (200)	C2 (0)
T3	A1B3C3		B3 (400)	C3 (30)
T4	A2B1C2	A2 (*Festuca arundinacea*)	B1 (0)	C2 (0)
T5	A2B2C3		B2 (200)	C3 (30)
T6	A2B3C1		B3 (400)	C1 (60)
T7	A3B1C3	A3 (*Cynodon dactylon* + *Lolium perenne*)	B1 (0)	C3 (30)
T8	A3B2C1		B2 (200)	C1 (60)
T9	A3B3C2		B3 (400)	C2 (0)

planted alone. *L. perenne* can support each other and obtain good growth (Desjardins et al., 2018; Zhang and Ji, 2019). *Z. matrella* and *C. dactylon* have fibrous roots, which give them a large root biomass, but creeping stems, which give them a low aboveground biomass and less turfgrass coverage.

The SDN soil was laid on the 10 blocks with a 10-cm thick. According to the soil bulk density in Table 1 and the dosages in Table 2, CA and GA_3 solutions with the required concentration were calculated and prepared, and then the solutions were evenly sprayed on each test plot by spraying the same volume of solution per unit area. CA was sprayed at the beginning of the experiment into SDN soil, while GA_3 was sprayed on turfgrass surface 15 days after seed germination. After that, the same dosage of CA or GA_3 was applied 15 days after each mowing. During the growth of turfgrass, except watering, all the plots were not added other artificial substances.

The turfgrass was first harvested 45 days after germination, and then harvested every two months, leaving 3-cm stubble heights after each harvesting. Five harvesting were carried out in October 2017, December 2017, February 2018, April 2018, and June 2018. Because the turfgrass were dormant in July and August, there was no harvest.

2.3. Sample collection

Each sampling was taken 1–3 days before each mowing. Two sampling units with a size of 1.5 m × 5 m were freely selected for each block. Collect the whole grasses and the corresponding root soils, and the whole grasses were separated into subsamples of roots and shoots. The roots and shoots were first thoroughly washed with tap water, and then washed with distilled water, drained with paper (Zhang and Ji, 2019). The length and biomass of fresh plant shoots and roots were measured with a tape and an electronic balance respectively (Desjardins et al., 2018). All the subsamples were separately dried in 105 °C oven for 30 min to kill the enzyme activity, and then dried in 60 °C oven for 24 h to volatilize water to a constant weight. For the soils, 0–10 cm depth of soil was collected with the cutting-ring method (Chen et al., 2021). All soil samples were air dried at room temperature and then ground to pass through a 100-mesh sieve after removing impurities. All plant and soil samples were stored in polyethylene bottles for analysis.

2.4. Sample analysis

Trace metals in soils were digested with a microwave digestion system (MARS6, CEM, United States) and the total amounts were determined by flame AAS (AAnalyst 800, PerkinElmer, United States). The operationally defined fractionation analysis of heavy metals in soil was carried out using a modified procedure proposed by the Community Reference Bureau (BCR), which includes four fractions, namely acid-extractable (BCR1), reducible (BCR2), oxidizable (BCR3) and the residual (Rauret et al., 1999). After extraction, the concentrations of each fraction were same as the total amount. In terms of total quantity measurement, the certified reference materials for plants and soil (GBW10016, GBW07430) were obtained from Institute of Geophysical and Geochemical Exploration, Chinese Academy of Geological Sciences for accuracy quality control. The measured concentrations of Cu, Zn, Pb, Cd, and Ni in GBW10016 were 18.83 ± 0.96, 50.15 ± 1.44, 1.47 ± 0.09, 0.0666 ± 0.0002 and 3.47 ± 0.39 mg kg^{-1}, respectively, with the relative standard deviation ranged in 2.64%–6.00%; while these indicators in GBW07430 were 30.80 ± 1.23, 97.36 ± 3.53, 57.90 ± 2.45, 0.235 ± 0.029 and 28.88 ± 0.92 mg kg^{-1}, respectively, presented acceptable relative standard deviations in the range of 1.24%–7.42%.

2.5. Evaluating the bioconcentration and translocation ability of trace metals

Bio-concentration factor (BCF) is an important indicator to evaluate the repair ability of a plant. The BCF is calculated as the ratio of metal concentration in plant root to metal concentration in soil (Ghazaryan et al., 2019a). The calculation formula is as follows:

$$\mathrm{BCF} = \frac{\text{Metal content in plant shoot, mg kg}^{-1}}{\text{Metal content in soil, mg kg}^{-1}} \tag{1}$$

The most intuitive evaluation for phytoremediation is how much trace metals are removed from the soil by transferring trace metals to the aerial parts of plants (Wu et al., 2021). Therefore, the annual phytoextraction amount (APEA) is proposed to assess phytoremediation efficiency and is calculated by the following equation:

$$\mathrm{APEA} = \sum_{i=1}^{5} C_i \times \mathrm{biomass}_i \tag{2}$$

Where C_i represents the trace metals concentrations (mg kg^{-1}) in the aboveground of turfgrass in each mowing. Biomass $_i$ indicates the aboveground biomass per unit area of turfgrass mowed every two months (kg m^{-2}). The APEA (mg m^{-2} a^{-1}) is simplified as a sum of five mowing in 10 months of due to the negligible growth in the remaining two months as mentioned previously.

2.6. Data processing and statistical analysis

The trace metals concentrations in soils and plants are expressed as a dried weight. The experimental data is processed by adopting Microsoft Excel 2016 for analysis of the means, standard deviation, coefficient of variation, range analysis of orthogonal test, as well as for drawing analysis. IBM SPSS Statistics 25 (SPSS Inc. IBM Corporation, Armonk, NY, USA) was used for statistical analysis and regression analysis. One-way analysis of variance (ANOVA) was performed to compare the means of different treatments.

3. Results and discussion

3.1. Growth performance of turfgrass under treatments

Plant height, root length, and biomass are the most basic indexes in plant morphology and are direct manifestations of plant growth performance. As shown in Table S1, turfgrass grew well, reaching 14–23 cm in height during 45 days of growth, including new growth after each mowing. It could be seen that T6 treatment had the most advantageous effect on turfgrass growth with the largest root mass and root length, while T3 treatment had the largest aboveground biomass and T9 treatment had the largest plant height. As a remediation plant, a large biomass facilitates extraction of trace metals, and a well-developed root system is also key to acquire trace metals (Wei et al., 2021).

The range analysis of the orthogonal experiment for plant growth characteristics manifested that Factor A had the greatest effect on root length and plant height, followed by CA treatment and GA_3 foliar spray (Fig. S1, Table S2). The influence degree of three factors on turfgrass biomass was Factor B > Factor A > Factor C from high to low (Table S2).

As reported, the growth performance of different turfgrasses species varies greatly, for example, their plant height ranges from 10 cm to 110 cm and biomass is from n to n × 100 mg cm^{-2} (Cui et al., 2018, 2021). In addition, CA had a significant promoting effect on the growth performance of turfgrass, especially on biomass, which increased with the increase of application amount. Similar results had been reported that adding CA was beneficial to increase the biomass of *Juncus effusus* L. (Najeeb et al., 2009), and to increase shoot and root length of *Brassica napus* L. (Zaheer et al., 2015). It could be attributed to the facilitation of CA to release of soil nutrients, such as available phosphorus from soil minerals (Jones, 1998), thus promoted plant growth. However, GA_3 played little influence on turfgrass growth in the study. Usually, GA_3 is involved in many process in plant growth, and exogenously applying was used to protect the photosynthetic apparatus against heavy metal stress (Bulak et al., 2014). Considering the well growth of turfgrass in this experiment, the effect of GA_3 on promoting plant growth was probably too weak to show up.

3.2. Accumulation of trace metals in turfgrass

The reduction of trace metals in soil can be realized by mowing the lawn and separating it from the field (Habiba et al., 2015). The concentrations of trace metals in turfgrass varied greatly, manifesting as large error bars, shown in Fig. 1, indicating that the uptake of trace metals of turfgrass had great differences in different time/season (Fig. S2). Usually, plants growing at higher temperatures have a higher translocation rate (the ratio of total metal content in shoot to total metal content in whole plants) for trace metals due to higher leaf transpiration (Pourghasemian et al., 2013; Ma et al., 2017). However, another study reported that concentrations of most elements were higher in grasses in winter than in summer (Rea et al., 2021). This is probably because those cool-season grass grasses, such as tall fescue and ryegrass, have optimum growth temperature in 10–20 °C, and in summer, when the temperature exceeds 30 °C, their growth will slow down and even enter a dormant state. In addition, turfgrass grows better when they are maintained at a suitable mowing height. Therefore, the growth of turfgrass was affected by seasonal changes and human mowing in the study, and these factors could also affect its behavior of enriching trace metals. However, the relationship between the number and timing of mowing and the accumulation of trace metals in turfgrass is still unclear.

The effects of treatments on the extraction capacity of turfgrass varied with different trace metals. The accumulation of Ni was most obvious. Treatments T2, T3, T6, T8 and T9 significantly increased Ni concentration in roots, and T3, T8 and T9 increased concentration Ni in leaves. For Cu, Zn and Cd, only a few treatments achieved significant differences. Treatment T5, T6 and T9 significantly increased Cu concentration both in roots and aerial parts; Treatment T3 and T9 only increased Zn concentration in roots; and treatment T3 and T9 significantly increased the Cd concentration in roots and aerial parts, but treatment T6 significantly decreased the concentrations of Zn and Cd in aerial parts. Pb was the only metal that did not change significantly in roots and aerial parts. Li et al. (2012) used stable Pb isotopes tracing technology showed that plants can take Pb from the atmosphere, and Pb concentration in $PM_{2.5}$ was 0.241 ± 0.144 μg m^{-3} in Chinese cities and was second only to Zn (Shen et al., 2021). Therefore, the main source of lead in turfgrass is probably atmospheric Pb rather than soil Pb, which could explain the unchanged Pb concentration in turfgrass.

According to the range analysis of orthogonal test (Table S3) and ANOVA analysis results, for the concentration of trace metals in turfgrass shoots, Factor A had the greatest influence degree on Cu, Zn and Pb (45.5% ~ 56.3%), reaching the significant level ($p < 0.05$), and the intercropping of *C. dactylon* + *L. perenne* showed the optimum promotion, indicating the complementarity of turfgrass was beneficial to the extraction of heavy metals. Previous studies also proved that intercropping of *Festuca arundinacea*, *Medicago sativa*, and *Salix miyabeana* was more effective in absorbing trace metals such as Cd, Cu, Pb, and Zn, than individual monocrop (Desjardins et al., 2018). The GA_3 spraying played dominant (48.8%) and significant ($P < 0.05$) effect on Cd concentration. It has been reported that GA_3 helps plants to synthesize proline and phenolic compounds to relieve Cd stress (Ali and Hadi, 2015). CA applying had the predominant influence on Ni concentration (74.6%), which reached the significant level ($p < 0.05$) and increased with increasing dosage. CA can complex with trace metals, increasing the dissolution of trace metals in the soil, and facilitating the absorption and enrichment of plant roots (Gerhardt et al., 2017).

For the concentration of trace metals in turfgrass roots (Table S4), the CA applying played a dominant and significant ($p < 0.05$) influence on Cu, Cd, and Ni concentration (41.6%–51.8%), which were increased monotonically with CA dosage, while Factor A produced predominant influence on the concentrations of Zn and Pb (76.9% and 72.9%) but not reached the significance level. However, the GA_3 spraying had little and insignificant effect on the bioaccumulation of trace metals in turfgrass root, which might be related to the way of foliar spray.

Among all treatments, T9, the combination of *C. dactylon* + *L. perenne* and CA 400 mg kg^{-1}, was outstanding in promoting the uptake of trace metals with the significantly high concentrations of Cu, Zn, Pb and Ni both in roots and aerial parts of turfgrasses. In addition, T3 treatment, the turfgrass culturing pattern of *Z. matrella* + *L. perenne,* CA 400 mg kg^{-1}, and 30 mg kg^{-1} GA_3, Cd and Ni accumulation reached the maximum of 0.44 mg kg^{-1} and 5.94 mg kg^{-1}, and increased by 54.5% and 40.1% compared to the CK, respectively. In terms of concentration in plants, these two treatments suggested great potential in promoting phytoremediation of trace metals.

3.3. The change of total trace metals in SDN soils during remediation

The change of trace metals concentration in SDN soils is the most intuitive manifestation of the remediation efficiency. During the observation period of planting, the changes of trace metals concentration in the SDN soil of each turfgrass planting every two months are shown in Fig. 2. The figure shows the concentrations of each trace metals of the SDN soil sampled few days before each mowing. During observation, the concentrations of Cd, Zn, Pb, Cu, and Ni in control group and treatment group decreased by 52%–73%, 32%–53%, 28%–45%, 13%–22%, and 8%–20%, respectively, implied that planting turfgrass significantly reduced the concentrations of trace metals in SDN soil. There were few cases for some treatments of individual elements, especially Pb, the monitoring values of the same treatment in the second sampling were even higher than those in the corresponding first sampling. This could be attributed to the uneven product mixing of the SDN soil and the restabilization of the SDN soil aggregates after their first using in landscaping (Jelusic and Lestan, 2014; Huang et al., 2017; Zhou et al., 2020).

According to the range analysis of orthogonal test (Table S5) and ANOVA analysis of trace metals of five sampling in SDN soil, the influences of Factor B on concentrations of all metals in SDN soil were dominant (58.9% ~ 86.9%) and significant ($P < 0.05$), which made the concentration of trace metals in turfgrass increased with the increase of CA dose. Li et al. (2021b) show that the higher the concentration of activator, the better the extraction of trace metals. Factor A shared 30.3%–32.1% influence on the concentration of Cu, Zn and Ni, but only the effects on Cu and Ni reached the significance ($p < 0.05$), suggesting the selectivity to trace metals by different turfgrass species or planting method. GA_3 spraying had very small and insignificant influence on the concentration of trace metals in SDN soils (3.6% ~ 11.3%). The reason was because GA_3 is more to enhance the stress resistance of plants to trace element stress to promote plant growth, while the plant growth-promoting micro-organisms reduces the extraction of trace metals in the soil (Singh et al., 2021).

In addition, sharp decreases were found in Zn, Pb and Cd between the first/second (Cd) or the second/third sampling (Zn and Pb) (Fig. 2 b, c and d), which was unlikely to be caused by plant uptake, but probably due to the rapid migration of trace metals in the SDN soil caused by acid

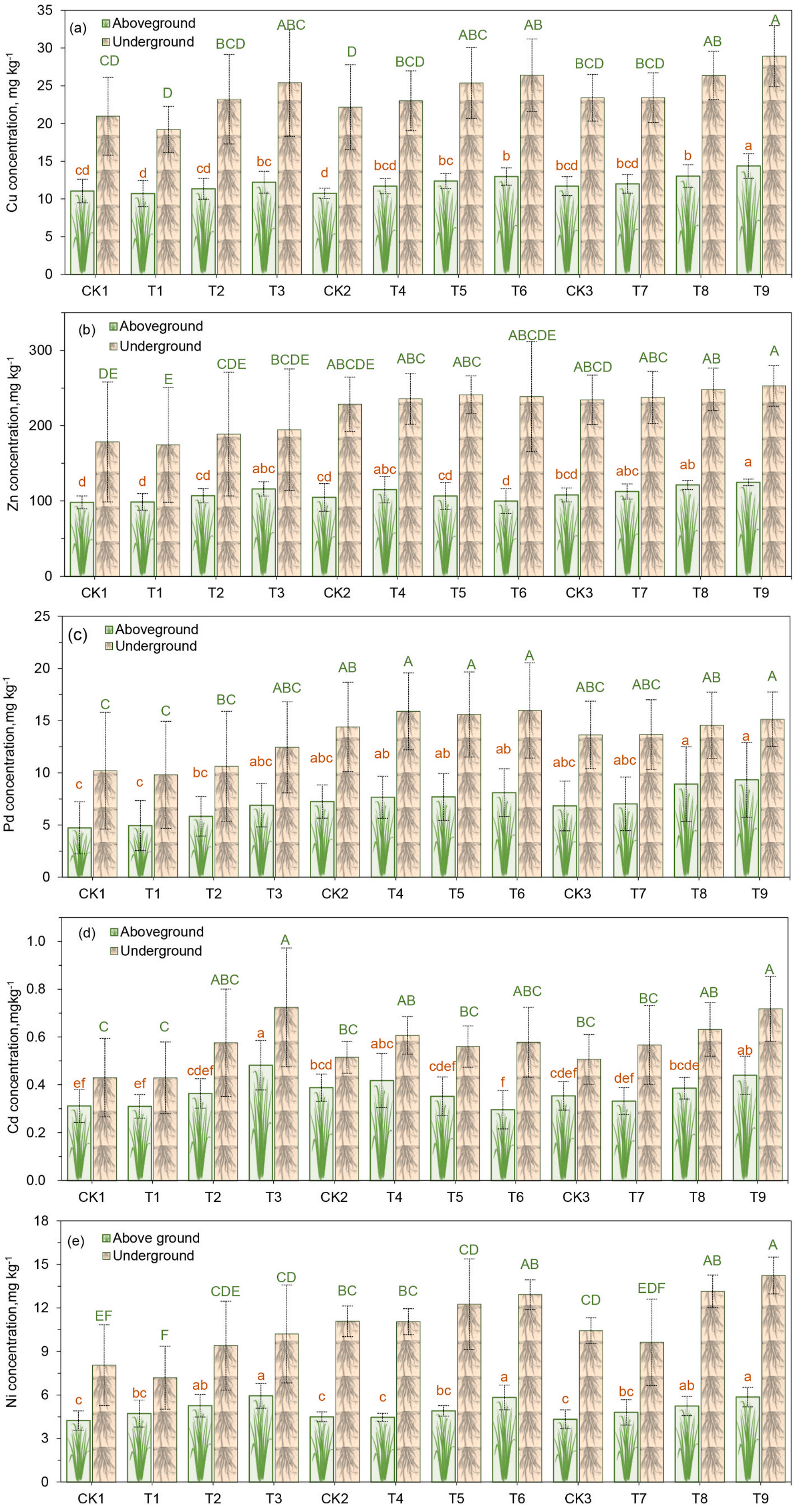

Fig. 1. The concentrations of trace metals (a) Cu, (b) Zn, (c) Pb, (d) Cd, and (e) Ni in the plants. The column is the average metal concentrations of every two months from October 2017 to June 2018, and the error bar represents the standard deviation. The treatments are listed in Table 2. Columns with the different uppercase and lowercase letters indicate that the differences between the treatments are significant, $p < 0.05$.

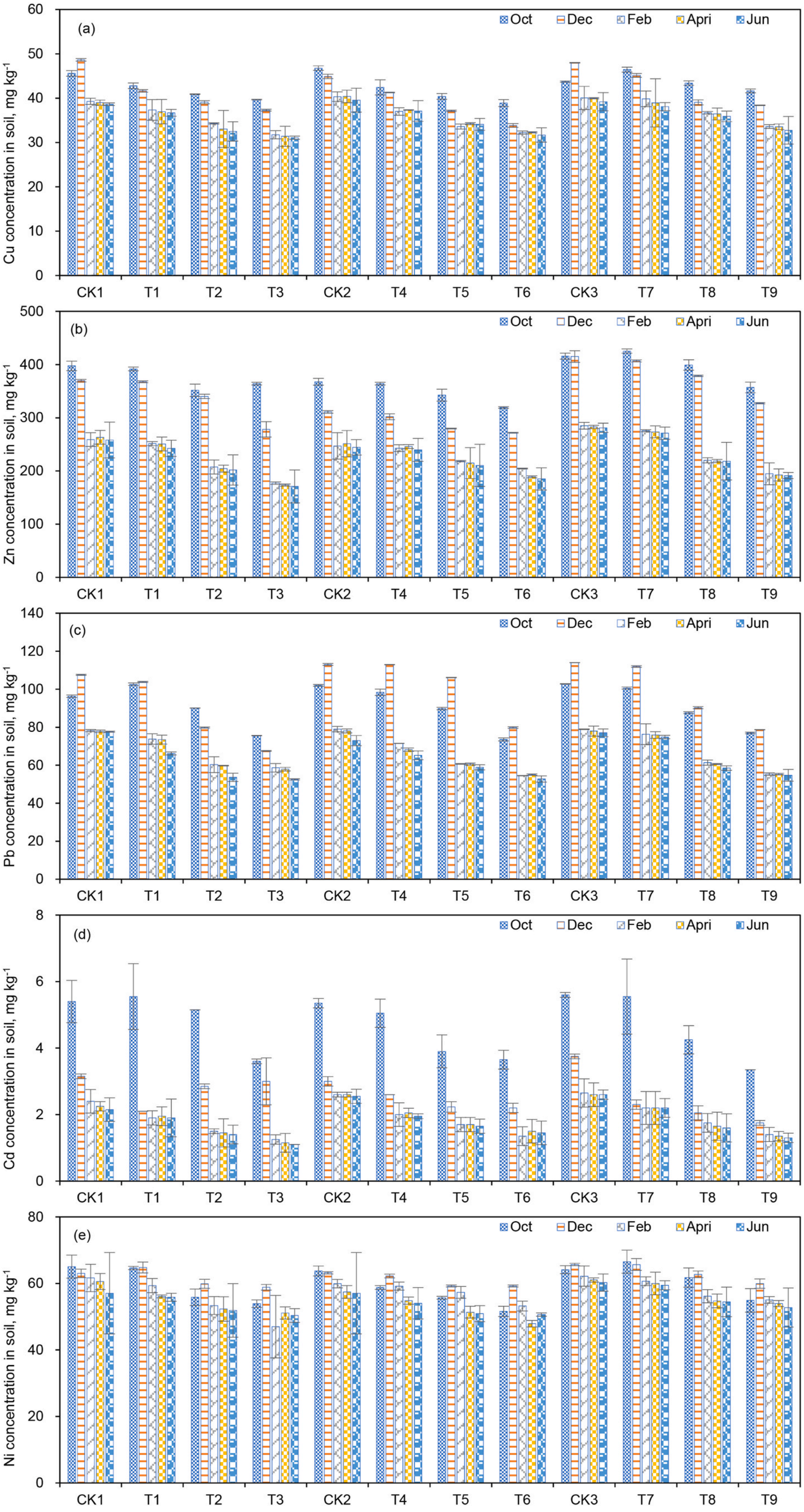

Fig. 2. The effect of treatments on the total concentration (a–e) of trace metals in soils. The column is the average of monitoring results of duplicates in October 2017, December 2017, February 2018, April 2018 and June 2018, respectively. And the error bar of total concentration represents the standard deviation. The treatments are listed in Table 2. Columns with the different letters indicate that the differences between the treatments are significant, $p < 0.05$.

rain washing under the open condition, so that plants could not absorb them in time (Jelusic and Lestan, 2014; Gill et al., 2017). These metals were reported had a high activity in compost sludge (Zhao et al., 2017). For example, the aerobic composting decreases the contents of the residue fraction for Pb, Zn, and Cd but significantly increases the contents of the total mobile fractions for Zn and Pb (Liu et al., 2007). Therefore, the reduction of trace metals concentration in SDN soil in the open field could not be completely attributed to the uptake of turfgrass. At the same time, when sludge compost is used in landscaping land, some trace metals with high activity have leaching risk, which needs to be paid attention to (Li et al., 2021a).

3.4. The speciation distribution of trace metals in SDN soil after remediation

The forms of trace metal greatly decides their mobility and bioavailability in soil, and also affects their phytoremediation efficiency by turfgrass (Ahmadipour et al., 2015). Usually, the bioavailability of heavy metals in BCR1, BCR2, BCR3, and the residue fraction decreased successively. The results of BCR analysis of trace metals for initial SDN soil are listed in Table 1. The five trace metals were all dominated in the residue fraction, and then Cu and Ni were secondary dominated in BCR3, Zn and Cd were in BCR2, while the proportions of Pb in BCR2 and BCR1were very close. According to most researches which point out that the fraction of BCR1 is the most active form of trace metals (Lu et al., 2019). The proportion of BCR1 of the five trace metals in SDN soil ranged in 2.9%–16.7%, in the decrease order of $Zn > Cd > Pb, Ni \gg Cu$. Among them, the proportions of Zn, Cd, Pb, and Ni were close to or even greater than 10%, represented a relative high activity (Yang et al., 2013). This was consistent with the speculation about leaching of Zn, Cd, and Pb in SDN soil at the beginning of land use mentioned in the previous section.

As shown in Fig. 3, after turfgrass growing and compared to the respective CK, most treatments increased the proportion of Zn, Pb, and Cu in the residual fraction of SDN soil, while the percentages of Ni (T2, T3, T6, T8, and T9) and Cd (T1, T2, T4, T5, and T6) in the residual fraction were decreased; In fraction of BCR1, the proportion of Pb and Cu were reduced, while the percentages of Ni and Zn were mainly increased, and that of Cd were mostly decreased. In the fraction of BCR2, the majority treatments reduced the proportion of Zn and Pb, meanwhile, most treatments raised the proportion of Cd, Cu and Ni. For the fraction of BCR3, the proportions of Cu, Zn and Ni were decreased under most treatments, while the ratio of Cd and Pb increased.

The orthogonal analysis (Table S6) and ANOVA analysis results on BCR1 of trace metals suggested that, among the three factors, Factor A exhibited the dominant influence on BCR1 of Cd, Zn, and Pb (42.4%–56.1%). Furthermore, different turfgrasses showed different activation advantages to the three trace metals, though only BCR1 of Zn and Cd presented significantly different among turfgrass strategies. Factor B played a dominant influence on the proportion of BCR1 of Ni (53.8%), with the maximum proportion appeared at 200 mg kg^{-1}, which was significantly higher than that of the untreated ($p < 0.05$). Factor C had a

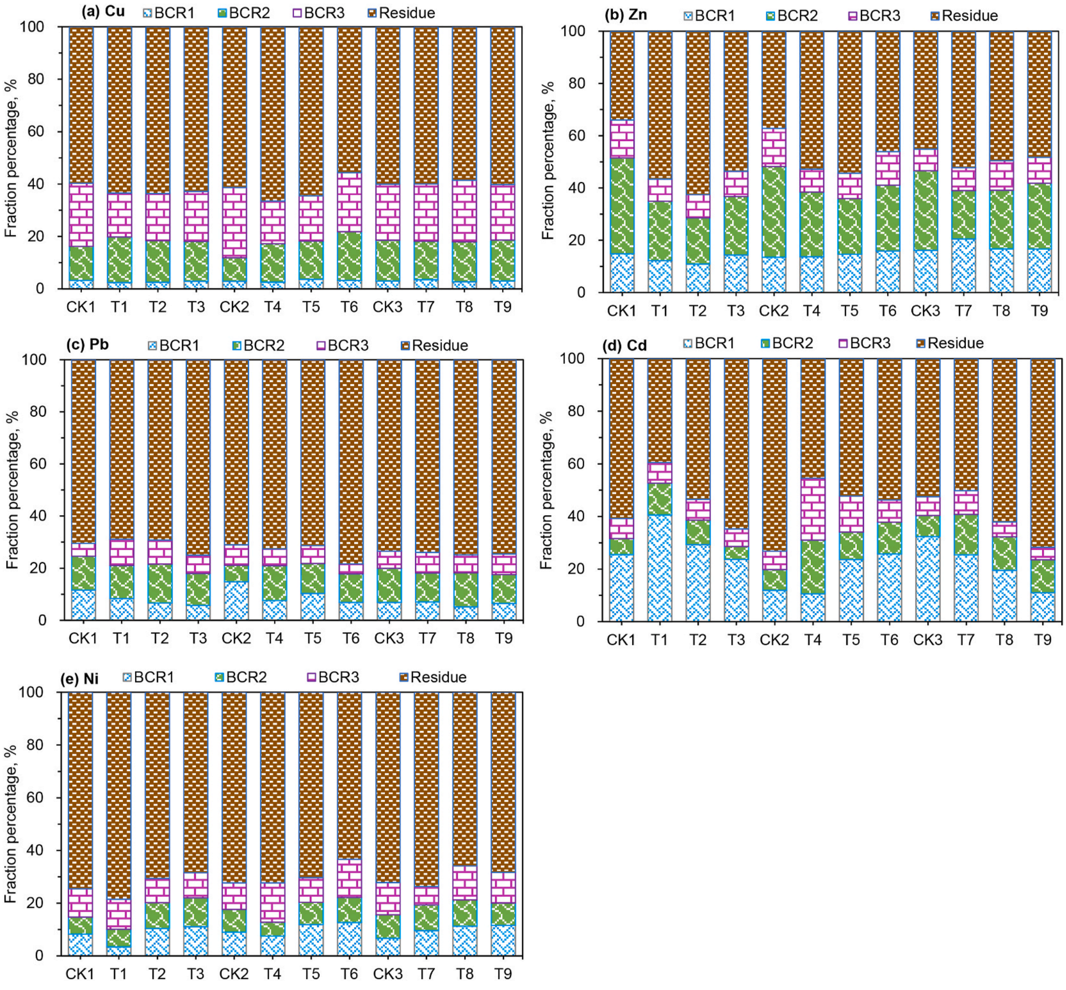

Fig. 3. The effect of treatments on fraction distribution of trace metals (a) Cu, (b) Zn, (c) Pb, (d) Cd, and (e) Ni in soils. Each sub-column is the average of duplicates in June 2018, the end of experimental observation. The fraction analysis is conducted as the improved BCR sequential extraction scheme including acid extractable fraction (BCR1), reducible fraction (BCR2), oxidizable fraction (BCR3) and the residual. The treatments are listed in Table 2.

dominant (46.9%) influence on the proportion of BCR1 of Cu, however there were insignificant differences among the three spraying strategies of GA_3. The well-developed root system and its root exudates of turfgrass play an important role in the fraction distribution of trace metals directly or indirectly by increasing the solubility of trace metal or stimulating the growth of rhizobacteria (Luo et al., 2008; Ma et al., 2011). Citric acid participates in the tricarboxylic acid cycle (TCA cycle, metabolic pathways ubiquitous in mitochondria of organisms), as an intermediate, which is common in plant roots and root exudates. The process of citric acid secretion by plant roots not only promotes the metabolism of root cells, but also provides energy for absorbing trace metals (Jones, 1998), while GA_3 is a common phytohormone produced by rhizobacteria (Ma et al., 2011). CA directly increases the dissolution of trace metals by forming complexes with trace metals or reduce soil pH. However, in this study, increasing the level of CA only increased the proportion of BCR1 of Cu and Ni, but decreased that of BCR1 of Pb and Cd. This indicated that the effect of CA on the fraction distribution of trace metals is complex, and may be related to plant species. It was reported that CA could also act indirectly by interacting with soil microbe or altering root growth (Bulak et al., 2014). Auxin has been shown to be involved in the improvement of phytoremediation, such as uptake of Cd by *Brassica juncea* L (Chen et al., 2020), however, there is no literature suggesting that auxin can affect the speciation of trace metals in soil.

Therefore, the activities of Pb, Cu, and Zn in SDN soils tended to lower by transforming them from fraction of high activity to that of low activity (For examples, Cu from BCR1 to BCR2, Pb from BCR1 and BCR2 to BCR3 and Residue), which were mainly influenced by the turfgrass culturing pattern or GA_3. Meanwhile, the activities of Ni (T2, T3, T6, T8, and T9) and Cd (T1, T2, T4, T5, and T6) were elevated by converting them from the residual to the fractions of BCR1 and BCR3 or the fractions of BCR2 and BCR3, and mainly driven by CA treatment and the turfgrass culturing pattern, respectively. The promotion on Ni was mainly enhanced by CA treatment, as reported in the literature (Najeeb et al., 2009; Ehsan et al., 2014). In addition, the intercropping of *Z. matrella* + *L. perenne* showed the highest proportion of Cd in BCR1, indicated that intercropped turfgrass enhanced Cd activity by means of compensatory, which showed great potential in phytoremediation of cadmium.

3.5. Efficiency assessment of turfgrass phytoremediation

3.5.1. Enhanced bioaccumulation of trace metals in turfgrass

Bioconcentration factor (BCF) has been used to describe the ability of turfgrass root for accumulating trace metals from the soil. The higher value means the stronger phytoremediatation ability of the turfgrass (Ghazaryan et al., 2019a). This is one of the bases for selecting turfgrasses for this study, in which some trace metals have BCF values greater than 1. For example, BCF values of *Z. matrella* range from 1.09 to 1.86 for Cu, Zn, and Ni (Xu et al., 2012); *L. perenne* shows a BCF value of 1.78 for Zn and 7.99 for Cd (Gu et al., 2013), *C. dactylon* has a BCF value of up to 5.67 for Cu (Sekabira et al., 2011), while *F. arundinacea* has satisfied BCF values of 3.73, 12.5 and 7.39 for Pb, Cd, and Ni, respectively (Steliga and Kluk, 2020). However, in this study, the BCF values of each metal in turfgrass of three culturing patterns were less than 1, might due to different growth environment compared to these literatures, such as regrowth produced by mowing every two months, and characteristic of SDN soil of high pH, CEC, and SOM. Previous works have demonstrated soil with high pH, CEC and organic matter tended to decrease the metal accumulation capacities in plants (Zhou et al., 2014; Ghazaryan et al., 2019b). In fact, BCF is a variable depending on different biological conditions and environmental exposure (McGeer et al., 2003). However, the BCF values of Cu, Zn, Pb, Cd, and Ni under treatments were elevated by 1.4–1.5, 1.2–1.7, 1.6–2.1, 1.4–2.6, and 1.5–1.6 times of their corresponding values in CK (Fig. S3), with some treatments reached significant levels, suggested that turfgrass had a good potential to improve the remediation effect of trace metals. Since the BCF value is the average value of five harvest samples, it is greatly affected by the season and the error bar is relatively large, but it can still be clearly seen that the extraction of trace metals in soil to the arial parts has a positive impact under the combined treatment of turfgrass.

Orthogonal analysis and ANOVA test (Table S7) analysis showed that Factor B had the predominant influence on BCF values of all tested metals. The difference between factors was extremely significant ($p < 0.01$), and BCF values of five metals increased with the increase of CA levels. CA has been identified as an effectively positive bio-reagent to boost the uptake of trace metals by root (Wu et al., 2010). In view of the results of previous studies that CA had a very limited effect on the fraction distribution of trace metals, we speculate that the promotion effect of CA on BCF was indirect rather than direct chelating. CA, as a carbon source, can directly promote microbial activity, rhizosphere microbial growth, and enzyme production (Ehsan et al., 2014; Zhang et al., 2015), thereby indirectly increasing plant growth and reducing trace metals stress (Mao et al., 2015; Liang et al., 2019). Second only to the factor of CA, the next one was Factor A mainly on Cu, Pb, Ni, and Factor C mainly on Zn and Cd. However, apart from Pb, turfgrass culturing pattern and GA_3 foliar spray did not reach significant levels.

3.5.2. Elevated removal efficiency of trace metals by mowing turfgrass

Different from other hyperaccumulation plants, turfgrass can be mowed several times to remove the absorbed trace metals, thus achieving the effective removal of trace metals (Zalacain et al., 2019). Therefore, the method was proposed to assess the removal efficiency of trace metals by mowing turfgrass, shown as Fig. 4. The APEA of trace metals from the turfgrasses decreased in the order: Zn of 249.0–273.2 mg $(m^2\ a)^{-1}$, Cu of 22.65–29.88 mg $(m^2\ a)^{-1}$, Pb of 12.00–16.85 mg $(m^2\ a)^{-1}$, Ni of 9.34–11.37 mg $(m^2\ a)^{-1}$, and Cd of 0.804–0.938 mg $(m^2\ a)^{-1}$. After treatments, the greatest APEA of Cd, Pb, Ni, Cu and Zn increased by 2.1, 2.05, 1.99, 1.81, and 1.6 times of the corresponding values of CK, respectively. Thereinto, the APEA of Cd were in the range of 0.87–1.76 mg/$(m^2 \cdot a)$, which were close to the reported value of the average Cd removal amount of 18.52 g/ha (1.852 mg/$(m^2 \cdot a)$) by forage mulberry (Jiang et al., 2021).

The range analysis of orthogonal test showed the contributions of each factor (Table 3). The influence of the three factors on all trace metals follows the rule of Factor B > Factor A > Factor C, that is, CA treatment had the greatest contribution, followed by turfgrass culturing pattern, while GA_3 spraying had the weakest effect. The differences of trace metals between different levels of CA treatment were extremely significant ($p < 0.01$), and APEAs of five metals increased with the increase of CA levels. Some studies showed that the application of CA not only increased the extraction amount of trace metals, but also significantly reduced the phytotoxicity of trace metals, which had a significant impact on the uptake and extraction of trace metals from soil by turfgrass arial parts (Agnello et al., 2014). The turfgrass culturing pattern had only significant effect on the APEA of Zn, and the intercropping of *Z. matrella* and *L. perenne* showed the biggest promotion on the APEAs of trace metals, with the except of Pb. This indicated that the toxic effects of trace metals and chelating agents on turfgrass and soil microorganisms, and the absorption ability of turfgrass to trace metals were quite different (Ultra et al., 2005). The effect of GA_3 was also not significant, and the combination of 30 mg kg^{-1} GA_3 sounded to be the optimum on APEAs of trace metals.

Shown as Fig. 4, Treatment T3 was the most outstanding, which was classified as the highest subset for all trace metals in the ANOVA test, why it was T3? The treatment combination included the intercropping of *Z. Matrella* and L. *Perenne*, 400 mg kg^{-1} CA, and 30 mg kg^{-1} GA_3. First, the intercropping of *Z. Matrella* and L. *Perenne* had the largest aerial biomass (Table S1 and Table S2), which provided a big carrier for removing trace metals by mowing (Cao et al., 2020; Sun et al., 2020). As a metal chelating reagent, CA can increase the mobility of trace metal in plants (Ma et al., 2020). In this study, it had a positive boost on the BCF and the APEAs of trace metals (Fig. S3 and Fig. 4). Meanwhile, CA also

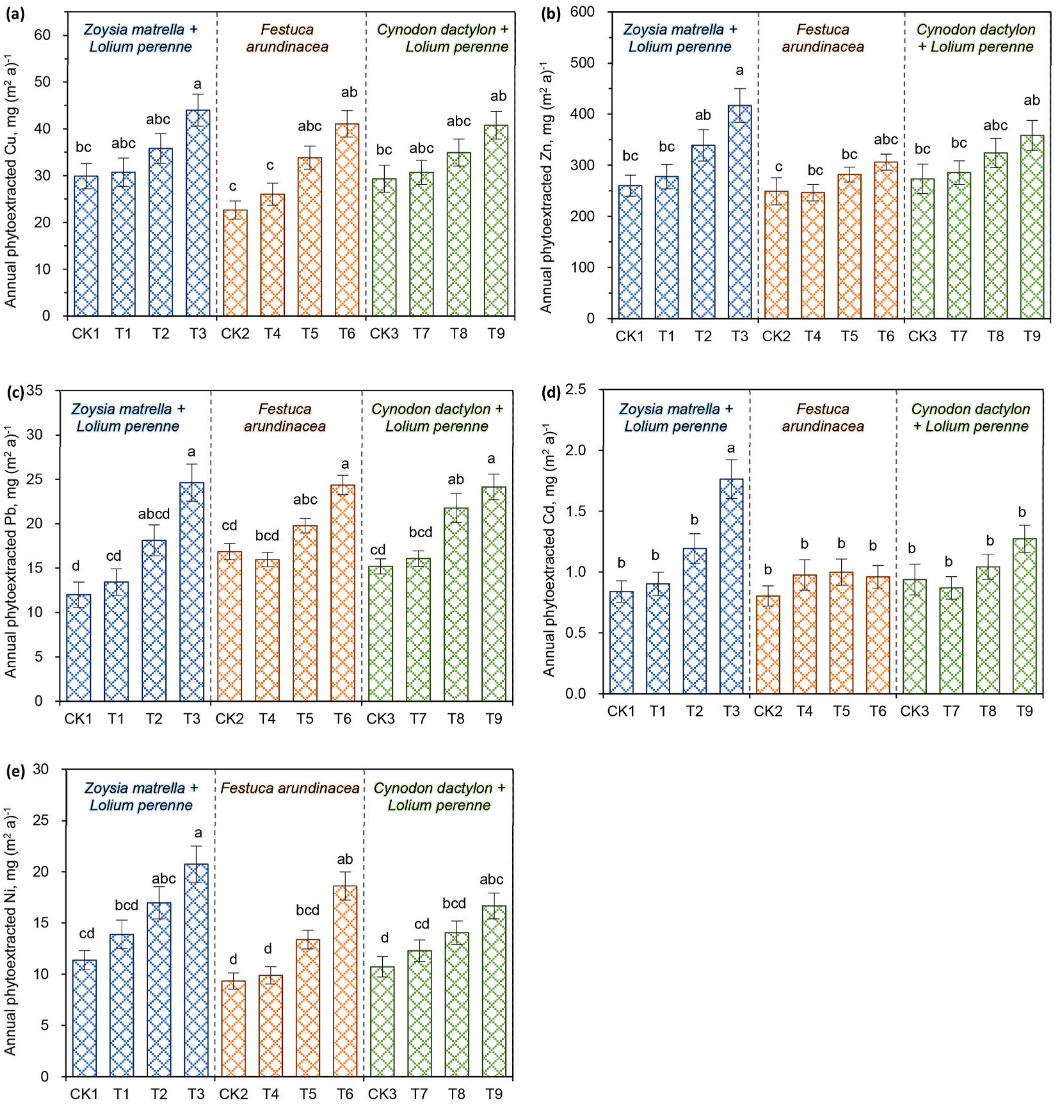

Fig. 4. Annual phytoextracted amount of trace metals by turfgrass growing in SDN soil. The column is the annual phytoextracted amounts, which is summed up from October 2017 to June 2018, and the error bar represents the standard deviation. The treatments are listed in Table 2. Different colors of columns represent different turfgrasses. Different letters indicate significant differences among the treatments, $p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

played a dominant promotion in the aerial part biomass, and reached the maximum at the dosage of 400 mg kg^{-1} (Table S1 and Table S2). Although the effect of auxin was not outstanding, the 30 mg kg^{-1} GA_3 produced the biggest aerial biomass (Table S2), the highest proportion of BCR1 of Cu, Zn, Pb and Ni (Table S6), and APEA for all trace metals (Table 3).

Correlation analysis showed that APEA of each trace metal were significantly correlated with BCF values (Table S8). This indicated that the regulatory means on turfgrass remediation had an important effect on the migration and accumulation behavior of trace metals between SDN soils and turfgrass. Among them, especially the CA regulatory means, which played a dominant role, had a positive promotion effect on plant extraction of all trace metals. This made the accumulation behavior of all trace metals from soil to turfgrass very similar, thus resulting the high correlation among BCF values and APEAs of trace metals. In addition, the correlations between the APEAs of trace metals and their concentration in BCR1 fractions, and SDN soil properties, were not significant in most cases. In this research, APEA is an accumulative value, BCR1 concentrations and SDN soil properties are state values, and could not accurately reflect the change of APEA. Similar findings had also been reported in Jelusic/Lestan's and Wang's research (Jelusic and Lestan, 2014; Wang et al., 2018).

4. Conclusion

In this study, a variety of treatments have been used to improve the remediation efficiency of turfgrasses on a trace metals-contaminated artificial soil derived from municipal sludge. Turfgrass species and culturing patterns had a decisive effect on plant height, root length and heavy metal concentration in turfgrass. CA treatment was prone to increase the aboveground biomass and the concentrations of most trace metals in turfgrasses, especially Ni through increasing the proportion of Ni in BCR1. The intercropping of *Z. matrella* and *L. perenne* presented the highest proportion of Ni in BCR1, which was due to the compensation of turfgrass. According to the range analysis based on orthogonal experiment, CA applying had a positive influence on BCF and APEA of trace metals. The highest APEA value of trace metals were found in the combination treatment of T3 (the intercropping of *Zoysia matrella* and *Lolium perenne*, application of CA 400 mg kg^{-1}, and spraying GA_3 30 mg kg^{-1}), thereinto, that of Cd was 2.1 times as much as that of control group. The research showed that, turfgrass has great potential in remediation of trace metals with under reasonable artificial control.

Table 3

Analysis on orthogonal test results of annual phytoextracted amount of trace metals.

Kvalue	Cu			Zn			Pb			Cd			Ni		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
k1	**36.83a**	29.14a	35.55a	**344.39b**	269.83a	302.60a	18.72a	15.15a	19.85a	**1.29a**	0.92a	0.97a	**17.19a**	12.02a	**15.52a**
k2	33.63a	34.84b	34.19a	278.04a	314.84b	314.47a	20.03a	19.89b	19.41a	0.98a	1.08a	1.15a	13.96a	14.80b	14.50a
k3	35.45a	**41.92c**	**36.16a**	322.60 ab	**360.37b**	**327.96a**	**20.65a**	**24.37c**	**20.16a**	1.06a	**1.33b**	**1.21a**	14.33a	**18.67c**	15.47a
R	3.19	12.78	1.97	66.36	90.54	25.37	1.93	9.22	0.75	0.31	0.42	0.24	3.24	6.65	1.01
Influence Degree	17.80%	**71.23%**	10.97%	36.41%	**49.68%**	13.92%	16.23%	**77.49%**	6.28%	31.87%	**43.11%**	25.01%	29.68%	**61.02%**	9.31%
	B > A > C					B > A > C			B > A > C			B > A > C			
Optimum	**A1B3C3**					**A3C3B3**			**A1B3C3**			**A1B3C1**			

Columns with the different letters indicate that the differences between the treatments are significant, $p < 0.05$.

Credit author statement

Feili Li: Reviewing and Editing, Supervision; **Hui Jin**: Data curation, Writing – original draft preparation; **Xingfei Wu**: Investigation, Methodology; **Yannian Liu**: Software; **Xiaoling Chen**: Visualization; **Jiade Wang**: Conceptualization, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

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References

Agnello, A.C., Huguenot, D., Van Hullebusch, E.D., Esposito, G., 2014. Enhanced phytoremediation: a review of low molecular weight organic acids and surfactants used as amendments. Crit. Rev. Environ. Sci. Technol. 44, 2531–2576.

Ahmadipour, F., Bahramifar, N., Mahmood Ghasempouri, S., 2015. Fractionation and mobility of cadmium and lead in soils of Amol area in Iran, using the modified BCR sequential extraction method. Chem. Speciat. Bioavailab. 26, 31–36.

Ali, N., Hadi, F., 2015. Phytoremediation of cadmium improved with the high production of endogenous phenolics and free proline contents in Parthenium hysterophorus plant treated exogenously with plant growth regulator and chelating agent. Environ. Sci. Pollut. Res. Int. 22, 13305–13318.

Bulak, P., Walkiewicz, A., Brzezinska, M., 2014. Plant growth regulators-assisted phytoextraction. Biol. Plant. (Prague) 58, 1–8.

Cao, X.R., Luo, J.P., Wang, X.Z., Chen, Z.Q., Liu, G.Q., Khan, M.B., Kang, K.J., Feng, Y., He, Z.L., Yang, X.E., 2020. Responses of soil bacterial community and Cd phytoextraction to a Sedum alfredii-oilseed rape (Brassica napus L. and Brassica juncea L.) intercropping system. Sci. Total Environ. 723, 138152.

Chen, L., Long, C., Wang, D., Yang, J.Y., 2020. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by Brassica juncea L. enhanced with exogenous application of plant growth regulators. Chemosphere 242, 125112.

Chen, Y., Liu, D., Ma, J., Jin, B., Peng, J., He, X., 2021. Assessing the influence of immobilization remediation of heavy metal contaminated farmland on the physical properties of soil. Sci. Total Environ. 781, 146773.

Cui, E., Cui, B., Fan, X., Li, S., Gao, F., 2021. Ryegrass (Lolium multiflorum L.) and Indian mustard (Brassica juncea L.) intercropping can improve the phytoremediation of antibiotics and antibiotic resistance genes but not heavy metals. Sci. Total Environ. 784, 147093.

Cui, T., Fang, L.C., Wang, M.K., Jiang, M., Shen, G.T., 2018. Intercropping of gramineous pasture ryegrass (Lolium perenne L.) and leguminous forage alfalfa (Medicago sativa L.) increases the resistance of plants to heavy metals. J. Chem. 2018, 7803408.

Dai, Y.N., A, D., Yang, Y., Tam, N.F.Y., Tai, Y.P., Tang, X.Y., 2016. Factors affecting behavior of phenolic endocrine disruptors, estrone and estradiol, in constructed wetlands for domestic sewage treatment. Environ. Sci. Technol. 50, 11844–11852.

Desjardins, D., Brereton, N.J.B., Marchand, L., Brisson, J., Pitre, F.E., Labrecque, M., 2018. Complementarity of three distinctive phytoremediation crops for multiple-trace element contaminated soil. Sci. Total Environ. 610–611, 1428–1438.

Ehsan, S., Ali, S., Noureen, S., Mahmood, K., Farid, M., Ishaque, W., Shakoor, M.B., Rizwan, M., 2014. Citric acid assisted phytoremediation of cadmium by Brassica napus L. Ecotoxicol. Environ. Saf. 106, 164–172.

Gerhardt, K.E., Gerwing, P.D., Greenberg, B.M., 2017. Opinion: taking phytoremediation from proven technology to accepted practice. Plant Sci. 256, 170–185.

Ghazaryan, K., Movsesyan, H., Ghazaryan, N., Watts, B.A., 2019a. Copper phytoremediation potential of wild plant species growing in the mine polluted areas of Armenia. Environ. Pollut. 249, 491–501.

Ghazaryan, K., Movsesyan, H., Ghazaryan, N., Watts, B.A., 2019b. Copper phytoremediation potential of wild plant species growing in the mine polluted areas of Armenia. Environ. Pollut. 249, 491–501.

Gill, L.W., Ring, P., Casey, B., Higgins, N.M.P., Johnston, P.M., 2017. Long term heavy metal removal by a constructed wetland treating rainfall runoff from a motorway. Sci. Total Environ. 601, 32–44.

Gou, J.Q., Strauss, S.H., Tsai, C.J., Fang, K., Chen, Y.R., Jiang, X.N., Busov, V.B., 2010. Gibberellins regulate lateral root formation in populus through interactions with auxin and other hormones. Plant Cell 22, 623–639.

Gu, C., Bai, Y., Tao, T., Chen, G., Shan, Y., 2013. Effect of sewage sludge amendment on heavy metal uptake and yield of ryegrass seedling in a mudflat soil. J. Environ. Qual. 42, 421–428.

Gul, I., Manzoor, M., Hashim, N., Shah, G.M., Waani, S.P.T., Shahid, M., Antoniadis, V., Rinklebe, J., Arshad, M., 2021. Challenges in microbially and chelate-assisted phytoextraction of cadmium and lead – a review. Environ. Pollut. 287, 117667.

Habiba, U., Ali, S., Farid, M., Shakoor, M.B., Rizwan, M., Ibrahim, M., Abbasi, G.H., Hayat, T., Ali, B., 2015. EDTA enhanced plant growth, antioxidant defense system, and phytoextraction of copper by Brassica napus L. Environ. Sci. Pollut. Res. 22, 1534–1544.

Hadi, F., Bano, A., Fuller, M.P., 2013. Augmented phytoextraction of lead (Pb^{2+})-polluted soils: a comparative study of the effectiveness of plant growth regulators, EDTA, and plant growth promoting rhizobacteria. Ann. Finance 17, 124–130.

Huang, B., Li, Z.W., Li, D.Q., Yuan, Z.J., Chen, Z.L., Huang, J.Q., 2017. Distribution characteristics of heavy metal(loid)s in aggregates of different size fractions along contaminated paddy soil profile. Environ. Sci. Pollut. Res. 24, 23939–23952.

Jelusic, M., Lestan, D., 2014. Effect of EDTA washing of metal polluted garden soils. Part I: toxicity hazards and impact on soil properties. Sci. Total Environ. 475, 132–141.

Jiang, Y.B., Jiang, S.M., Huang, R.Z., Wang, M., Cao, H., Li, Z.B., 2021. Phytoremediation potential of forage mulberry (Morus atropurpurea Roxb.) for cadmium contaminated paddy soils. Int. J. Phytoremediation 1–7.

Jones, D.L., 1998. Organic acids in the rhizosphere - a critical review. Plant Soil 205, 25–44.

Lestan, D., Luo, C.L., Li, X.D., 2008. The use of chelating agents in the remediation of metal-contaminated soils: a review. Environ. Pollut. 153, 3–13.

Li, F., Shao, L., Chen, Y., Zhang, G., Nie, Q., Jin, Z., 2021a. Leaching characteristic of potentially toxic metals of artificial soil made from municipal sludge compost. Chemosphere 270, 128632.

Li, F.L., Liu, C.Q., Yang, Y.G., Bi, X.Y., Liu, T.Z., Zhao, Z.Q., 2012. Natural and anthropogenic lead in soils and vegetables around Guiyang city, southwest China: a Pb isotopic approach. Sci. Total Environ. 431, 339–347.

Li, Y., Wang, Y., Khan, M.A., Luo, W., Xiang, Z., Xu, W., Zhong, B., Ma, J., Ye, Z., Zhu, Y., Duan, L., Liu, D., 2021b. Effect of plant extracts and citric acid on phytoremediation of metal-contaminated soil. Ecotoxicol. Environ. Saf. 211, 111902.

Liang, Y.Q., Wang, X.H., Guo, Z.H., Xiao, X.Y., Peng, C., Yang, J., Zhou, C., Zeng, P., 2019. Chelator-assisted phytoextraction of arsenic, cadmium and lead by Pteris vittata L. and soil microbial community structure response. Int. J. Phytoremediation 21, 1032–1040.

Liu, Y., Ma, L., Li, Y., Zheng, L., 2007. Evolution of heavy metal speciation during the aerobic composting process of sewage sludge. Chemosphere 67, 1025–1032.

Lu, J., Lu, H., Li, J., Liu, J., Feng, S., Guan, Y., 2019. Multi-criteria decision analysis of optimal planting for enhancing phytoremediation of trace heavy metals in mining sites under interval residual contaminant concentrations. Environ. Pollut. 255, 113255.

Luo, C.L., Shen, Z.G., Li, X.D., 2008. Root exudates increase metal accumulation in mixed cultures: implacications for naturally enhanced phytoextraction. Water Air Soil Pollut. 193, 147–154.

Ma, H., Li, X., Wei, M., Zeng, G., Hou, S., Li, D., Xu, H., 2020. Elucidation of the mechanisms into effects of organic acids on soil fertility, cadmium speciation and ecotoxicity in contaminated soil. Chemosphere 239, 124706.

Ma, N., Wang, W.L., Gao, J.Q., Chen, J., 2017. Removal of cadmium in subsurface vertical flow constructed wetlands planted with Iris sibirica in the low-temperature season. Ecol. Eng. 109, 48–56.

Ma, Y., Prasad, M.N., Rajkumar, M., Freitas, H., 2011. Plant growth promoting rhizobacteria and endophytes accelerate phytoremediation of metalliferous soils. Biotechnol. Adv. 29, 248–258.

Mao, L., Tang, D., Feng, H.W., Gao, Y., Zhou, P., Xu, L.R., Wang, L.M., 2015. Determining soil enzyme activities for the assessment of fungi and citric acid-assisted phytoextraction under cadmium and lead contamination. Environ. Sci. Pollut. Res. 22, 19860–19869.

McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J., Green, A., 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: implications for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem. 22, 1017–1037.

Najeeb, U., Xu, L., Ali, S., Jilani, G., Gong, H.J., Shen, W.Q., Zhou, W.J., 2009. Citric acid enhances the phytoextraction of manganese and plant growth by alleviating the ultrastructural damages in Juncus effusus L. J. Hazard Mater. 170, 1156–1163.

Oladoye, P.O., Olowe, O.M., Asemoloye, M.D., 2022. Phytoremediation technology and food security impacts of heavy metal contaminated soils: a review of literature. Chemosphere 288, 132555.

Pourghasemian, N., Ehsanzadeh, P., Greger, M., 2013. Genotypic variation in safflower (Carthamus spp.) cadmium accumulation and tolerance affected by temperature and cadmium levels. Environ. Exp. Bot. 87, 218–226.

Rauret, G., López-Sánchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A., Quevauviller, P., 1999. Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. J. Environ. Monit. 1, 57–61.

Rea, M., Johansen, M., Payne, T., Hirth, G., Hondros, J., Pandelus, S., Tucker, W., Duff, T., Stopic, A., Green, L., Pring, A., Lenehan, C.E., Popelka, R.l.S., 2021. Radionuclides and stable elements in vegetation in Australian arid environments: concentration ratios and seasonal variation. J. Environ. Radioact. 234, 106627.

Rostami, S., Azhdarpoor, A., 2019. The application of plant growth regulators to improve phytoremediation of contaminated soils: a review. Chemosphere 220, 818–827.

Sarret, G., Vangronsveld, J., Manceau, A., Musso, M., D`Haen, J., Menthonnex, J.-J., Hazemann, J.-L., 2001. Accumulation forms of Zn and Pb in Phaseolus vulgaris in the presence and absence of EDTA. Environ. Sci. Technol. 35, 2854–2859.

Sekabira, K., Oryem–Origa, H., Mutumb, G., Kakudidi, E., Basamba, T.A., 2011. Heavy metal phytoremediation by *Commelina benghalensis* (L) and *Cynodon dactylon* (L) growing in Urban stream sediments. Int. Plant Physiol. Biochem. 38, 133–142.

Shen, M., Xu, H., Liu, S., Zhang, Y., Zhang, N., Zhou, J., Chow, J.C., Watson, J.G., Cao, J., 2021. Spatial distribution of PM2.5-bound elements in eighteen cities over China: policy implication and health risk assessment. Environ. Geochem. Health 21, 10653.

Sidhu, G.P.S., Bali, A.S., Singh, H.P., Batish, D.R., Kohli, R.K., 2020. Insights into the tolerance and phytoremediation potential of Coronopus didymus L. (Sm) grown under zinc stress. Chemosphere 244, 125350.

Singh, H., Bhat, J.A., Singh, V.P., Corpas, F.J., Yadav, S.R., 2021. Auxin metabolic network regulates the plant response to metalloids stress. J. Hazard Mater. 405, 124250.

Steliga, T., Kluk, D., 2020. Application of Festuca arundinacea in phytoremediation of soils contaminated with Pb, Ni, Cd and petroleum hydrocarbons. Ecotoxicol. Environ. Saf. 194, 110409.

Sun, Y., Liu, M., Song, M.H., Tian, Y.Q., Xu, X.L., 2020. Interactions between neighboring native and alien species are modulated by nitrogen availability. Rhizosphere 16, 100242.

Ultra, V.U., Yano, A., Iwasaki, K., Tanaka, S., Kang, Y.M., Sakurai, K., 2005. Influence of chelating agent addition on copper distribution and microbial activity in soil and copper uptake by brown mustard (Brassica juncea). Soil Sci. Plant Nutr. 51, 193–202.

Wang, G.Y., Zhang, S.R., Zhong, Q.M., Xu, X.X., Li, T., Jia, Y.X., Zhang, Y.Z., Peijnenburg, W., Vijver, M.G., 2018. Effect of soil washing with biodegradable chelators on the toxicity of residual metals and soil biological properties. Sci. Total Environ. 625, 1021–1029.

Wei, Z.H., Le, Q.V., Peng, W.X., Yang, Y.F., Yang, H., Gu, H.P., Lam, S.S., Sonne, C., 2021. A review on phytoremediation of contaminants in air, water and soil. J. Hazard Mater. 403, 123658.

Wu, D., Yu, X., Lai, M., Feng, J., Dong, X., Peng, W., Su, S., Zhang, X., Wan, L., Jacobs, D. F., Zeng, S., 2021. Diversified effects of co-planting landscape plants on heavy metals pollution remediation in urban soil amended with sewage sludge. J. Hazard Mater. 403, 23855.

Wu, G., Kang, H., Zhang, X., Shao, H., Chu, L., Ruan, C., 2010. A critical review on the bio-removal of hazardous heavy metals from contaminated soils: issues, progress, eco-environmental concerns and opportunities. J. Hazard Mater. 174, 1–8.

Xu, J.Q., Yu, R.L., Dong, X.Y., Hu, G.R., Shang, X.S., Wang, Q., Li, H.W., 2012. Effects of municipal sewage sludge stabilized by fly ash on the growth of Manilagrass and transfer of heavy metals. J. Hazard Mater. 217–218, 58–66.

Yang, S.L., Zhou, D.Q., Yu, H.Y., Wei, R., Pan, B., 2013. Distribution and speciation of metals (Cu, Zn, Cd, and Pb) in agricultural and non-agricultural soils near a stream upriver from the Pearl River, China. Environ. Pollut. 177, 64–70.

Zaheer, I.E., Ali, S., Rizwan, M., Farid, M., Shakoor, M.B., Gill, R.A., Najeeb, U., Iqbal, N., Ahmad, R., 2015. Citric acid assisted phytoremediation of copper by Brassica napus L. Ecotoxicol. Environ. Saf. 120, 310–317.

Zalacain, D., Martinez Perez, S., Bienes, R., Garcia Diaz, A., Sastre-Merlin, A., 2019. Turfgrass biomass production and nutrient balance of an urban park irrigated with reclaimed water. Chemosphere 237, 124481.

Zhang, H., Chen, X.P., He, C.Q., Liang, X., Oh, K., Liu, X.Y., Lei, Y.R., 2015. Use of energy crop (Ricinus communis L.) for phytoextraction of heavy metals assisted with citric Acid. Int. J. Phytoremediation 17, 632–639.

Zhang, Y., Ji, H.B., 2019. Physiological responses and accumulation characteristics of turfgrasses exposed to potentially toxic elements. J. Environ. Manag. 246, 796–807.

Zhao, B., Liu, A., Wu, G., Li, D., Guan, Y., 2017. Characterization of heavy metal desorption from road-deposited sediment under acid rain scenarios. J. Environ. Sci. (China) 51, 284–293.

Zhao, S., Lian, F., Duo, L., 2011. EDTA-assisted phytoextraction of heavy metals by turfgrass from municipal solid waste compost using permeable barriers and associated potential leaching risk. Bioresour. Technol. 102, 621–626.

Zhou, H., Zhou, X., Zeng, M., Liao, B.H., Liu, L., Yang, W.T., Wu, Y.M., Qiu, Q.Y., Wang, Y.J., 2014. Effects of combined amendments on heavy metal accumulation in rice (Oryza sativa L.) planted on contaminated paddy soil. Ecotoxicol. Environ. Saf. 101, 226–232.

Zhou, Q.X., Liu, Y.X., Li, T., Zhao, H.Z., Alessi, D.S., Liu, W.T., Konhauser, K.O., 2020. Cadmium adsorption to clay-microbe aggregates: implications for marine heavy metals cycling. Geochem. Cosmochim. Acta 290, 124–136.