

Effects of Different Densities on Cadmium Accumulation of *Cerastium glomeratum*

Lijin Lin¹, Chunyang Liu², Jun Shi³ and Ming'an Liao^{2,*}

¹Institute of Pomology and Olericulture, Sichuan Agricultural University, 611130 Chengdu, Sichuan, China

²College of Horticulture, Sichuan Agricultural University, 611130 Chengdu, Sichuan, China

³Mianyang Academy of Agricultural Sciences, 621023 Mianyang, Sichuan, China

Abstract. The effects of different densities on cadmium (Cd) accumulation of *Cerastium glomeratum* were investigated through a pot experiment. The results showed that the root, stem, leaf and shoot biomass of single *C. glomeratum* decreased with the increase of densities of *C. glomeratum* seedlings (1, 2, 3, 4 and 5 seedlings in one pot), and the Cd contents in stems, leaves and shoots of *C. glomeratum* increased. The Cd extraction by whole plant of single *C. glomeratum* increased when the density was less than 2, and decreased more than that. The Cd extraction by stems, leaves, shoots and whole plant of *C. glomeratum* in each pot increased with the increase of densities of *C. glomeratum* seedlings. The maximums of the Cd extraction by stems, leaves, shoots and whole plant of *C. glomeratum* in each pot were 110.55, 81.65, 192.20 and 572.35 µg/pot, which increased by 367.05% ($p < 0.05$), 348.63% ($p < 0.05$), 359.04% ($p < 0.05$) and 243.59% ($p < 0.05$), respectively, compared with 1 seedling in one pot. Therefore, highly density could improve phytoremediation efficiency of *C. glomeratum* in Cd-contaminated soil.

1 Introduction

In agricultural production, the reasonable planting density can increase the yield of crop in per unit area, and improve the using efficiency of land [1-2]. Because of the competition between individual plants for light, water and fertilizer resources, high planting density could result in lower crop yield of per plant, and decrease the yield of crop in per unit area; low planting density can increase single crop yield, but the yield per unit area may reduce because of the small number of individual plant [3-4]. For hyperaccumulator plant, the biomass of per unit area is directly affect their remediation efficiency on heavy metal contaminated soil, and may affect the absorption of heavy metals in soil [5]. The agronomic control measure is an effective increasing yield way, and by regulating the planting density can improve remediation efficiency of hyperaccumulator [6-7]. An appropriate planting density increases the shoot biomass of *Sedum plumbizincicola*, and increases the amount of zinc and cadmium (Cd) absorption by shoot of *Sedum plumbizincicola*, which is benefit to phytoremediation of contaminated agricultural soils [8]. Improving the planting density of *Brassica juncea* can enhance the enrichment of Cd by plant, promoting the remediation to Cd-contaminated soil [9].

Cerastium glomeratum belongs to Caryophyllaceae, which is an annual herb of common weed growing in winter and spring [10]. In a preliminary study, we found that *C. glomeratum* is a Cd accumulator plant [11]. However, the biomass of *C. glomeratum* is small, needing some methods to increase the biomass of *C. glomeratum* for improving the ability of phytoremediation. In this study, the effects of different densities on Cd accumulation of *C. glomeratum* were investigated through a pot experiment, to provide a new method to remediate Cd-contaminated soil of farmland in winter and spring.

2 Materials and method

2.1 Materials

The inceptisol soil samples were collected from Ya'an campus farm of the Sichuan Agricultural University (29°59'N, 102°59'E), China, in February 2014. The basic properties of the soil were the same as reference [12]. *Cerastium glomeratum* seedlings with two pairs of euphyllas were collected from the Ya'an campus farm (from uncontaminated soil) in March 2014.

2.2 Experimental design

The experiment was conducted at the Ya'an campus farm from February to April in 2014. The soil samples were air-dried and passed through a 5-mm sieve. Three kilograms of the air-dried soil was weighed into each polyethylene pot (15 cm high, 18 cm in diameter). Cd was added to soils as CdCl₂·2.5H₂O at 10 mg/kg, and the soil moisture was maintained at 80% of field capacity for 1 month. The density treatments in the experiment were 1, 2, 3, 4 and 5 seedlings of *C. glomeratum*, and each treatment was replicated three times using a completely randomized design with 10-cm spacing between pots. *C. glomeratum* seedlings with corresponding density were transplanted into each pot and the soil moisture content was maintained at 80% of field capacity from the time the plants were transplanted into the pots until the time the plants were harvested.

2.3 Sample analysis

After *C. glomeratum* had matured (after 30 d), the plants were then gently removed from the soil. The roots and shoots of *G. parviflora* were harvested and washed with tap water. The roots were immersed in 10 mM/L HCl for 10 min to remove Cd adhering to the root surface. Then,

the treatments and analyses of plants were described as in reference [12]. Statistical analyses were performed using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference at a 5% confidence level. The translocation factor (TF) is defined as Cd content in shoot/ Cd content in root [13].

3 Results and discussion

3.1 Biomass of *C. glomeratum*

With the increase of densities of *C. glomeratum* seedlings, the root, stem, leaf and shoot biomass of single *C. glomeratum* decreased (Table 1). When the densities were 2, 3, 4 and 5 seedlings in one pot, the root biomass decreased by 10.83% ($p < 0.05$), 24.95% ($p < 0.05$),

37.98% ($p < 0.05$) and 44.77% ($p < 0.05$) respectively, compared with 1 seedling in one pot, and the shoot biomass decreased by 22.53% ($p < 0.05$), 24.62% ($p < 0.05$), 29.28% ($p < 0.05$) and 31.42% ($p < 0.05$) respectively, compared with 1 seedling in one pot. These results indicate that high density could inhibit the growth of *C. glomeratum*, which because the competition was between individual plants in limited space. However, the reasonable density could improve the total biomass in unit area, which is consistent with other studies [14-15]. The root/ shoot ratio of *C. glomeratum* increased at first and decreased later with the densities, and the maximum root/ shoot ratio was the density of 2 (Table 1), indicating that density inhibited growth of *C. glomeratum* roots. So, under Cd stress, the root proportion decreased the resistance of *C. glomeratum*.

Table 1. Biomass of *C. glomeratum*

Densities	Roots (g/plant)	Stems (g/plant)	Leaves (g/plant)	Shoots (g/plant)	Root/ shoot ratio
1	0.545±0.006a	1.498±0.011a	0.797±0.007a	2.295±0.018a	0.237
2	0.486±0.004b	1.141±0.008b	0.637±0.004b	1.778±0.013b	0.273
3	0.409±0.006c	1.104±0.006c	0.626±0.006b	1.730±0.011c	0.236
4	0.338±0.008d	1.086±0.007cd	0.537±0.008c	1.623±0.016d	0.208
5	0.301±0.007e	1.066±0.008d	0.508±0.010d	1.574±0.018e	0.191

3.2 Photosynthetic pigment contents of *C. glomeratum*

With the increase of densities of *C. glomeratum* seedlings, the contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid in *C. glomeratum* decreased (Table 2). When the densities were 2, 3, 4 and 5 seedlings in one pot, the chlorophyll a content in *C. glomeratum* decreased by 4.91% ($p > 0.05$), 10.27% ($p < 0.05$), 11.34% ($p < 0.05$) and 11.70% ($p < 0.05$) respectively, the chlorophyll b content decreased by 2.33% ($p > 0.05$), 11.33% ($p > 0.05$), 12.33% ($p > 0.05$) and 11.67% ($p > 0.05$) respectively, the total chlorophyll content decreased by 4.37% ($p > 0.05$), 10.49% ($p < 0.05$), 11.55% ($p < 0.05$) and 11.69% ($p < 0.05$) respectively, and the carotenoid content decreased by 6.25% ($p < 0.05$), 11.00% ($p < 0.05$), 12.25% ($p < 0.05$) and 14.50% ($p < 0.05$) respectively, compared with 1 seedling in one pot. The other study shows that the carotenoid can receive the surplus energy of excited chlorophyll molecule, and avoids formatting the singlet oxygen, which plays the protective effect of light [16]. So, the high density decreased carotenoid content in *C. glomeratum* suggesting that the damage of *C. glomeratum* by light enhanced with the increase of growing density. With the increase of densities of *C. glomeratum* seedlings, there was no obvious changes in the chlorophyll a/b (Table 2). The chlorophyll a/b was ranked as: 3 seedlings > 4 seedlings > 1 seedling > 5 seedlings > 2 seedlings.

3.3 Cd content in *C. glomeratum*

With the increase of densities of *C. glomeratum* seedlings, the Cd content in roots of *C. glomeratum* increased when the density was less than 3, and decreased more than that (Table 3). The maximum of Cd content in roots was 319.80 mg/kg at the density of 3. However, the Cd contents in stems, leaves and shoots of *C. glomeratum* increased. The maximums of Cd content in stems, leaves and shoots were 20.74, 32.14, 24.42 mg/kg respectively. When the densities were 2, 3, 4 and 5 seedlings in one pot, the Cd content in roots increased by 43.29% ($p < 0.05$), 39.76% ($p < 0.05$), 35.26% ($p < 0.05$) and 10.38% ($p > 0.05$) respectively, compared with 1 seedling in one pot, and the Cd content in shoots increased by 6.96% ($p < 0.05$), 17.43% ($p < 0.05$), 24.51% ($p < 0.05$) and 33.88% ($p < 0.05$) respectively, compared with 1 seedling in one pot. The other study shows that the intercropping significantly increased the cadmium contents in each organ of two ecotypes of *Bidens pilosa* compared with their monoculture respectively [15], which is consistent with this experiment. The translocation factor (TF) of *C. glomeratum* decreased when the density less than 2, and increased more than that (Table 3), indicating that density promoted Cd transporting from roots to shoots of *C. glomeratum* in high density, which could be benefit to improve the phytoremediation ability of *C. glomeratum*.

Table 2. Photosynthetic pigment contents of *C. glomeratum*

Densities	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
1	1.120±0.012a	0.300±0.017a	1.420±0.028a	3.733	0.400±0.008a
2	1.065±0.020ab	0.293±0.005a	1.358±0.025ab	3.635	0.375±0.006b
3	1.005±0.027bc	0.266±0.024a	1.271±0.051b	3.778	0.356±0.009bc
4	0.993±0.013c	0.263±0.008a	1.256±0.005b	3.776	0.351±0.004c
5	0.989±0.042c	0.265±0.027a	1.254±0.069b	3.732	0.342±0.012c

Table 3. Cd contents in *C. glomeratum*

Densities	Roots (mg/kg)	Stems (mg/kg)	Leaves (mg/kg)	Shoots (mg/kg)	TF
1	228.82±15.81b	15.80±0.28b	22.84±0.93c	18.24±0.50c	0.080
2	327.88±17.14a	16.06±1.33b	25.70±0.71bc	19.51±1.11bc	0.060
3	319.80±21.50a	17.16±1.19b	28.92±1.53ab	21.42±1.29abc	0.067
4	309.50±14.85a	18.25±1.06ab	31.72±1.81a	22.71±1.28ab	0.073
5	252.58±10.49b	20.74±1.78a	32.14±1.22a	24.42±1.57a	0.097

3.4 Cd extraction by single *C. glomeratum*

The Cd extraction by roots of single *C. glomeratum* increased when the density was less than 2, and decreased more than that (Table 4). The Cd extraction by roots of single *C. glomeratum* was ranked as: 2 seedlings > 3 seedlings > 1 seedling > 4 seedlings > 5 seedlings. The Cd extraction by stems of single *C. glomeratum* was ranked as: 5 seedlings > 1 seedling > 4 seedlings > 3 seedlings > 2 seedlings, and by leaves was 1 seedling > 3 seedling > 4 seedlings > 2 seedlings > 5 seedlings. When the densities were 2, 3, 4 and 5 seedlings in one pot, the Cd extraction by shoots of single *C. glomeratum* were lower than 1 seedling treatment. The Cd extraction by whole plant of single *C. glomeratum* was ranked as: 2 seedlings > 3 seedlings > 1 seedling > 4 seedlings > 5 seedlings. When the densities were 2 and 3 seedlings in one pot, the Cd extraction by whole plant increased by 16.48% ($p < 0.05$) and 0.76% ($p > 0.05$) respectively, and when the densities were 4 and 5 seedlings in one pot, the Cd extraction by whole plant decreased by 15.08% ($p < 0.05$) and 31.28% ($p < 0.05$) respectively, compared with 1 seedling in one pot. Therefore, the densities of 2 and 3 seedlings improved the phytoremediation ability of single *C. glomeratum*, and the density of 2 was the best.

3.5 Cd extraction by *C. glomeratum* in each pot

When the densities were 2, 3, 4 and 5 seedlings in one pot, the Cd extraction by roots of *C. glomeratum* in each pot was higher than 1 seedling in one pot, and ranked as: 4

seedlings > 3 seedlings > 5 seedlings > 2 seedlings > 1 seedling (Table 5). With the increase of densities of *C. glomeratum* seedlings, the Cd extraction by stems, leaves, shoots and whole plant of *C. glomeratum* in each pot increased. The maximums of the Cd extraction by stems, leaves, shoots and whole plant of *C. glomeratum* in each pot were 110.55, 81.65, 192.20 and 572.35 µg/pot, which increased by 367.05% ($p < 0.05$), 348.63% ($p < 0.05$), 359.04% ($p < 0.05$) and 243.59% ($p < 0.05$), respectively, compared with 1 seedling in one pot. So, highly density could improve phytoremediation efficiency of *C. glomeratum* in Cd-contaminated soil.

4 Conclusions

With the increase of densities of *C. glomeratum* seedlings (1, 2, 3, 4 and 5 seedlings in one pot), the root, stem, leaf and shoot biomass of single *C. glomeratum* decreased, as did in chlorophyll content, and the Cd contents in stems, leaves and shoots of *C. glomeratum* increased. The maximums of Cd content in stems, leaves and shoots were 20.74, 32.14, 24.42 mg/kg respectively. The Cd extraction by whole plant of single *C. glomeratum* increased when the density was less than 2, and decreased more than that. The Cd extraction by stems, leaves, shoots and whole plant of *C. glomeratum* in each pot increased with the increase of densities of *C. glomeratum* seedlings. The maximums of the Cd extraction by stems, leaves, shoots and whole plant of *C. glomeratum* in each pot were 110.55, 81.65, 192.20 and 572.35 µg/pot. Therefore, highly density could improve

phytoremediation efficiency of *C. glomeratum* in Cd- contaminated soil.

Table 4. Cd extraction by single *C. glomeratum*

Densities	Roots ($\mu\text{g/plant}$)	Stems ($\mu\text{g/plant}$)	Leaves ($\mu\text{g/plant}$)	Shoots ($\mu\text{g/plant}$)	Whole plant ($\mu\text{g/plant}$)
1	124.71 \pm 7.33b	23.67 \pm 0.25a	18.20 \pm 0.58a	41.87 \pm 0.83a	166.58 \pm 8.15b
2	159.35 \pm 6.94a	18.32 \pm 1.39c	16.37 \pm 0.34b	34.69 \pm 1.73b	194.04 \pm 8.66a
3	130.80 \pm 6.99b	18.94 \pm 1.22bc	18.10 \pm 0.79a	37.04 \pm 2.01b	167.84 \pm 8.99b
4	104.61 \pm 2.39c	19.82 \pm 1.03bc	17.03 \pm 0.70ab	36.85 \pm 1.73b	141.46 \pm 4.12c
5	76.03 \pm 1.37d	22.11 \pm 1.73ab	16.33 \pm 0.30b	38.44 \pm 2.02ab	114.47 \pm 3.39d

Table 5. Cd extraction by *C. glomeratum* in each pot

Densities	Roots ($\mu\text{g/pot}$)	Stems ($\mu\text{g/pot}$)	Leaves ($\mu\text{g/pot}$)	Shoots ($\mu\text{g/pot}$)	Whole plant ($\mu\text{g/pot}$)
1	124.71 \pm 7.33d	23.67 \pm 0.25e	18.20 \pm 0.58e	41.87 \pm 0.83e	166.58 \pm 8.15d
2	318.70 \pm 13.87c	36.64 \pm 2.77d	32.74 \pm 0.68d	69.38 \pm 3.45d	388.08 \pm 17.32c
3	392.40 \pm 20.96ab	56.82 \pm 3.65c	54.30 \pm 2.38c	111.12 \pm 6.02c	503.52 \pm 26.98b
4	418.44 \pm 9.56a	79.28 \pm 4.10b	68.12 \pm 2.80b	147.40 \pm 6.90b	565.84 \pm 16.46a
5	380.15 \pm 6.86b	110.55 \pm 8.63a	81.65 \pm 1.48a	192.20 \pm 10.11a	572.35 \pm 16.97a

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