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Growth response and threshold concentration of *Agrostis stolonifera* to Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} stress

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Abstract: By sand cultural method, the experiment was conducted to investigate growth response and threshold concentration of *Agrostis stolonifera* to Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} stress. It was found that rate of seed germination decreased with increasing supply of Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+}. Heavy metal supply at concentration $\leqslant$100 mg/L increased plant height of *A. stolonifera*, whereas Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} supply higher than 100 mg/L decreased plant height. Cu^{2+} inhibited root growth significantly, and root length decreased with increasing Cu^{2+} supply. Compared with the control, root length of *A. stolonifera* was lowered by 93.75% at Cu^{2+} concentration of 600 mg/L. Aboveground biomass increased at Cu^{2+}, Zn^{2+} or Pb^{2+} concentration $\leqslant$200 mg/L, but decreased with increasing concentrations when their concentrations were higher than 200 mg/L. Chlorophyll content increased at Cu^{2+}, Zn^{2+} concentration $\leqslant$100 mg/L or Cd^{2+}, Pb^{2+} concentration $\leqslant$200 mg/L, but tended to decrease at Cu^{2+}, Zn^{2+} concentration $\geqslant$100 mg/L or Cd^{2+}, Pb^{2+} concentration $\geqslant$200 mg/L. Only Cd^{2+} significantly lowered chlorophyll content of *A. stolonifera* by 43.55% at concentration of 600 mg/L. Synthetical effect of heavy metal on *A. stolonifera* growth indicated that *A. stolonifera* was most sensitive to Cu^{2+} stress with low threshold, but the effect of Zn^{2+} stress on *A. stolonifera* growth was the least.

Key words: heavy metal stress; *Agrostis stolonifera*; growth response; threshold concentration

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Nowadays heavy metal contamination is prevalent throughout the world, mainly coming from various human activities such as mining, smelting, manufacturing processes of metals, vehicle emissions, and dumping of industrial wastes (Charkhabi *et al.*, 2005; Aksoy *et al.*, 2005; Lin *et al.*, 2005; Speir *et al.*, 2003). On this aspect, researchers have conducted extensive and thorough work (Shu *et al.*, 2004; Chander *et al.*, 2001; Chen *et al.*, 2003). Heavy metals have a low biodegradability and high persistence in the environment (Liao, 1992). Their high toxicity on biological organisms, especially to human beings through food chain has been drawn great attention in recent years. However, heavy metal contamination on crops and vegetables has received increasing attention (Athar *et al.*, 2002; Du *et al.*, 2003; Moreno *et al.*, 2002; Cui *et al.*, 2003), and phytoremediation of heavy metal-polluted soil has become a hot issue in the world (Lasat 2002; Tandy *et al.*, 2004; Walker *et al.*, 2004; Kayser *et al.*, 2000). Cu^{2+} and Zn^{2+} are essential elements for plant growth and development, but may be harmful at high concentrations. Cd^{2+} and Pb^{2+} are not essential elements in metabolic processes in plants, they can accumulate to concentrations that are toxic or lethal to plants. Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} pollution is common in urban environment with rapid industrial devel-

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opment. What is needed to point out, in urban ecosystem, turfgrass plays an important role in alleviating contamination and protecting environment (Chen *et al.*, 1991). But heavy metal toxicity on turfgrass has not been paid much attention (Cui *et al.*, 2004). *Agrostis stolonifera* is one of the common turfgrasses used for ornamental lawn and sports turf. It is planted and applied in large areas, has great values of beautifying environment and serving society (Chen *et al.*, 1991). The objective of the current study was to investigate growth responses and threshold concentrations of *A. stolonifera* to Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress. It could provide a scientific basis for regulating standard of heavy metal concentration in lawn irrigation water in China and for turfgrass remediation of heavy metal-polluted places.

1 Materials and methods

1.1 Materials

Sand was used as turfgrass medium. It was sieved, rinsed with distilled water repeatedly, then oven-dried at 105 ℃ for 1 h and then 80 ℃ for 8 h. *Agrostis stolonifera* L was chosen as plant material. Heavy metal treatments were conducted by solving $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $3CdSO_4 \cdot 8H_2O$ or $Pb(NO_3)_2$. Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} treatment concentrations were all 0, 100, 200, 300, 400, 500 and 600 mg/L respectively (Zhao *et al.*, 2002a).

1.2 Methods

1.2.1 Turfgrass culture and heavy metal stress 70 g sand medium was loaded into culture dishes in diameter of 9 cm and 100 seeds of *A. stolonifera* were even sown in each dish. At the same time, above heavy metal solutions with equal volumes were added to each dish respectively until the solution immersed the seeds, with 0 mg/L used as the control(CK). Each treatment was replicated three times. After seed germination of *A. stolonifera*, turfgrass culture was conducted in light incubator for 15 d under the following conditions: 30 ℃ during the day and 25 ℃ at night, relative humidity of 50%~70%, light culture in 24 h. Watering was performed with equal amount daily throughout the experiment to maintain adequate medium moisture.

1.2.2 Index measurment Seed germination rate was recorded daily until the seedling stage. 10 random seedlings per dish were chosen to determine plant height and their average was assumed as the plant height of the dish. After the experiment was fulfilled, plants of each dish were mowed, washed and then oven dried at 105 ℃ for 1 h to kill cells, followed by 80 ℃ for approximately 8 h to constant weight. They were weighed in dry weight to determine aboveground biomass. The dried samples were extracted with 80% acetone to determine chlorophyll content (Li, 2000). 0.02 g dried plant leaf was precisely weighed and put into mortar. A little quartz sand and calcium carbonate was added. Then the compound was ground and extracted fully with 80% acetone. It was placed in darkness for 3~5 min, then filtrated into 25 mL volumetric flask and fixed volume with 80% acetone. The extract was determined absorbance at wavelength 663 nm and 645 nm and chlorophyll content was calculated. The roots of each dish were washed fairly free of sand. 10 random plants were selected to determine root length, and the longest fibrous root was assumed as the root length of the plant. The root lengths of 10 plants were averaged.

1.2.3 Data evaluation and analysis Synthetical effect index (*SEI*) was applied to evaluate the synthetical effect of Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} on *A. stolonifera* growth(Duo *et al.*, 1999). The *SEI* can be calculated by the following formula: $SEI = (\Sigma Gr + \Sigma Br + \Sigma Rr + \Sigma Cr)/4$, where ΣGr is the sum of difference of relative percentage of seed germination rate with Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} each stress concentration and control, ΣBr is the sum of difference of relative percentage of aboveground biomass with Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} each stress concentration and control, ΣRr is the sum of difference of relative percentage of root length with Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} each stress concentration and control, and ΣCr is the sum of difference of relative percentage of chlorophyll content with Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} each stress concentration and control (Duo *et al.*, 1999).

Threshold concentration (*TC*) is an index to indicate the sensitivity of plants to pollutant stress (Zhao *et al.*, 2002b). In current study, as the representatives of aboveground and underground growth indices, aboveground biomass and root length were chosen as growth indices to calculate threshold concentration to each heavy metal stress. The synthetical threshold concentration can be calculated by: $TC=(B_{r30}+R_{r30})/2$, where R_{r30} is the threshold concentration of each heavy metal when aboveground biomass of *A. stolonifera* decreased 30% of the control, R_{r30} is the threshold concentration of each heavy metal when root length of *A. stolonifera* decreased 30% of the control (Zhao *et al.*, 2002b).

Analysis of variance was performed on all the data sets. The least significant difference was applied for multiple comparison. Statistical analysis of the data was performed using SPSS statistical package (SPSS 12.0).

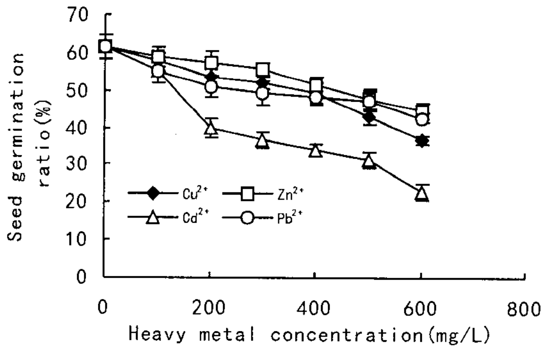

Fig. 1 Effects of heavy metal stress on seed germination of *A. stolonifera*

2 Results

2.1 Effects of heavy metal on seed germination

The addition of Cu^{2+}, Zn^{2+}, Cd^{2+} or Pb^{2+} led to a reduction in seed germination rate across the treatments, and germination rate decreased as heavy metal concentration increased (Fig. 1). Among four heavy metals, Cd^{2+} inhibited seed germination of *A. stolonifera* significantly, especially at high concentrations. At Cd^{2+} concentration of 600 mg/L, seed germination rate decreased by 63.04% as compared with control. Significant differences ($P<0.01$) were found in seed germination rate among treatments of each heavy metal. For Cu^{2+} stress, the result of further multiple comparison was 0^{A}, 100^{AB}, 200^{ABC}, 300^{ABC}, 400^{BC}, 500^{CD}, 600^{D} (mg/L), among which different letters denoted significant difference ($P<0.01$) in seed germination rate between Cu^{2+} concentrations. For Zn^{2+}, Cd^{2+} and Pb^{2+} stress, the results were 0^{A}, 100^{A}, 200^{AB}, 300^{ABC}, 400^{ABC}, 500^{BC}, 600^{C} (mg/L); 0^{A}, 100^{A}, 200^{B}, 300^{B}, 400^{B}, 500^{BC}, 600^{C} (mg/L) and 0^{A}, 100^{AB}, 200^{ABC}, 300^{BC}, 400^{BC}, 500^{BC}, 600^{C} (mg/L) respectively.

2.2 Effects of heavy metal on plant height

Except Pb^{2+} stress, the differences in plant height of *A. stolonifera* were statistically significant among treatments of other three heavy metals ($P<0.01$, Fig. 2). Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} supply at concentrations $\leqslant 100$ mg/L promoted plant height growth, but inhibited it at concentrations >100 mg/L. As compared with control, plant height decreased by 40.72%, 31.22%, 48.87% and 19.91% respectively at Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} concentration of 600 mg/L. Similar to seed germination, Cd^{2+} inhibited plant height growth significantly at 600 mg/L. Analysis of variance indicated the results of 0^{BC}, 100^{AB}, 200^{ABC}, 300^{AB}, 400^{AB}, 500^{C}, 600^{C} (mg/L) for Cu^{2+} stress, 0^{AB}, 100^{AB}, 200^{A}, 300^{AB}, 400^{AB}, 500^{AB}, 600^{B} (mg/L) for Zn^{2+} stress and 0^{A}, 100^{A}, 200^{ABC}, 300^{AB}, 400^{AB}, 500^{AB}, 600^{B} (mg/L) for Cd^{2+} stress in which different letters showed significant difference ($P<0.01$) in plant height between stress concentrations of each heavy metal.

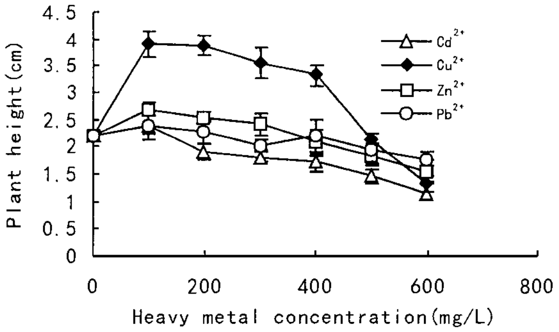

Fig. 2 Effects of heavy metal stress on plant height of *A. stolonifera*

2.3 Effects of heavy metal on aboveground biomass

Fig. 3 showed the results of shoot biomass under different stress concentrations of four heavy metals. As compared with the control, Cu^{2+}, Zn^{2+}, Pb^{2+} increased aboveground biomass at concentrations $\leqslant 200$ mg/L, but aboveground biomass decreased with increasing stress concentrations when concentration was higher than 200 mg/L. However, for Cd^{2+} stress, the maximum shoot biomass was observed at concentration of 100 mg/L. Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress at high concentration clearly reduced shoot biomass. As compared with the control, the shoot biomass of *A. stolonifera* decreased by 40. 00%, 30. 00%, 30. 30% and 20. 20% respectively at Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress concentration of 600 mg/L. After the maximum biomass, significantly negative correlation lied between biomass(*Br*) and stress concentration(*C*) of each heavy metal. The regressive equations for Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress were $Br_{Cu^{2+}} = 0.162 - 0.0002C_{Cu^{2+}}$, $r = 0.9948^{**}$; $Br_{Zn^{2+}} = 0.210 - 0.0002C_{Zn^{2+}}$, $r = 0.9825^{**}$; $Br_{Cd^{2+}} = 0.1653 - 0.0002C_{Cd^{2+}}$, $r = 0.9955^{**}$ and $Br_{Pb^{2+}} = 0.178 - 0.0002C_{Pb^{2+}}$, $r = 0.9815^{**}$, respectively. Analysis of variance showed that there was significant difference ($P<0.01$) in biomass among treatments of each heavy metal. The results of multiple comparison were 0^{AB}, 100^{AB}, 200^{A}, 300^{AB}, 400^{AB}, 500^{AB}, 600^{B} (mg/L) for Cu^{2+} stress, 0^{AB}, 100^{AB}, 200^{A}, 300^{AB}, 400^{AB}, 500^{AB}, 600^{B} (mg/L) for Zn^{2+} stress, 0^{ABC}, 100^{A}, 200^{AB}, 300^{ABC}, 400^{ABC}, 500^{BC}, 600^{C} (mg/L) for Cd^{2+} stress and 0^{AB}, 100^{AB}, 200^{A}, 300^{AB}, 400^{AB}, 500^{AB}, 600^{B} (mg/L) for Pb^{2+} stress in which different letters denoted significant difference ($P<0.01$) in aboveground biomass between stress concentrations of each heavy metal.

2.4 Effects of heavy metal on root length

Except Cd^{2+} stress, there were significant differences in root length among treatments of other three heavy metals ($P<0.01$). Zn^{2+}, Cd^{2+} and Pb^{2+} stress showed the same tendency, namely, root length increased first and then decreased, and the maximum appeared at concentration of 100 mg/L. However, Cu^{2+} inhibited root growth of *A. stolonifera* greatly, moreover, root length decreased significantly with increasing Cu^{2+} supply. As compared with control, root length decreased by 93. 75% at Cu^{2+} concentration of 600 mg/L(Fig. 4). After the maximum, negative correlation was found between root length (Rr) and stress concentration(C) of Zn^{2+}, Cd^{2+} and Pb^{2+}. The regressive equations for Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress were $Rr_{Cu^{2+}} = 0.85 - 0.0016C_{Cu^{2+}}$, $r = 0.8940^{**}$; $Rr_{Zn^{2+}} = 2.02 - 0.002C_{Zn^{2+}}$, $r = 0.8915^{**}$; $Rr_{Cd^{2+}} = 1.5193 - 0.0011C_{Cd^{2+}}$, $r = 0.9606^{**}$; $Rr_{Pb^{2+}} = 1.934 - 0.0018C_{Pb^{2+}}$, $r = 0.9088^{**}$ respectively. Analysis of variance showed the following results: 0^{A}, 100^{B}, 200^{C}, 300^{CD}, 400^{D}, 500^{D}, 600^{E} (mg/L) for Cu^{2+} stress, 0^{B}, 100^{A}, 200^{B}, 300^{B}, 400^{B}, 500^{B}, 600^{B} (mg/L) for Zn^{2+} stress and 0^{B}, 100^{A}, 200^{B}, 300^{B}, 400^{B}, 500^{B}, 600^{B} (mg/L) for Pb^{2+} stress in which different letters denoted significant difference ($P<0.01$) in root length between stress concentrations of each heavy metal.

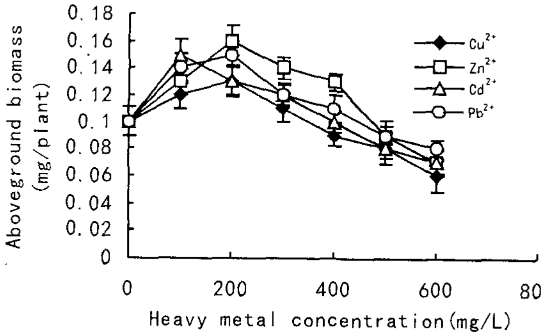

Fig. 3 Effects of heavy metal stress on aboveground biomass of *A. stolonifera*

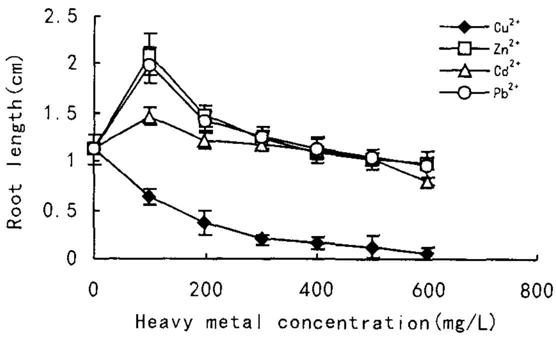

Fig. 4 Effects of heavy metal stress on root length of *A. stolonifera*

2.5 Effects of heavy metal on chlorophyll content

Significant differences were found in chlorophyll content among treatments of each heavy metal ($P<0.01$, Fig. 5). Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress at low concentrations increased chlorophyll content. But at high concentrations chlorophyll content decreased, probably because chlorophyll synthesis was hindered or degradation was accelerated. The specific mechanism is further needed to study (Cui *et al.*, 2004; Xu *et al.*, 2006). The maximum of chlorophyll content appeared at concentration of 100 mg/L for Cu^{2+} and Zn^{2+} stress, and at 200 mg/L for Cd^{2+} and Pb^{2+} stress. At stress concentration of 100 mg/L, Cu^{2+} and Zn^{2+} increased chlorophyll content by 77.94% and 57.59% respectively when compared with control. However, at high stress concentration, only Cd^{2+} considerably decreased chlorophyll content. As compared with control, chlorophyll content decreased by 43.55% at Cd^{2+} concentration of 600 mg/L. Analysis of variance showed the results of 0^{CD}, 100^{A}, 200^{B}, 300^{BC}, 400^{CD}, 500^{CD}, 600^{D} (mg/L) for Cu^{2+} stress, 0^{C}, 100^{A}, 200^{A}, 300^{AB}, 400^{AB}, 500^{ABC}, 600^{BC} (mg/L) for Zn^{2+} stress, 0^{AB}, 100^{AB}, 200^{A}, 300^{AB}, 400^{BC}, 500^{BC}, 600^{C} (mg/L) for Cd^{2+} stress and 0^{CD}, 100^{AB}, 200^{A}, 300^{BC}, 400^{BCD}, 500^{BCD}, 600^{D} (mg/L) for Pb^{2+} stress in which different letters showed significant difference ($P<0.01$) in chlorophyll content between stress concentrations of each heavy metal.

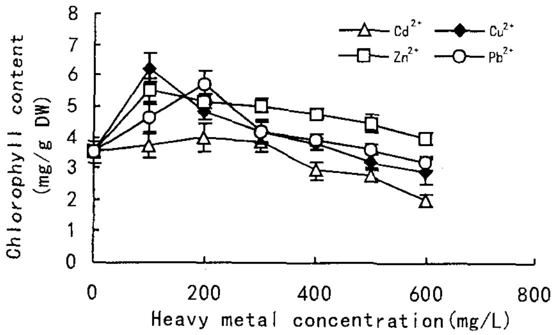

Fig. 5 Effects of heavy metal stress on chlorophyll contents of *A. stolonifera*

2.6 Synthetical effect of heavy metal on *A. stolonifera* growth

In order to evaluate synthetical effect of each heavy metal on *A. stolonifera* growth, synthetical effect index (*SEI*) was applied. In general, plant height was positively correlated with aboveground biomass, so it was not included in the formula of *SEI*. Table 1 showed that the negative effect of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} stress on *A. stolonifera* growth decreased in the order of $Cu^{2+}>Cd^{2+}>Pb^{2+}>Zn^{2+}$. *A. stolonifera* was most sensitive to Cu^{2+} stress because Cu^{2+} inhibited root growth greatly. But *A. stolonifera* had relatively high ecological threshold to Zn^{2+}.

2.7 Threshold concentration of heavy metal

In the research, aboveground biomass and root length were chosen as aboveground index and underground index to calculate threshold concentration of each heavy metal. When inhibited rate of aboveground biomass or root length was 30%, the concentration of each heavy metal was defined as the threshold concentration of the index (Zhao *et al.*, 2002b). Table 2 showed the synthetical threshold concentration of heavy metals increased in the order $Cu^{2+}<Cd^{2+}<Pb^{2+}<Zn^{2+}$. It could be proved by the results of *SEI*. *A. stolonifera* had relatively low ecological threshold to Cu^{2+} stress.

Table 1 Synthetical effect index of *A. stolonifera* to four heavy metal ions

Heavy metal	ΣGr	ΣBr	ΣRr	ΣCr	*SEI*	Order
Cu^{2+}	-123.33	-10.00	-460.71	117.19	-119.21	4
Zn^{2+}	-85.29	120.00	104.46	225.79	91.24	1
Cd^{2+}	-242.36	50.00	4.46	-50.72	-59.66	3
Pb^{2+}	-121.18	90.00	96.43	120.06	46.33	2

Table 2 Threshold concentration under stress of Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}

Heavy metals	Threshold concentration of each index (mg/L)		*TC* (mg/L)	Order
	B_{r30}	R_{r30}		
Cu^{2+}	460.00	41.25	250.63	4
Zn^{2+}	700.00	618.00	659.00	1
Cd^{2+}	476.50	668.45	572.48	3
Pb^{2+}	540.00	638.89	589.44	2

3 Discussion and conclusion

In the current study, with metal concentration increased, plants appeared to be thin, but showed no symptoms of phytotoxicity, which agreed with Sopper's (1989) observations. So *A. stolonifera* had strong tolerance to the four heavy metals. It was probably correlated with turfgrass properties. In view of growth indices, germination rate decreased with the increase of four heavy metal concentrations, only Cd^{2+} affected germination rate markedly. Except that root length of *A. stolonifera* decreased as Cu^{2+} concentration increased, the effects of four heavy metal ions on plant height, root length, biomass and chlorophyll content showed the same tendency, namely, heavy metals at low concentrations ($\leqslant$100 mg/L or 200 mg/L) had positive effect, above indices appeared maximum, but after the maximum, they decreased with the increase of heavy metal concentrations.

From the angle of applied value of turfgrass, root growth and greenness are of vital importance because root development would affect turfgrass growth and greenness would directly affect ornamental properties of turfgrass. The basic deleterious effect of Cu^{2+} on growth is related to the root system. Faust *et al.* (2000) reported that root development of Penncross creeping bentgrass decreased as Cu^{2+} concentration increased. Similarly, in the present study, Cu^{2+} inhibited root elongation of *A. stolonifera* significantly, root length decreased by 93.75% at Cu^{2+} concentration of 600 mg/L when compared with control. So Cu^{2+} would probably limit turf establishment of *A. stolonifera*. Analysis on synthetical effect of heavy metals and threshold concentration showed the same results. *A. stolonifera* was most sensitive to Cu^{2+} stress due to marked inhibition of root growth. However, *A. stolonifera* was tolerant to Zn^{2+} with high concentrations, which could be confirmed by other report (Palazzo *et al.*, 2003). So in practical application, irrigation of turfgrass should strictly control concentration of Cu^{2+}. Due to high toxicity of Cd^{2+} and Pb^{2+}, their concentrations should be regulated low, but concentration of Zn^{2+} may be higher.

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匍茎翦股颖对 Cu^{2+}、Zn^{2+}、Cd^{2+} 与 Pb^{2+} 胁迫的生长响应与阈限浓度

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摘　要：采用砂培法，研究了匍茎翦股颖对 Cu^{2+}、Zn^{2+}、Cd^{2+} 与 Pb^{2+} 胁迫的生长响应及阈限浓度，结果表明：种子萌发率随着4种重金属浓度的增加而下降。对株高的影响是当重金属浓度小于100 mg/L时会促进株高生长，高于100 mg/L则产生抑制作用。Cu^{2+} 显著抑制根系生长，并随浓度的增加抑制效应愈加显著；在 Cu^{2+} 浓度为600 mg/L时匍茎翦股颖的根长比对照下降了93.75%。Cu^{2+}、Zn^{2+}、Pb^{2+} 浓度小于200 mg/L时会促进地上生物量的增加，但高于200 mg/L时，地上生物量会随着3种重金属的增加而减少。Cu^{2+}、Zn^{2+} 浓度小于100 mg/L或 Cd^{2+}、Pb^{2+} 浓度小于200 mg/L会增加叶绿素的含量，高浓度会降低叶绿素的含量；Cd^{2+} 在浓度为600 mg/L时显著降低叶绿素含量，与对照相比，下降了43.55%。匍茎翦股颖生长的综合效应分析表明，匍茎翦股颖对 Cu^{2+} 胁迫最敏感，具有较低的阈限浓度，而 Zn^{2+} 胁迫对匍茎翦股颖的生长影响最小，阈限浓度相对较高。

关键词：重金属胁迫；匍茎翦股颖；生长响应；阈限浓度

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为进行物种多样性保护研究提供有益的旁证。为对五列木种群适应特性以及该种群对于群落构建的生态学意义等问题进一步展开研究提供有益帮助。为五列木种群保护和管理提供依据。保护珍稀濒危植物是维持生物多样性必不可少的措施之一，也是扩大该种群的有效途径，通过研究五列木种群生态特征，可为五列木种群保护和管理提供依据。由于五列木种群Ⅲ级小树最多，发展潜力大，因此，五列木种群的年龄结构呈发展型。人为干扰轻，种群发展趋势良好，因此深入开展研究和保护是必要的。且重在保护，尤其是保护其生境，使种群呈良性发展。怎样进行保护呢？笔者认为除了对五列木的资源状况、生境和群落特征进行研究外，还需进一步弄明白五列木种群的濒危机制、濒危过程、该物种生存所需的条件等。

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