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Original Research

Effects of Physiological Integration and Fertilization on Heavy Metal Remediation in Soil by a Clonal Grass

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Abstract

In this paper, ramets of an annual clonal grass *Digitaria sanguinalis* were subjected to rhizome severing and heavy metal pollution to determine the effects of physiological integration on growth and heavy metal accumulation traits. The negative effect of pollution on survival of offspring ramets was modified by the presence of a stolon connection. Generally, pollution negatively affected growth of offspring ramets and integrated parents. Offspring ramets in polluted soils and connected parents had higher metal contents than those outside polluted soils. In offspring, pollution and rhizome severing reduced the translocation factor (TF) of copper but pollution increased TF of zinc. The results implied that strengthened resource supply with physiological integration was likely to alleviate heavy metal stress to a greater extent. Therefore, connected clones were induced to three levels of fertilization and four heavy metal pollution treatments, studying to what extent fertilization benefited plants. The application of fertilizer to the parents slightly increased the survival rate of connected offspring. The clones produced more biomass with increasing fertilizer intensity. Fertilization resulted in less biomass allocation to roots, but the specific effect of heavy metal led to more investment to root. Fertilization promoted heavy metal accumulation and positively affected TF through integration. The suggested appropriate utilization of fertilizer in connected clones could compensate for damage induced by heavy metal to the whole system. This method should be of great potential use for remediation of heavy metals in soils by clonal plants.

Keywords: fertilization, growth, heavy metal accumulation, physiological integration

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Introduction

Heavy metals with densities larger than 5 g/cm^3 are widely distributed in the environment. With rapid economic development and population increase, large areas of land in the world have been contaminated by heavy metals due to anthropogenic disturbance, e.g., pesticide utilization, fertilization, fossil fuel burning, mining, wastes from metal industries, land application of sewage sludge, and so on [1-4]. As an inorganic contaminant, heavy metal in soils cannot be degraded. Once entering the soil, the toxicity of these contaminants is persistent, which not only disturbs vegetation growth in polluted soils, but also threatens animal and human health through the food chain [5-8].

Phytoremediation is one kind of technology to remedy heavy metal pollution in soil [9]. Heavy metals accumulated in soil can gradually transfer from soil to roots and spread to stems, leaves, and other constituent parts of plants, which are mainly influenced by soil texture and properties, ambient conditions, plant species, and physiology [10, 11].

Hyperaccumulator plants used for phytoremediation generally have small target biomass, low efficiency, and long circle. Interference by heavy metals with uptake of necessary ion, absorption of water, and nutrients from soils might be problems as critical for plant survival and growth as direct toxic influence of the heavy metals [12]. Besides, heavy metals inhibit plant photosynthesis, respiration, degeneration of main cell organelles, and damage lipid structures of plasma membrane [13]. All these together constrict the practical application of phytoremediation.

Clonal growth is a widespread characteristic among plants that helps them persist in harsh environments [14, 15]. Horizontal runners (e.g., stolons, rhizomes, or roots) between ramets of clonal plants allow translocation of resources, such as water, carbohydrates, minerals, and other substances [16, 17]. This case-named physiological integration has been proven to enhance the tolerance of ramets to different environmental stress, including trampling [14], resource shortages [17], shading [18], sand burial, and related wind erosion [16]. Some clonal-plants have been confirmed to have excellent adaptability to heavy metal pollution by special clonal characteristics [19-22]. Translocation of contaminants from ramets in the patchily polluted habitat might spread toxins throughout the whole clones [19], which could be important for successful survival of clonal plants under contamination stress.

In heavy metal-polluted soils, fertilizer application does not only provide nutrients supporting plant growth, but might also alter the speciation of heavy metals in soils and thus bioavailability [23, 24]. Inhibition of resource absorption resulting from heavy metals could be mediated by extra fertilization. Heterogeneity of pollutant-stress can be an important environmental factor for clonal plants. It is supposed that fertilization to ramets might increase fitness of whole clones exposed to heterogeneous pollution and associated heavy metal accumulation throughout physiological integration.

There is no research on how to use special traits such as physiological integration of clonal-plants to strengthen the process of heavy metal remediation. In this paper, we investigate the growth and heavy metals accumulation responses of connected and disconnected fragments of *Digitaria sanguinalis* clonal grass living in heterogeneous patches (unpolluted *vs.* heavy metal-polluted soils). Furthermore, we study how different intensities of fertilization induced to parent ramets integrated with offspring in pollution affect growth and heavy metals accumulation traits of whole clones.

The following questions are addressed:

1) To what extent are effects of heavy metal pollution induced to offspring ramets ameliorated by physiological integration?
2) How does pollution of offspring ramets affect growth and heavy metal accumulation of integrated parents throughout integration?
3) To what extent does fertilization exerted on parent ramets confer benefits to connected offspring in terms of survival, growth, and heavy metal accumulation?

Materials and Methods

Focal Species and Study Sites

Digitaria sanguinalis L. (Gramineae) is a clonal annual herb that can reach a height of 80 cm and a stem diameter of 3 mm. This quick-growing species is a rhizome-connected clonal species with horizontally extended rhizomes on which new ramets are produced. High resistance to drought and shortage of nutrients allows for wide distribution in China. Usually it is regarded as a weed in farmlands and orchards [25].

In September of 2013 we collected seeds of *D. sanguinalis* from five sites within an area of 2 km^2 in a suburb of Qingdao, Shandon Province, China. The average annual temperature is 13°C and the average annual humidity is 73%. These sites were at least 200 m between each other. In each site the mother plants from where the seeds came were not integrated. The pot experiment was conducted in a greenhouse at Qingdao Agricultural University in China from August to October 2014. The mean diurnal temperature during the experiment was 25ºC and average humidity was 40%.

Experimental Design

On 10 July 2014, seeds of *D. sanguinalis* were sown in the field for germination and vegetative propagation. Parent ramets were these first seedlings germinated from seeds. After one month growth, the plants started producing offspring ramets on horizontal rhizomes. On 12 August, 240 pairs of fragments of similar sizes were selected from the stocks and transplanted to plastic pots (31 cm×12 cm×13 cm; effective volumes 3.83 L) in the greenhouse. Each fragment consisted of one parent ramet and one connected offspring. The pots were divided into two chambers equally with plastic films and waterproof tape. Parent and offspring ramets were rooted individually in different chambers. The soil used in this experiment was collected from the surface layer of 20 cm in the farmland. pH was 7.13, total N was 0.28 g kg^{-1}, total P was 0.96 g kg^{-1}. The background values of copper and zinc were 8.83±1.22 mg/kg and 28.98±2.01 mg/kg, respectively.

About one week after transplantation, 216 fragments were selected and randomly assigned to different treatments in a factorial design at the beginning of the experiment. There were nine replications of each treatment. For the purpose of determining the effects of physiological integration, the rhizomes between parent and offspring ramets were severed in half (connection *vs.* disconnection). In these connected clones, parent ramets were subjected to three levels of slow-release fertilizer ($14N–14P_2O_5–14K_2O$, 3-4 months, Osmocote Exact; the Scotts Company, Ohio, the USA): control (no fertilizer, F_1), moderate level (two grams, F_2), and high level (six grams, F_3). Offspring ramets were exerted to different copper and zinc pollution: control (no extra pollutants were added, P_1), moderate pollution (400 mg Cu kg^{-1} soil or 500 mg Zn kg^{-1} soil, P_2 or P_3), and severe pollution (400 mg Cu kg^{-1} soil and 500 mg Zn kg^{-1} soil in one chamber, P_4). Though copper and zinc are essential micronutrients for plants, high levels are toxic for photosynthesis, respiration, or nitrogen absorption [26, 27]. They are common pollutants in Chinese farmland. Both Cu and Zn were added as sulphate ($CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$ in Bodi, Tianjin, China). Pollutant concentrations used in this study were decided according to the environmental quality standard for soils in China. During the greenhouse experiment, parent and offspring ramets were watered regularly to avoid water stress.

Measurements and Data Processing

Survival and Growth

Throughout the experiment, mortality was recorded separately for parent and offspring ramets every day. If parent or offspring ramets in one fragment died, the whole clone (parent + offspring ramets) was abandoned.

On 6 October 2014 all parent and offspring ramets were harvested individually. Ramets were divided into aboveground shoots and underground roots and washed carefully. Dry biomass were determined after drying at 70°C for 48 h. Total biomass of parent reamets, offspring ramets, and the whole clones were calculated. The root:mass ratio was separately calculated as root mass to total mass for parent and offspring ramets.

Heavy Metal Content

After biomass measurement, samples were ground with a ball mill (DECO-PBM-V-4L, Changsha, Hunan Province, China) and dried to constant weight. The homogenized samples were hydrolyzed using H_2O_2/HNO_3 at the ratio of 1:4 (*v* / *v*) in a Teflon crucible (effective volume 100 ml) on an electrical heating panel (MWJ-3020, Wuxi, Jiangsu Province, China). Copper and zinc contents in the extracts were analyzed by inductively coupled plasma-optical emission spectrometry (Optima 8000, Perkin Elmer, Massachusetts, the USA). Translocation factor (TF in short) of offspring ramets, reflecting the transport and distribution of heavy metals in plants, was calculated as follows [12]:

$$TF = (\text{shoot mass} \times \text{shoot metal content}) / (\text{root mass} \times \text{root metal content}) \quad (1)$$

Statistical Analysis

A generalized linear model was used to analyze the effect of rhizome severing, heavy metal pollution, and fertilization on ramet survival. Data were examined using Levene's test for equality of variance and the Shapiro-Wilk test for normality. If necessary, data were Ln-transformed to meet the requirements of ANOVA. Two-way ANOVA was used to test the effects of heavy metal pollution and physiological integration on growth and heavy metal accumulation traits. In disconnected plants, we randomly chose at most nine survived fragments for analysis. In order to determine to what extent increasing fertilizer intensity benefited offspring ramets in pollution, two-way ANOVA was used with pollution intensity and fertilizer intensity as main factors. One-way ANOVA was used to test the differences in growth among parents, offspring, and whole clones, and in metal accumulation traits among parent and offspring. SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) was employed for all the data analyses. Here $P<0.05$ was chosen as significance level.

Results

Survival

All the parent ramets survived at the end of the experiment. Heavy metal pollution had a strong negative effect on the survival of offspring ramets ($\chi^2 = 46.935$, $P<0.001$; Fig. 1), which was modified by the presence of a stolon connection to parent ramets ($\chi^2 = 23.468$, $P<0.001$; Fig. 1). Application of fertilizer to the parent ramets slightly increased survival rates of connected offspring ($\chi^2 = 6.739$, $P = 0.034$; Fig. 1).

Responses Between Connected and Disconnected Clones

Growth

Heavy metal pollution decreased shoot mass, root mass, and associated total mass in both parent and offspring ramets except for parents disconnected with offspring, as reflected by significant connection × pollution effect (Table 1, Fig. 2). Consequently, pollution significantly reduced total biomass of whole clones (parent + offspring

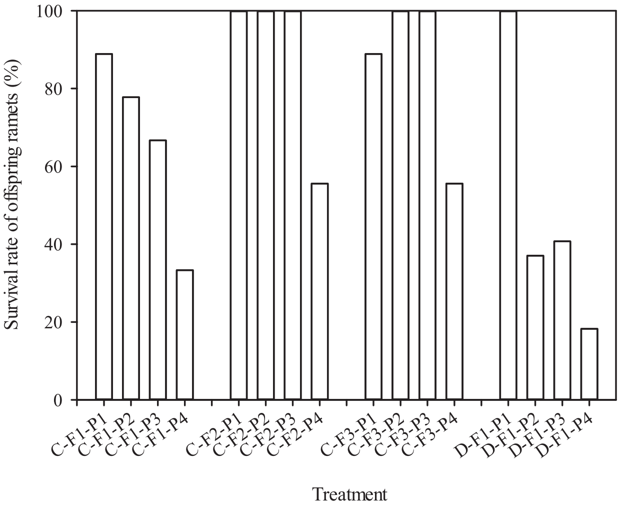

Fig. 1 Survival rate of offspring ramets subjected to rhizome severing (disconnected) or not (connected), different intensities of fertilizer (F1, F2 and F3) and heavy metal pollution (P1, P2, P3 and P4).

ramets) in connection (ANOVA effect of connection $F_{1, 48}$=11.189, P=0.002; pollution $F_{3, 48}$=37.925, P<0.001; connection × pollution $F_{3, 48}$=5.842, P=0.002). However, neither connection nor pollution had effect on biomass allocation to root and shoot (Table 1, Fig. 3).

Heavy Metal Accumulation

Parent ramets connected with offspring ramets growing in contaminated soils exhibited higher mean content of Cu and Zn than disconnected parents (Table 2; Figs 4a, b, c, d). Offspring ramets living in contaminated

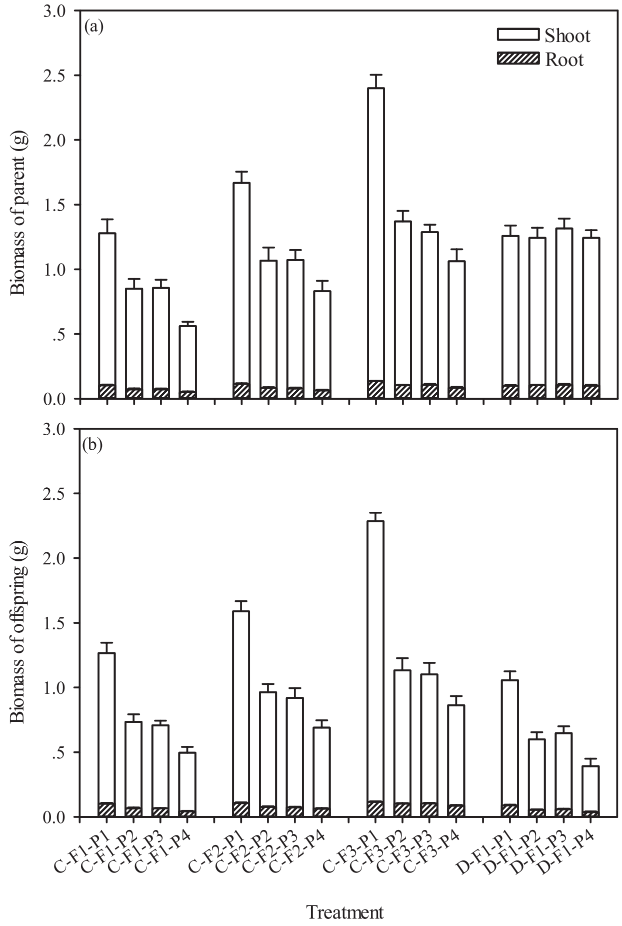

Fig. 2 Shoot biomass and root biomass of parent (a) and offspring (b) ramets subjected to rhizome severing (disconnected) or not (connected), different intensities of fertilizer (F1, F2 and F3) and heavy metal pollution (P1, P2, P3 and P4). Data are mean±SE without transformation.

Table 1. Results of two-way analyses of variance (ANOVA) for effects of rhizome connection (C), different pollution intensity (P), and their interactions (C×P) on growth traits of parent and offspring ramets.

Effect	*df*	Shoot mass		Root mass		Total mass		Root mass ratio	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Parent									
C	1	30.654	<0.001	32.887	<0.001	34.155	<0.001	0.375	0.543
P	3	4.850	0.005	4.057	0.012	5.302	0.003	0.125	0.945
C×P	3	4.815	0.005	5.250	0.003	5.371	0.003	0.054	0.983
Error	48								
Offspring									
C	1	5.933	0.019	5.012	0.030	6.077	0.017	0.760	0.388
P	3	35.952	<0.001	31.914	<0.001	36.928	<0.001	2.2311	0.088
C×P	3	0.483	0.695	0.177	0.911	0.465	0.708	0.140	0.935
Error	48								

If necessary, data were Ln-transformed before analysis.

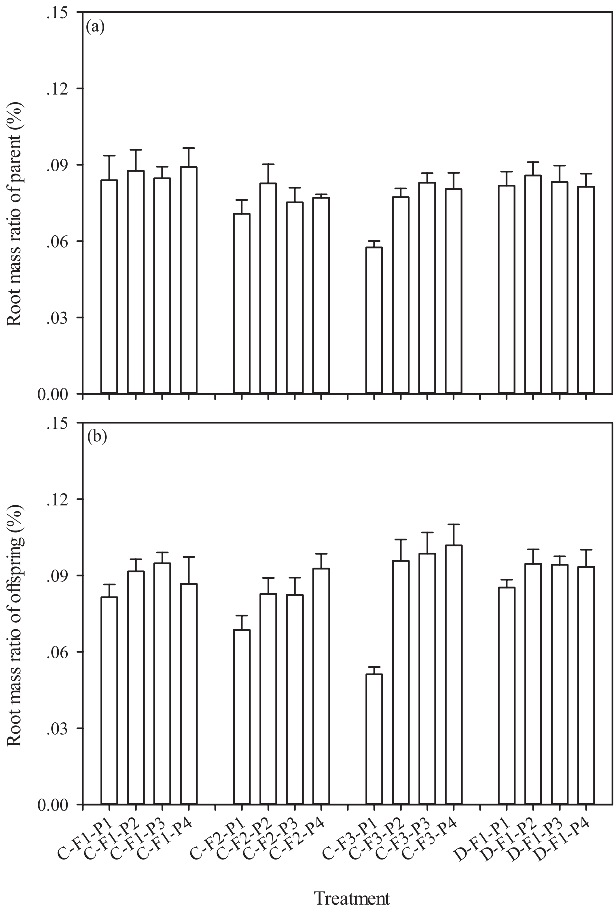

Fig. 3 Root mass ratio of parent (a) and offspring (b) ramets subjected to rhizome severing (disconnected) or not (connected), different intensities of fertilizer (F1, F2 and F3) and heavy metal pollution (P1, P2, P3 and P4). Data are mean± SE without transformation.

soils had higher heavy metal contents in shoots and roots than those out of contamination (Table 2; Figs 4e, f, g, h). Heavy metal contents in ramets varied with different pollution intensities (Table 2, Fig. 4). Pollution decreased translocation factor (TF in short) of Cu but increased TF of Zn, and rhizome severing slightly reduced TF of Cu in offspring ramets (Table 2, Fig. 5). That shows different accumulation style of copper and zinc in *D. sanguinalis.*

Effects of Fertilization in Connected Clones

Growth

In the connected clones, both parent (one-way ANOVA, shoot $F_{11, 75}$=24.818, P<0.001; root $F_{11, 75}$=12.031, P<0.001; total $F_{11, 75}$=25.688, P<0.001) and offspring ramets (one-way ANOVA, shoot $F_{11, 75}$=34.440, P<0.001; root $F_{11, 75}$=13.584, P<0.001; total $F_{11, 75}$=34.300, P<0.001) produced more shoot mass, root mass, and total mass obviously with increasing fertilizer intensity (Table 3, Fig. 2). Fertilization increased total biomass of whole clones (ANOVA effect of fertilizer $F_{2, 75}$=61.311, P<0.001), but pollution decreased them (ANOVA effect of pollution $F_{3, 75}$=108.071, P<0.001). Utilization of fertilizer resulted in less biomass allocation to roots than shoots in both parent and offspring ramets without pollution (Table 3, Fig. 3). Conversely, the specific effect of heavy metal pollution led to more investment to root (Table 3, Fig. 3).

Heavy Metal Accumulation

Fertilization promoted both growth and heavy metal accumulation in plants. Cu and Zn content of whole

Table 2. Results of two-way analyses of variance (ANOVA) for effects of rhizome connection (C), different pollution intensity (P), and their interactions (C×P) on heavy metal accumulation traits of parent and offspring ramets.

Effect	*df*	Shoot Cu content		Root Cu content		Shoot Zn content		Root Zn content		Cu translocation factor		Zn translocation factor	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Parent													
C	1	20.739	<u><0.001</u>	10.216	<u>0.002</u>	372.911	<u><0.001</u>	66.689	<u><0.001</u>	—	—	—	—
P	3	2.950	<u>0.042</u>	1.372	0.263	99.715	<u><0.001</u>	15.681	<u><0.001</u>	—	—	—	—
C×P	3	2.467	0.073	3.102	<u>0.035</u>	90.755	<u><0.001</u>	12.719	<u><0.001</u>	—	—	—	—
Error	48												
Offspring													
C	1	0.256	0.615	0.697	0.408	0.009	0.924	0.172	0.680	4.835	<u>0.033</u>	2.655	0.110
P	3	30.435	<u><0.001</u>	262.976	<u><0.001</u>	483.821	<u><0.001</u>	392.612	<u><0.001</u>	194.949	<u><0.001</u>	16.968	<u><0.001</u>
C×P	3	0.207	0.891	0.111	0.954	0.303	0.823	0.282	0.838	0.374	0.772	1.151	0.338
Error	48												

If necessary, data were Ln-transformed before analysis

Table 3. Results of two-way analyses of variance (ANOVA) for effects of different fertilizer intensity (F), pollution intensity (P), and their interactions (F×P) on growth traits of parent and offspring ramets in connection.

Effect	*df*	Shoot mass		Root mass		Total mass		Root mass ratio	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Parent									
F	2	40.448	<u><0.001</u>	24.277	<u><0.001</u>	45.520	<u><0.001</u>	3.355	<u>0.040</u>
P	3	55.421	<u><0.001</u>	28.410	<u><0.001</u>	57.761	<u><0.001</u>	2.662	0.054
F×P	6	3.744	<u>0.003</u>	0.096	0.997	3.519	<u>0.004</u>	1.005	0.429
Error	75								
Offspring									
F	2	36.933	<u><0.001</u>	32.420	<u><0.001</u>	38.870	<u><0.001</u>	1.082	0.344
P	3	89.861	<u><0.001</u>	60.772	<u><0.001</u>	89.868	<u><0.001</u>	10.871	<u><0.001</u>
F×P	6	5.320	<u><0.001</u>	1.538	0.178	4.585	<u>0.001</u>	2.516	<u>0.028</u>
Error	75								

If necessary, data were Ln-transformed before analysis

fragments, including parent (one-way ANOVA, root Cu $F_{11, 75}$=75.824, P<0.001; root Zn $F_{11, 75}$=58.801, P<0.001; shoot Cu $F_{11, 75}$=13.752, P<0.001; shoot Zn $F_{11, 75}$=53.810, P<0.001) and offspring (one-way ANOVA, root Cu F=12.416, P<0.001; root Zn F=48.878, P<0.001; shoot Cu F=46.530, P<0.001; shoot Zn F=51.951, P<0.001; Table 4; Fig. 4), significantly raised with increasing fertilizer intensities to parent. Pollution to offspring ramets directly increased metal content of contaminated ramets and connected parents (Table 4, Fig. 4). In offspring ramets, fertilization benefiting growth positively affected TF of both Cu and Zn through integration. Cu TF of offspring ramets rooting in polluted soils, especially the Cu- and combined pollution, were lower than those in unpolluted soils (Table 4, Fig. 5a). In Zn- and combined pollution, Zn TF of offspring ramets connected with parent receiving no and moderate intensity of fertilizer increased (Table 4: Fig. 5b). However, under a high level of fertilizer, pollutants caused a decrease in Zn TF (Table 4: Fig. 5b).

Discussion

Effects of Integration, Pollution, and Fertilization on Survival

Population growth of clonal plants in harsh conditions is mainly decided by successful colonization of offspring [28]. Heterogeneity of pollutant-stress – especially heavy metal – becomes an important environmental factor for clonal plants. In this research, we observed strong reduction in survival of offspring ramets caused by metal pollution and rhizome severing. In these connected clones, more offspring ramets under heavy metal stress survived on account of connection to fertilized parents. These results suggest that exchange of resources through physiological integration may to some extent determine the survival of vegetative offspring ramets under stress. It also indicates that more available resources are helpful for establishing clones in adverse conditions.

Effects of Physiological Integration on Pollution

Physiological integration has been proved to be associated with tolerance to many stress factors [14, 16, 17], but its effects on modifying responses to contamination stress in general (and in particular heavy metal) have received little attention. Feedback regulation between source and sink may lead to compensatory growth of photosynthesis efficiency in connected ramets without pollution [20, 23]. Support offered by ramets living in environments out of stress supplies excessive resources to these ramets in stressful environments and increases the fitness of the whole clone system [29]. In some species or under certain growing conditions physiological integration can decrease the fitness of plants [30, 31]. Metallic elements accumulated in ramets under pollution could be exported horizontally to other connected ramets and then transported both acropetally to leaves and basipetally to below-ground structures [19]. That implies that the biological hazard of heavy metal stress could spread between clone systems by physiological integration as the resources sharing, decreasing harm to suffering ramets.

Both Copper and Zinc are essential micronutrients to plants for regular growth. In this study, original Cu and Zn content added to soils were all far beyond threshold values and considered toxic to plants [26, 27]. Comparing with parents separated from polluted offspring, pollutants to offspring resulted in reduction of biomass in connected parents and associated whole clone biomass. This suggests that integration only mitigated the negative effect of serious contamination stress to a certain extent. In offspring ramets,

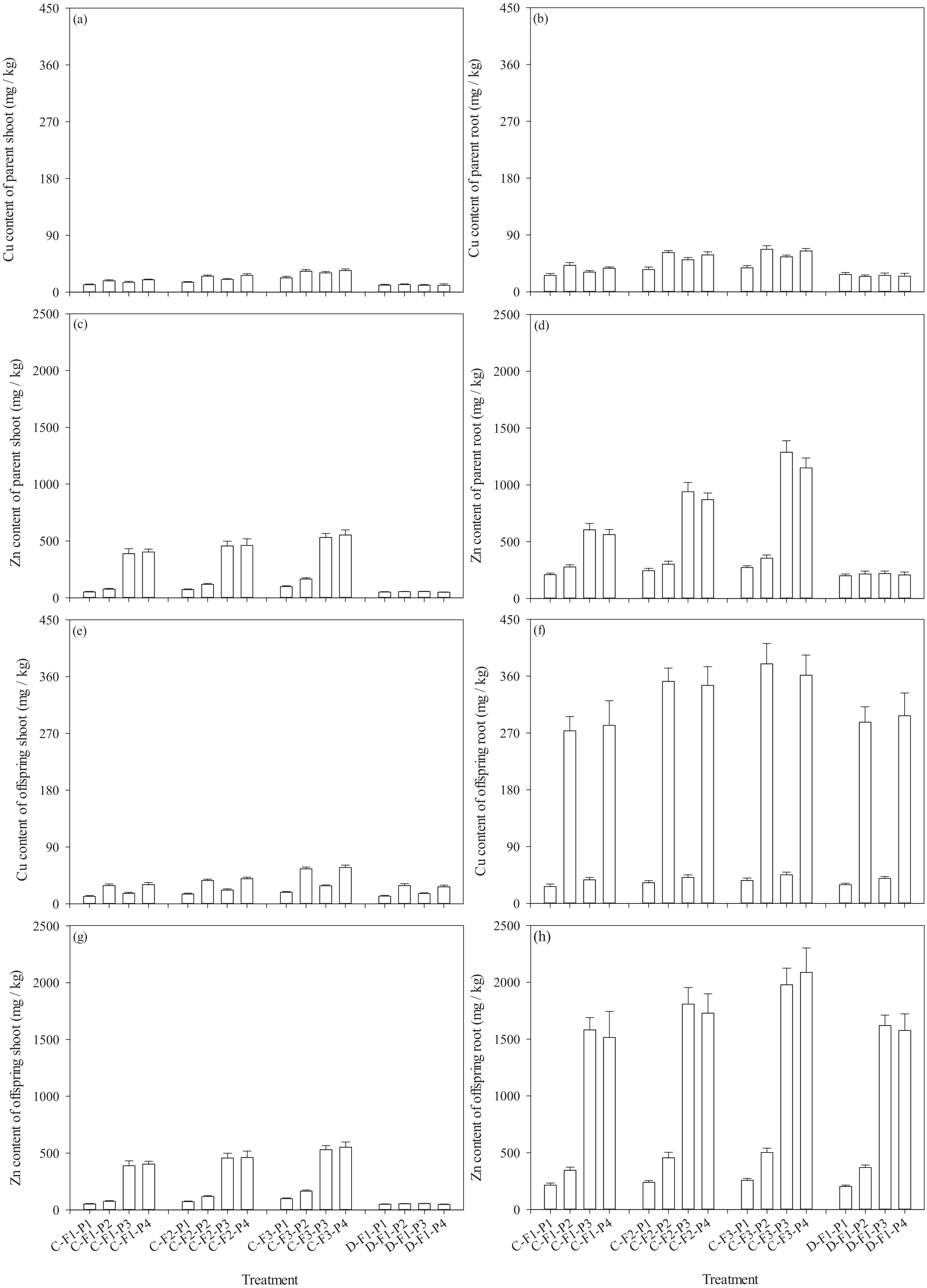

Fig. 4. Cu content of parent (a) and offspring (e) shoot, Cu content of parent (b) and offspring (f) root, Zn content of parent (c) and offspring (g) shoot, Zn content of parent (d) and offspring (h) root. These ramets were subjected to rhizome severing (disconnected) or not (connected), different intensities of fertilizer (F1, F2 and F3) and heavy metal pollution (P1, P2, P3 and P4). Data are mean±SE without transformation.

Table 4. Results of two-way analyses of variance (ANOVA) for effects of different fertilizer intensity (F), pollution intensity (P), and their interactions (F×P) on heavy metal accumulation traits of parent and offspring ramets in connection.

Effect	*df*	Shoot Cu content		Root Cu content		Shoot Zn content		Root Zn content		Cu translocation factor		Zn translocation factor	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Parent													
F	2	43.837	<0.001	27.076	<0.001	31.037	<0.001	29.371	<0.001	—	—	—	—
P	3	14.797	<0.001	22.339	<0.001	360.739	<0.001	191.156	<0.001	—	—	—	—
F×P	6	0.412	0.869	0.837	0.545	1.737	0.124	2.619	0.023	—	—	—	—
Error	75												
Offspring													
F	2	60.624	<0.001	6.136	0.003	26.303	<0.001	8.138	0.001	14.491	<0.001	8.249	0.001
P	3	107.731	<0.001	459.258	<0.001	479.341	<0.001	393.412	<0.001	289.280	<0.001	5.217	0.003
F×P	6	6.047	<0.001	0.215	0.971	0.578	0.747	0.295	0.937	1.909	0.090	2.427	0.034
Error	75												

If necessary, data were Ln-transformed before analysis

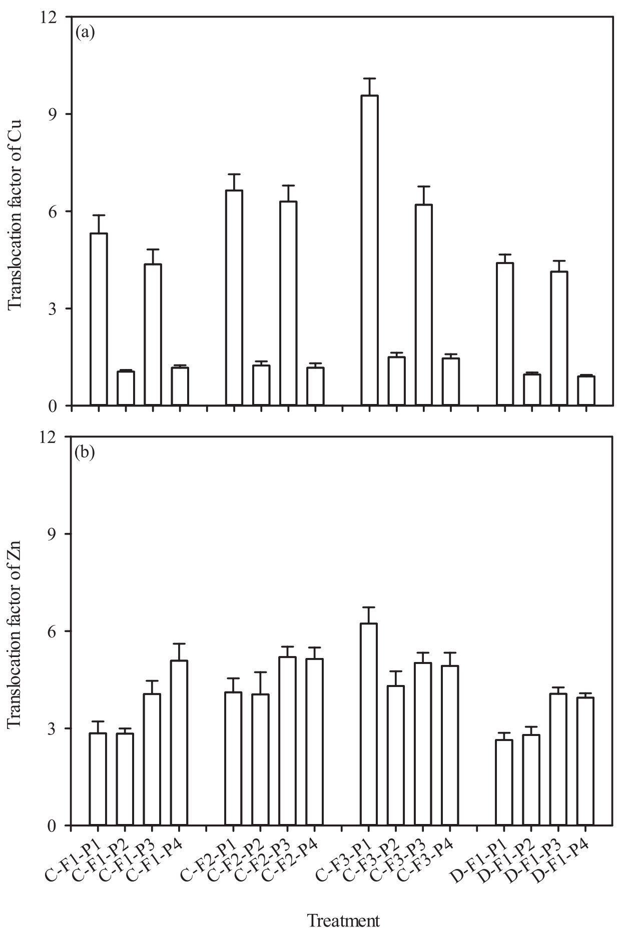

Fig. 5. Translocation factor of Cu (a) and Zn (b) of offspring ramets subjected to rhizome severing (disconnected) or not (connected), different intensities of fertilizer (F1, F2 and F3) and heavy metal pollution (P1, P2, P3 and P4). Data are mean±SE without transformation.

pollution marginally increased biomass allocation to roots (Table 1, $P = 0.088$). When subjected to heavy metal stress, it is likely that heavy metals reduced the ability of the plants to assimilate resources, e.g., carbon, water, or nutrients [13, 20]. More biomass allocation to roots may be related to the reduction in resource uptake and photosynthesis [20]. A possibly strengthened resource supply could alleviate the heavy metal stress to a greater extent.

We also found that parent ramets connected with offspring under contamination showed higher heavy metal content than disconnected parents. In the process of growth and pollutant accumulation, offspring obtained the benefit but parents paid the cost. Resources and pollutant mobility within the whole clone system required energy and increased cost [32]. Moreover, heavy metals exceeding a certain concentration had a direct toxic effect on plants. Excessive levels of Cu and Zn content on plants in this experiment increased the cost and reduced the fitness of whole clones, possibly by inhibiting resources uptake and energy flow [20, 33].

Effects of Fertilization in Connected Clones

As one of the traditional agronomic measures, the most profound effect of fertilization is increasing production and quality of plants. Fertilizing plants with heavy metal contamination can also strengthen their growth. Increasing biomass means higher total heavy metal amount accumulated in plants. Additionally, interaction between fertilization and heavy metal in soils directly changes pH and bioavailability of metallic elements in soils, and indirectly affects processes of physiological metabolism of plants, which are all associated with heavy metal absorption [24, 34].

In offspring ramets experiencing heavy metal stress, the mean total biomass with moderate and high fertilizer were about 1.33 and 1.61 times larger than those without fertilizer. In addition, the total Cu and Zn amounts of offspring averaged about 1.72 times and 2.72 times the control. This suggests that limited compensation from parent to offspring and fitness of whole clone could be strengthened by more available nutrients to the parent. In general, we also found more Cu and Zn accumulated in aboveground parts of plants with the application of fertilizer reflected by TF. Specific physiological mechanisms, such as increasing antioxidant enzymes levels, integrating metal to cell walls, complex forming with proteins or organic acids, and so on [13], enhance tolerance of plants suffering from heavy metal contamination and ensure normal growth. It is hypothesized that physiological integration and related fertilization could protect the plants to some extent against damage by affecting biochemical detoxification strategies to plants to resist heavy metal stress.

D. sanguinalis is a common clonal species in nature, with high capability of spreading in field and adaptation to the environment. This annual herb can be harvested three or four times per year, which means that in the growing period, favorable living conditions are possible to increase heavy metal accumulation in the clonal species and accelerate associated removal of heavy metal from soil. Besides, we disposed of connected clones and only used two ramets for the experiment. As a result of strong ability of clonal propagation, one clone system of *D. sanguinalis* could generate 8 to 18 ramets in natural conditions. This suggests that one clone system could occupy a large area of habitat for remediation. However, more studies on the relationship between fertilizer intensity, numbers of ramets generated, and heavy metal accumulation efficiency are needed.

Conclusions

Although some rhizomatous species fragments or clones in connection are known to be tolerant of heavy metal pollution [19-21], this study is the first to document that extra fertilization throughout physiological integration benefits survival, growth, and heavy metal accumulation traits associated with soil remediation. The interactive effects may be mediated by the ability for transportation of extra resources between connected clones, which compensates for the metal-induced damage to the whole clonal system. The results add to the understanding of clonal plant adaptation to heavy metal pollution. The method of inducing fertilization to clonal plants shall be of great potential use for the phytoremediation of heavy metals in soils.

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