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*Original Research*

# Varied Responses of Growth and Mineral Elements Concentrations in *Pennisetum ericanum* and *Festuca arundinacea* under Cd/Cu Addition

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**Abstract**

An experiment was carried out to compare cadmium and copper absorption and mineral nutrition accumulation in pennisetum and tall fescue in order to select an appropriate grass to remediate Cd/Cu-contaminated soil and explore their detoxification mechanisms of contamination by mineral elements. The biomass remained constant in tall fescue under each Cd addition level and increased in pennisetum until Cu reached 500 μM, whereas they dramatically decreased as the Cu or Cd solution increased, which was concurrent with quadratic regression model analysis. The Cd/Cu concentrations in tall fescue were mostly accumulated in the roots and were much higher than those in pennisetum. The extracted amount of Cd in the shoots and the total Cu concentrations of pennisetum were higher than the corresponding values in tall fescue at every Cd/Cu addition level. Negative correlations were observed between Cd and shoot Ca, Cu, K, Mg, and Zn, and root Cu and Na of tall fescue and the root K of pennisetum. The Cu concentration was negatively correlated with K and positively correlated with Na in tall fescue and pennisetum under the Cu treatments. As the Cd/Cu concentration in solution increased, K/Na values were significantly decreased in the roots of tall fescue under Cu stress and pennisetum under Cd/Cu stress, whereas they increased in the roots of tall fescue under Cd addition. In summary, pennisetum exhibited the greater biomass and Cd/Cu extraction; indicating it as a candidate energy grass for phytoextraction. The adjustment capacity of grass for K and Na might relate to the tolerance to Cd/Cu.

**Keywords**: cadmium, copper, bioenergy grass, translocation factor, mineral element

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## Introduction

Due to changes in the natural environment and human activities, such as metallurgical, petrochemical, agricultural, and mining activities, as well as urban sewage, heavy metal pollution has become a major problem in terms of environmental safety and human health [1-2]. Compared with other contaminants, Cd and Cu are easily bioaccumulated with little decomposition, which is harmful to both the soil and organisms. Cd, one of the most toxic heavy metals, can seriously damage plant metabolism, even in trace amounts [3]. Although Cu is a necessary element for plants, it will disturb the balance of the plant antioxidant defense system, and excessive amounts can influence photosynthesis [4]. Therefore, it is urgent to remediate Cd- and Cu-polluted soil in order to protect plants, animals, and humans from health risks.

Various technological solutions, including physical methods such as electro-osmosis, soil fracturing, and thermal decomposition have been applied to remediate contaminated soils; however, these methods are cumbersome and expensive [5-6]. Additionally, chemical methods such as coagulation-flocculation, oxidation, chemical precipitation, ion exchange, adsorption, and membrane filtration can lead to secondary pollution [7]. Compared with chemical and physical methods, phytoremediation, defined as the utilization of plants to remove or accumulate soil contaminants, is a cost-effective and environmentally safe technique. Phytoremediation has become popular in the past two decades as researchers have found plants with different accumulation capacities for different metal species [7-10]. However, the slow growth rate and lower biomass become limiting factors for hyper-accumulator utilization. Bioenergy grasses grow quickly and have higher biomass than other grasses, enormous root structures, and strong tolerance to marginal lands [11-12], which are in accordance with the suggestion by Valipour and Ahn [13] for plant species suitable for phytoremediation. Their huge biomass compensated for lower accumulating contaminants in plants and produced biomass for fiber without the conflict with food crops avoiding contaminant entry into the food chain [14-15].

Pennisetum has been reported to have strong tolerance to heavy metals and accumulate Cd and Zn in its shoots, suggesting its potential to serve as a Cd and Zn phytoremediation plant [16-17]. Previous studies have focused on contrasting the phytoremediation ability of one or two species of energy grasses under stress from one (Cd) or various contaminant species (Zn, Cr, Cu, Pb and Cd); however, few studies have been designed to compare bioenergy grass with other grasses having strong Cd stabilization ability, despite the potential of these studies to be more persuasive. Thus, tall fescue, which was reported to have strong tolerance to Cd and Pb stress and to stabilize most heavy metals in its root without any poisoning phenomena, was selected in this experiment [18-20]. Comparing the Cd/Cu accumulation concentrations in these two plants was considered more suitable for highlighting the advantage of bioenergy grass with respect to biomass and broad heavy metal adaptability.

The absorption of heavy metals has a competitive or synergistic effect with mineral nutrition, which could disturb various physiological and biochemical activities in plants. Cd has no known biological function in plants and enters through only divalent metal ion channels ($Ca^{2+}$, $Mg^{2+}$) or as cation transporters ($Zn^{2+}$, $Cu^{2+}$ or $Fe^{2+}$) [21-22]. Within plants, Cd then impacts plant nutrition absorption and disturbs the inner ionic equilibrium [23]. Many reports have examined the relationships between Cd and mineral elements, showing that Cd affected Ca, K, P, Mg, and Fe absorption; however, there are still no final conclusions. Plants have evolved sophisticated and tightly regulated homeostatic networks that control Cu uptake and delivery to target proteins and detoxification processes, as reported by Peñarrubia et al. [24]. Nevertheless, studies on the effect of excess Cu on mineral element accumulation are scarce. Furthermore, to screen for plants suitable for heavy metal phytoremediation, the selective absorption ability of Cd/Cu regulated by mineral elements in different plant species should be examined. Because some researchers have found that halophyte species display specific adaptations to Cd stress, the phytoremediation ability of halophyte has drawn more attention [25-27]. Researchers have noticed the characteristics of osmotic adjustment capacity by nontoxic organics, such as proline, soluble sugar, and soluble protein in non-halophytes under Cd toxicity [28-29]; however, it is unknown whether the heavy metal tolerance was related to inorganic ions such as K and Na in non-halophyte plants. Thus, comparing the characteristics of nutritional element absorption, especially K and Na, under Cd/Cu stress would be helpful for determining the alleviation mechanism of heavy metal toxicity in selected plants.

Therefore, the objectives of this study are to compare the Cd and Cu adaptation and accumulation traits in pennisetum (P) and tall fescue (F) during the seedling stage, to demonstrate which of pennisetum or tall fescue has higher Cd/Cu tolerance and absorption, and to provide support and academic evidence for the further utilization of bioenergy grasses in contaminated areas. In addition, we attempt to explain the detoxification mechanism by contrasting the differences in nutritional element absorption.

## Materials and Methods

### Experimental Design

Hydroponic culture was designed for this experiment to exclude the different absorption, mobility, and retention characteristics of the metal and nutrition elements in soil. The experiment was conducted in a greenhouse at China Agricultural University. Two species of grasses were selected: a tall fescue (*Festuca arundinacea* Schreb.) cultivar named "Escalade" and pennisetum (*Pennisetum americanum* (L.) Schum). These grasses were provided by the Grass Seed Quality Inspection Center of China Agricultural University. Cd was evaluated at 4 levels with three replications: Cd (0, 5, 20, 100 μM); and Cu is a necessary element for plant growth, so the lowest level was added as 0.25 μM of ½-strength Hoagland nutrient solution as control check (CK), hence Cu concentration was set as 4 level (CK, 50, 200, 500 μM).

## Plant Materials and Growth Conditions

Seeds of tall fescue cultivar and pennisetum were immersed in an $H_2O_2$ solution for 20 minutes, rinsed thoroughly with tap water, and then washed with deionized water three times. Then the seeds were sown in plastic pots filled with silica sand. The pots were placed in sunlight and stored at room temperature. The pots were watered once daily to keep the sand humidified until germination, and then the plants were watered twice. Fifteen days later, five seedlings with a strip of sponge were rolled up and fastened to a polystyrene plank and transferred to plastic containers. Each container was filled with 2 L ½-strength Hoagland nutrient solution. Each container housed five seedlings for each treatment. In total, 48 pots were placed in the China Agricultural University greenhouse at 20/28ºC. The nutrient solution was changed once every three days. Before Cd treatment, the seedlings were grown in nutrient solution for 4 weeks. The Cd-treated plants were harvested after Cd treatment for 3 weeks. The Cu were added in solution as follows: $Cu(SO_4)\cdot 5H_2O$ at 4 levels of CK, 50, 200, and 500 μM, respectively. The temperature conditions and nutrition solution were the same as for Cd addition. The seedlings of tall fescue and pennisetum were grown for 6 weeks in solution before Cu treatment. The Cu-treated plants were harvested after Cu treatment for 3 weeks.

## Mineral Element Analysis

The aboveground parts were cut using scissors, and the roots were washed with tap water, immersed in deionized water three times, and then dried using absorbent paper. Then the above- and below-ground plant parts were placed in an oven at 65ºC for 48 hours and then weighed. The final biomass data are shown for the six plants per pot. The dried materials were milled (<0.5mm) and digested by $HNO_3$ and $H_2O_2$ (3:1 v/v) in a microwave-accelerated reaction system (MarsX; CEM) under a three-step digestion process. The cadmium (Cd), copper (Cu), calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), iron (Fe), sodium (Na), and zinc (Zn) concentrations were determined by inductively coupled plasma-mass spectrometry (ICP-MS; model 7700; Agilent Technologies). Agilent technologies standards were used to ensure the accuracy of chemical analysis.

## Data Analysis

The metal concentration per plant was used to evaluate the plant Cd and Cu phytoextraction ability using the following equation [30]:

$$\begin{aligned} &\text{Heavy metal concentration per plant} \\ &= \text{metal concentration of dry pant tissue} \\ &\times \text{dry biomass of per pot} \end{aligned}$$

The Translocation Factor (TF) was calculated to evaluate the phytoremediation efficiency using the following equation [17]:

$$\text{Translocation factor} = \frac{\text{metal concentration of dry plant shoot}}{\text{metal concentration of dry plant root}}$$

The Cd, Cu, and mineral element concentrations were subjected to one-way ANOVA using the least significant difference (LSD) to analyze significant differences between treatments ($P<0.05$). Correlation analyses were conducted between the Cd/Cu and nutrition concentrations. The data were analyzed using SPSS17.0 statistics software for Macintosh (IBM).

# Results

## Biomass

The growth responses of the two grasses to different Cd and Cu levels are shown in Fig. 1. The shoots and

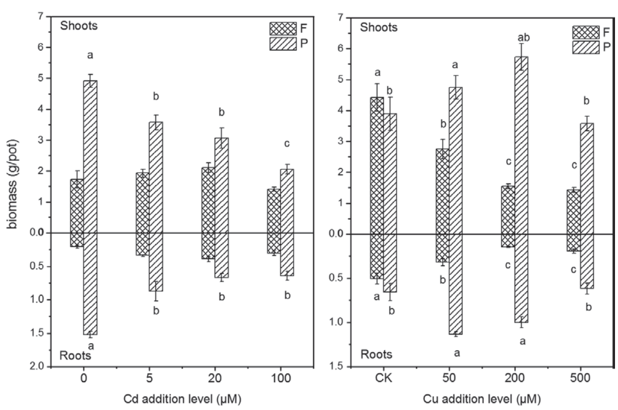


Fig. 1. Shoot and root dry biomasses of tall fescue and pennisetum under Cd and Cu treatments. F represent tall fescue, P represent pennisetum. Bars above the columns represent standard errors (n = 3). Different letters in the bar diagram mean significantly difference at 0.05 levels under different Cd or Cu additional levels for the same plant species. It is same to the below figures and tables.

Table 1. Linear regressions of the variables shoot and root biomass with Cd and Cu (X) addition levels in solution of tall fescue and pennisetum.

| | Plant species | | Intercept | X | $X^2$ | Sig | $R^2$ |
|---|---|---|---|---|---|---|---|
| Cd | F | Shoot | 1.755 | 0.023 | 0.000 | 0.055 | 0.516 |
| | | Root | 0.252 | 0.009 | $-8.811\times10^{-5}$ | 0.109 | 0.425 |
| | P | Shoot | 4.443 | -0.089 | 0.001 | 0.004 | 0.751 |
| | | Root | 1.285 | -0.041 | 0.000 | 0.023 | 0.611 |
| Cu | F | Shoot | 3.263 | -0.004 | $2.644\times10^{-5}$ | 0.001 | 0.822 |
| | | Root | 0.463 | -0.002 | $3.716\times10^{-6}$ | 0.002 | 0.800 |
| | P | Shoot | 4.724 | 0.004 | $-1.342\times10^{-5}$ | 0.188 | 0.341 |
| | | Root | 0.851 | 0.002 | $-5.289\times10^{-6}$ | 0.050 | 0.526 |

F represented tall fescue, P represented pennisetum (as in the following tables)

roots biomass of these grasses were significantly affected by Cd and Cu addition ($P<0.05$), except the root biomass of pennisetum and tall fescue under Cd stress ($P>0.05$). The shoots biomass of pennisetum and shoots and roots of tall fescue significantly decreased with an increase in the Cd and Cu contents in solution, respectively. Surprisingly, the biomass of tall fescue kept stable when the Cd concentration was below 100 µM, and the same trend appeared in the roots of pennisetum as the Cd contents in solution increased. The shoots and roots biomass of pennisetum first increased then decreased with solution Cu increasing.

For the 5, 20, and 100 µM Cd addition levels, compared with the control, the shoot biomass of pennisetum decreased by 27%, 38%, and 58%, respectively, and the root biomass of pennisetum was diminished by 43%, 56%, and 58%, respectively. Compared with the control treatment, the tall fescue shoots biomass decreased by 38%, 65%, and 68%, and the root biomass decreased by 37%, 71%, and 61% for the low, medium, and high Cu addition levels, respectively. The shoot biomass of tall fescue ranged from 1.41 for 100 µM Cd to 2.11 for 20 µM Cd addition. In the case of pennisetum, the shoot biomass ranged from 3.57 for 500 µM Cu to 5.73 for 200 µM Cu addition, and the root biomass ranged from 0.62 for 500 µM Cu to 1.13 for 50 µM Cu addition. The biomass data reflected that the two grasses had opposite Cd/Cu tolerance: low and medium addition levels of Cu could promote pennisetum growth, whereas Cd had no obvious toxicity effect on tall fescue growth – even under the highest Cd addition levels.

Quadratic regression models for the shoot and root biomass of pennisetum and tall fescue under Cd and Cu addition were evaluated (Table 1). The confidence level for each model exceeded 95% (i.e., $P<0.05$), except for the root of tall fescue under Cd stress and that of pennisetum under Cu stress. The sensitivity of the biomass to Cd/Cu addition was shown by comparing the absolute values of the $X^2$ coefficient estimates (Table 1). Therefore, among the shoot and root biomass, the sensitivity of tall fescue and pennisetum

Table 2. Concentrations of Cd and Cu in tall fescue and pennisetum under different Cd and Cu addition levels (mg/kg DW).

| | | F | | | P | | |
|---|---|---|---|---|---|---|---|
| | | Shoot | Root | TF | Shoot | Root | TF |
| Cd | 0 | 0.3±0.1d | 13.7±11.9d | 0.02b | 1.0±0.9d | 0.4±0.3c | 20.0a |
| | 5 | 55.5±5.0c | 896.2±47.6c | 0.06ab | 68.9±6.7c | 120.7±16.6b | 0.57b |
| | 20 | 84.4±9.3b | 1060.6±149.6b | 0.08a | 93.0±3.4b | 281.2±59.2b | 0.33b |
| | 100 | 166.8±9.0a | 2471.6±171.1a | 0.07ab | 149.9±9.4a | 1533.6±64.7a | 0.12c |
| Cu | CK | 16.4±1.5d | 174.4±45.0d | 0.09a | 18.9±2.5c | 60.0±2.0c | 0.31a |
| | 50 | 45.1±2.5c | 1039.4±92.8c | 0.04b | 33.2±0.9c | 868.4±96.0b | 0.04b |
| | 200 | 73.3±12.5b | 2093.8±122.7b | 0.03b | 95.2±3.2a | 1005.6±40.0b | 0.09b |
| | 500 | 113.5±6.4a | 4123.4±236.6a | 0.03b | 105.3±9.9a | 2056.9±65.6a | 0.05b |

Different letters in the same column represent significant differences at 0.05 level to Cd was as follows: tall fescue < pennisetum in the shoots, and tall fescue > pennisetum in the roots. The trend in the sensitivity of the shoots to Cu was tall fescue > pennisetum, whereas the trend in the roots was the exact opposite. Moreover, the roots were less sensitive than the shoots.

## Cd and Cu Concentrations

The Cd and Cu concentrations measured in the shoots and roots of tall fescue and pennisetum under different Cd and Cu addition treatments are shown in Table 2. The Cd and Cu concentrations in the shoots and roots of the two plants were significantly different among the various Cd/Cu additional levels ($P$<0.05) and increased as the contaminant level in the solutions increased ($P$<0.05). The highest Cd/Cu concentrations were observed in the tissues of tall fescue.

The shoot Cd concentrations in tall fescue were similar to those in pennisetum at every Cd addition level, and the largest Cd accumulation amount was 166.8 mg/kg. The Cd concentrations in the roots of tall fescue were 1.6-7.4 times higher than those in pennisetum. The Cu concentrations in the aboveground part of tall fescue were higher than those in pennisetum, except for the pennisetum under 200 µM Cu addition, which had a 30% higher Cu concentration than tall fescue. However, the Cu concentrations in the roots of tall fescue were much higher than those in pennisetum at every Cu addition level. Furthermore, the root Cd/Cu concentrations in the two grasses were substantially higher than those in the shoots. The Cd accumulation in the roots was on average 94% and 75% of the total concentration in tall fescue and pennisetum, respectively; the corresponding Cu concentration ratios in the roots were 97% and 94%.

The TF was calculated to further confirm the Cd/Cu accumulation in the shoots, and all the values were less than 1, indicating that the three grasses stabilized Cd/Cu mainly in the belowground parts. The TF of the grasses decreased as the Cd/Cu solution concentration increased, and the highest TF was observed in pennisetum.

## Extracted Cd and Cu Concentrations

The extracted Cd and Cu concentrations in each pot of tall fescue and pennisetum detected under different Cd and Cu additional levels are shown in Fig. 2. The extracted Cd and Cu concentrations in each pot of these grasses significantly increased as the solution Cd/Cu concentration increased; the roots extracted Cd/Cu concentrations were higher than the shoots concentrations under every Cd/Cu addition level, except for the extracted Cd concentration in pennisetum. The total extracted Cd concentration in tall fescue was higher than that in pennisetum. However, the Cd extracted from the pennisetum shoots was higher than that extracted from tall fescue, increasing by 129%, 60%, and 31% under the 5, 20, and 100 µM Cd addition levels, respectively.

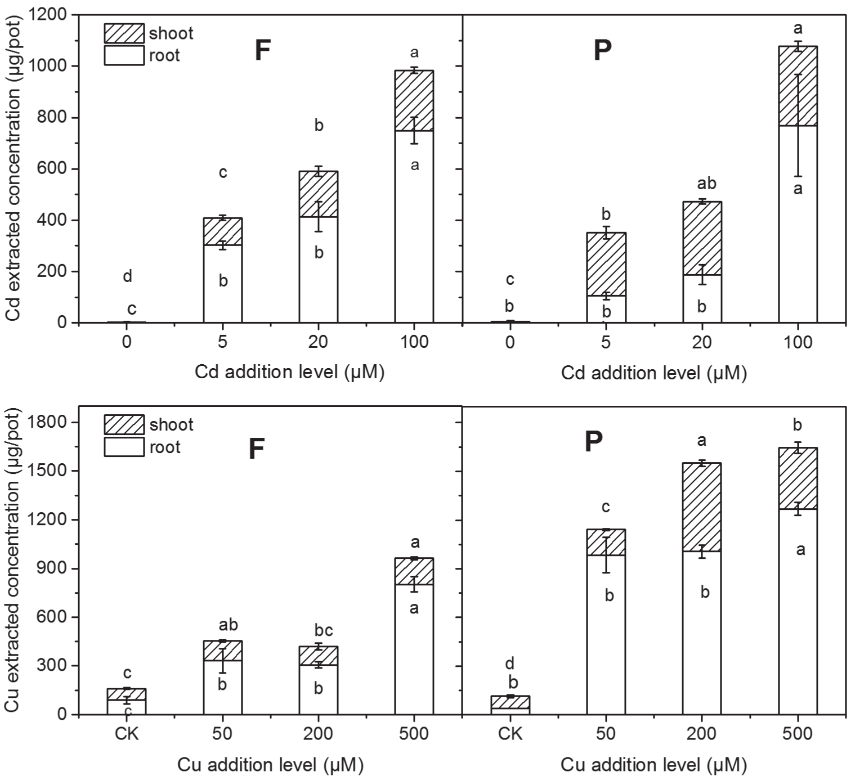


Fig. 2. Shoot and root Cd/Cu extracted concentration of tall fescue and pennisetum under Cd and Cu treatments.

### Concentrations of Macroelements, Middleelements, and Microelements

The macroelement concentrations (Ca, K, Mg, and P) in tall fescue and pennisetum receiving Cd/Cu treatments are shown in Fig. 3. In the Cd and Cu treatment, the concentrations of Ca, K, Mg, and P significantly decreased in the shoots of tall fescue as the Cd concentration increased, except K and P under Cd treatment, and its root K concentrations decreased as the Cu content in solution increased. For pennisetum, the shoot Ca concentration significantly decreased as the Cd content in solution increased, and the same result was observed in the roots for the K concentration with Cd addition and K, Mg, and P concentrations with Cu addition. The concentrations of Ca and P in the roots

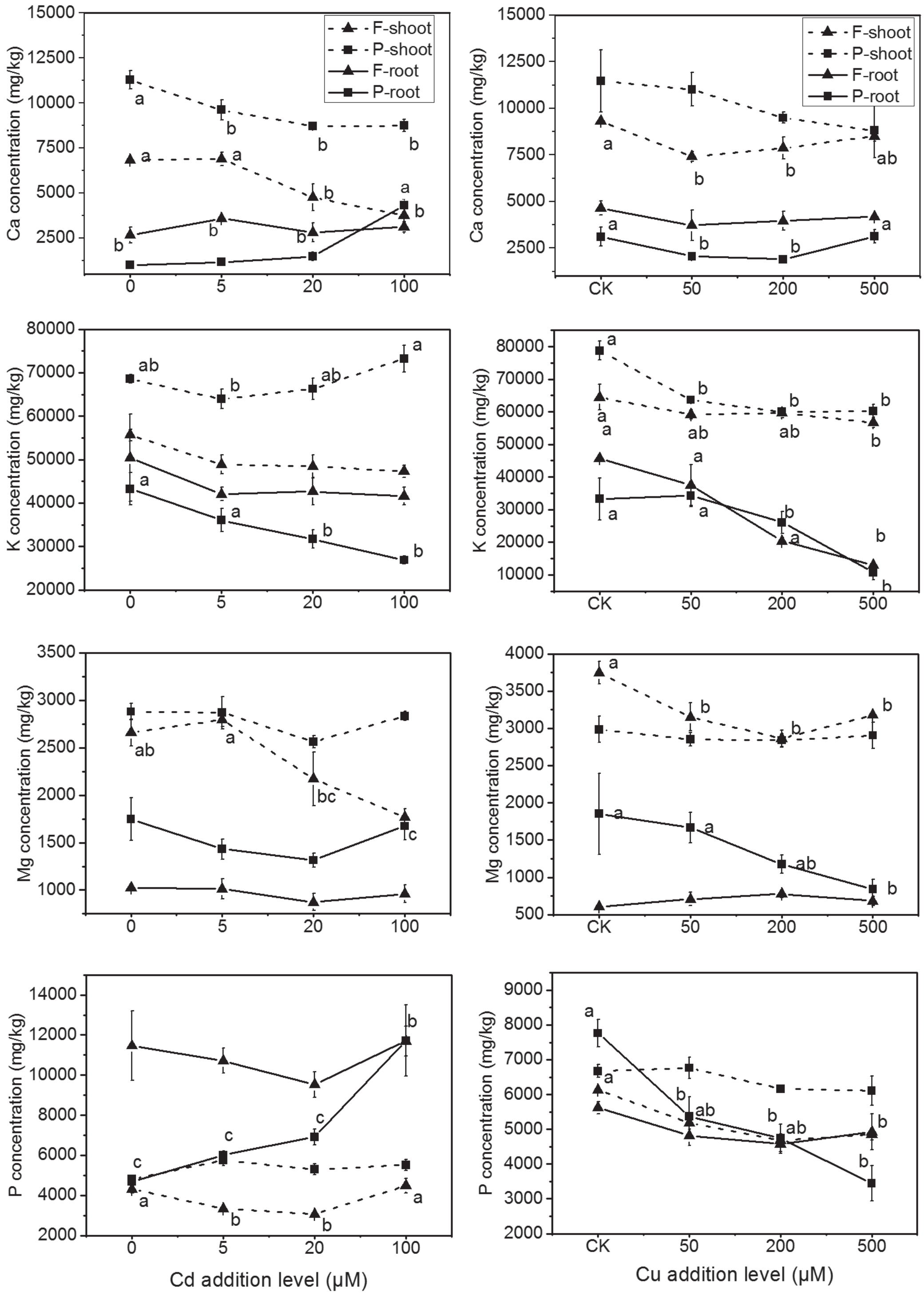


Fig. 3. The macroelement concentrations (Ca, K, Mg, P) in tall fescue and pennisetum under Cd/ Cu treatments. The triangle refer to tall fescue, the square refer to pennisetum; the dotted line refer to shoots and solid line refer to roots. It is same to the below figures.

under 100 μM Cd addition were significantly higher than those at the other Cd addition levels.

The microelement concentrations (Fe, Na, Zn and Cu) in tall fescue and pennisetum under Cd/Cu treatments are shown in Fig. 4. As the solution Cd/Cu increased, the shoot Zn and Fe concentrations in tall fescue decreased, whereas there were no significant differences in the levels of the other elements in the shoots of these two grasses. For the belowground plant parts, the Fe and Na concentrations in pennisetum with Cd treatment and the Na concentration in tall fescue and pennisetum with Cu treatment increased as the solution Cd/Cu concentration increased. However, opposite trends were observed for the Na and Zn concentrations in tall fescue with Cd addition and the Zn concentration in pennisetum with Cu addition.

The values of K/Na in tall fescue and pennisetum under Cd/Cu treatments are shown in Fig. 5. These results proved to be particularly interesting. The K/Na values in the shoots of tall fescue and

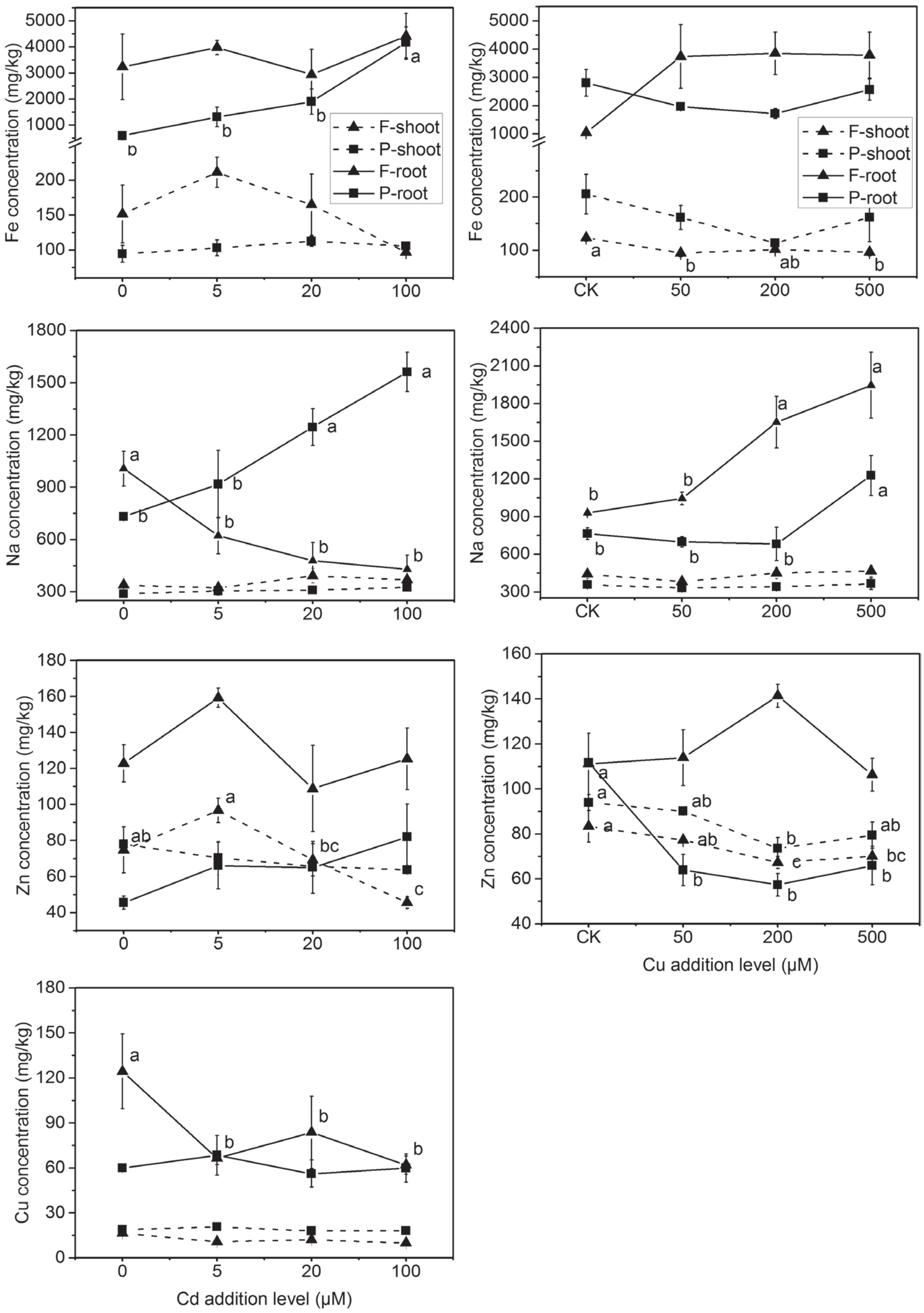


Fig. 4. The microelement concentrations (Fe, Na, Zn, Cu) in tall fescue and pennisetum under Cd/ Cu treatments.

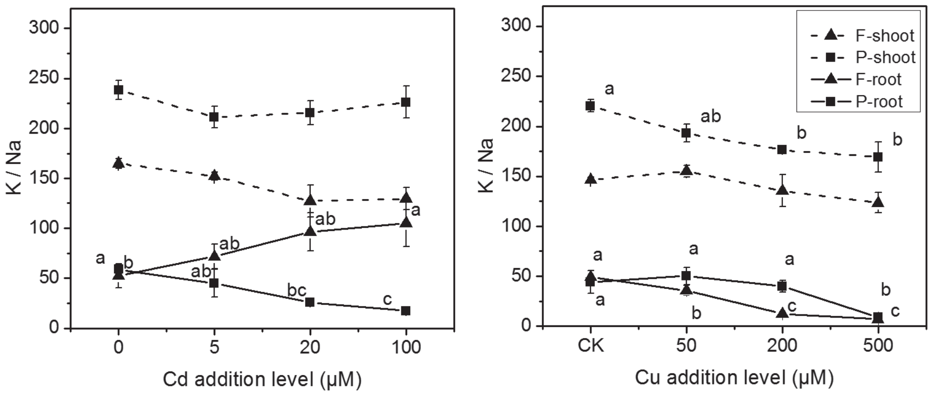


Fig. 5. The K/Na value in tall fescue and pennisetum under Cd/ Cu treatments.

pennisetum receiving Cd treatment were not significantly different among the four Cd levels, whereas the values in the pennisetum roots decreased; by contrast, the K/Na values in the roots of tall fescue increased with the solution Cd concentration. For the Cu treatment, the K/Na values decreased as the addition level was increased, and this decrease was sharper in tall fescue than pennisetum.

## The Relationship between Cd/Cu and Mineral Element

The correlation between Cd and Cu and the nutritional element concentrations (Ca, K, Mg, P, Fe, Na, Zn, and Cu) and K/Na value in the shoots and roots is shown in Table 3. In the shoots, Cd was negatively correlated with Ca, K, Mg, Zn, Cu, and K/Na in tall fescue, and negatively correlated with Cd in pennisetum. In the roots, Cd had negative relationships with Na and Cu and positive relationships with K/Na in tall fescue, as well as negative relationships with K and K/Na and significantly positive relationships with Ca, P, Fe, Na, and Zn in pennisetum. In the shoots, Cu was negatively correlated with K, Zn, and K/Na in pennisetum and Zn in fescue. In the roots, Cu was positively correlated with Na and negatively correlated with K, Mg, P, and K/Na in pennisetum, and negatively correlated with K and K/Na and positively correlated with Na in fescue. All the significantly correlated relationships showed linear regressions with the Cd/Cu concentrations in tall fescue and pennisetum.

# Discussion

## Plant Growth

Cd, as the most toxic element in the soil environment, significantly inhibited plant growth. In the present study, the shoots biomass of pennisetum was higher than that of tall fescue, although it decreased with an increasing Cd concentration in solution. In contrast, the biomass of tall fescue exhibited no significant difference under various Cd stress scenarios. Pennisetum was reported as biomass decreasing at Cd concentrations higher than 30 mg/kg [17], and biomass-inhibiting effects were reported for other grasses like *Chrysopogon Zizanioides*, *Lolium perenne*, *Panicum virgatum*, and the Cd hyper-accumulator *Bidens tripartite* [30-31]. Tall fescue was observed to have a strong tolerance to Cd

Table 3. Pearson correlation coefficient between Cd/Cu and mineral element concentration of tall fescue and pennisetum.

| | | | Ca | K | Mg | P | Fe | Na | Zn | Cu | K/Na |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cd | F | Shoot | -0.758** | -0.667* | -0.700* | 0.226 | -0.443 | 0.377 | -0.606* | -0.736** | -0.660* |
| | | Root | 0.252 | -0.437 | -0.054 | 0.289 | 0.468 | -0.602* | 0.062 | -0.651* | 0.633* |
| | P | Shoot | -0.716* | 0.422 | -0.055 | 0.447 | 0.329 | 0.424 | -0.465 | -0.151 | -0.112 |
| | | Root | 0.956** | -0.653* | 0.197 | 0.907** | 0.951** | 0.676* | 0.787** | 0.177 | -0.607* |
| Cu | F | Shoot | 0.069 | -0.509 | -0.528 | -0.507 | -0.343 | 0.322 | -0.619* | / | 0.484 |
| | | Root | 0.059 | -0.896** | 0.176 | -0.248 | 0.433 | 0.824** | -0.005 | / | -0.886** |
| | P | Shoot | -0.475 | -0.695* | -0.078 | -0.563 | -0.311 | 0.221 | -0.777** | / | -0.789** |
| | | Root | 0.192 | -0.812** | -0.732* | -0.861* | 0.023 | 0.668* | -0.590 | / | -0.774** |

*The coefficient is significant at 0.05 level, **The coefficient is significant at 0.01 level under 80 mg/kg without any toxic symptoms or biomass reduction [20]. The performances of tall fescue and pennisetum under Cd addition were in accordance with previous studies.

Cu is an essential element for plant growth, as it is a component of metabolic enzymes and participates in a variety of physiological metabolic activities. Fertilization with Cu in the appropriate concentration range could promote plant growth, but when the concentration is over a certain range, Cu can induce membrane damage and destruction, change the activity of antioxidant enzymes and redox levels in the cell, and be toxic to plant development [4]. Our results verified that the biomass of pennisetum initially increased and then decreased under Cu addition, which was similar to the results obtained by Arduini et al. for *Pinus pinea*, *Pinus pinaster,* and *Fraxinus angustifolia* seedlings [32]. However, the biomass of tall fescue directly decreased when the Cu content in solution was more than 200 μM; this trend was contrary to the phenomenon observed in Cd stress.

The biomass directly reflected that tall fescue has strong tolerance to Cd and sensitivity to Cu, whereas the opposite relationships regarding the tolerance/sensitivity to Cd/Cu were detected in pennisetum. These results were in accordance with the trend in the absolute values of $X^2$ of the quadratic regression models for the shoots of these grasses. However, the absolute values of $X^2$ of the quadratic regression models for the roots showed the opposite relationship, which may be due to the roots, which are more sensitive to Cd/Cu stress, coming into contact with the contaminants in solution first and being able to regulate the physiological and biochemical activity to immediately protect themselves from toxicity. Thus, the absolute values of $X^2$ could directly reflect the tolerance to Cd/Cu in tall fescue and pennisetum in shoots biomass, the values in root indicating root sensitivity to heavy metals for preparing in advance to fight Cd/Cu stress.

## Cd and Cu Accumulation and Translocation

The highest shoot Cd concentrations in tall fescue and pennisetum were 166.8 and 171 mg/kg, respectively, under 100 μM Cd addition, whereas the shoot Cu concentrations of the two grasses were much lower than the criterion under all Cu treatments. Hence, tall fescue and pennisetum could be regarded as Cd hyperaccumulators in seriously Cd-polluted soils. Their root Cd/Cu concentrations were higher than the minimum standard under all Cd/Cu addition levels, thus suggesting that both tall fescue and pennisetum could be used as phytostabilizers for Cd/Cu-polluted soils. The results were consistent with previous reports, which found that the accumulated Cd concentrations in the roots of pennisetum and tall fescue were higher than those in the shoots [33-34]. Similar results were found in other studies on *Arundo donax*, *Miscanthus sacchariflorus*, and *Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1 under stress from various heavy metals [35-36]. Retention of the contaminants in the plant root is regarded as a main strategy for alleviating heavy metal toxicity because roots secrete organic matter (organic acids) to chelate heavy metals or undergo acidification of the rhizosphere to promote heavy metal dissolution and absorption. Most vascular herbaceous species prefer to chelate metal ions in the root cell wall, except for hyperaccumulators, which prefer to accumulate metals in the shoots [37-38].

Although the TFs of the these grasses were lower than 1 and showed a decreasing trend as the Cd/Cu contents in solution increased, the TFs of pennisetum from 0.11 and 0.58 were much higher than those of tall fescue and were similar to the results (0.18-0.55) reported by Zhang et al. [17]. The Cd TF of tall fescue ranged from 0.06 to 0.08, which was lower than the results reported by Xu, with Cd TF values of tall fescue were 0.1 and 0.2 at 40 and 80 mg/kg, respectively [20]. This finding may be caused by the different growth periods and growth substrates. The TFs of tall fescue were kept stable and lower than that of pennisetum irrespective of Cd additional levels, implying that pennisetum had the ability to be a promising Cd phytoextracted plant. In addition, the TFs of Cd were higher than those of Cu in the grasses under corresponding contaminate levels, possibly because the roots of the grasses retain more Cu in the root structures, such as the cell wall, or Cd is a stronger stimulator of transporter protein expression during the root xylem loading process [39-40]. As expected, due to the large biomass of pennisetum, which compensated the lower absolutely shoot Cd and Cu accumulated concentrations, its shoot Cd and Cu concentrations were kept similar or higher than tall fescue in each plant irrespective of the solution Cd/Cu concentrations.

## Na, K, and other Mineral Elements Absorption

In this experiment, as the Cd/Cu solution increased, increasing trends in Na and decreasing trends in K were observed in the roots of tall fescue and pennisetum under Cd/Cu addition, except for the roots of tall fescue under Cd addition. Hemelraad [41] found that in freshwater clams, Cd led to a dramatic decrease in Na during the first 2-8 weeks of the treatment period, and then the Na content stabilized declined, and K started to increase in the last four weeks. Researchers found that during ion absorption and transportation processes, Na could decrease K absorption [42], and excess Na could damage the plasma membrane, leading to K efflux [43]. In this study, it was inferred that Cd/Cu had antagonistic effects with Na and then influenced K absorption. A considerable decrease in the K/Na value, which was used as a representation of the degree of ion damage and salinity tolerance, could cause metabolic abnormality, increase plant cell membrane permeability, and decrease antioxidant enzyme activities [44-46]. With an increase in solution Cd/Cu, the root K/Na value was observed to significantly decrease in this study, and the plant leaves begin to lose water and undergo chlorosis, especially under the highest Cd/Cu stress. Hence, the unbalance between K and Na could be one of the reasons that Cd and Cu induce oxidative stress and produce reactive oxygen species [47-48]. In contrast, the Na concentration decreased and the K concentration remained stable in the roots of tall fescue under Cd addition; additionally, there was no competitive inhibition between Na and K or obvious poisoning phenomenon. Thus, the strong Cd tolerance mechanism of tall fescue was regulated by selectively absorbing the ions into the roots and compartmentalizing the contaminants in belowground parts to diminish their toxicity to aboveground parts, which was similar to the mechanism of plant resistance to salinity [49].

Cd, which does not have a known biological function in plants, enters plant cells via cation channels of Ca and Mg or transporters of other divalent cations such as Zn, Cu, or Fe [50]. Zhang reported that Cd could compete with the transporters of element ions in plants as the concentration of Cd in soil increases, which induces the antagonistic interaction between Cd and element ions [17]. In our study, Cd had a negative relationship with the Ca, Mg, Zn, and Cu concentrations in the shoots of tall fescue and Ca in pennisetum, suggesting that Cd is absorbed in shoots by the Ca and Mg channels and Zn, Cu carriers, which was supported by previous reports. However, positive correlations were observed between Ca, P, Fe, Na, and Zn content and Cd concentration in the roots of pennisetum. Combined with the lower Cd concentration in the roots, these results indicated that in the competition with Cd, these elements were stronger, which inhibited Cd absorption [51]. In addition, P was found to reduce Cd toxicity by forming a Cd-phosphate precipitant and stimulating organic acid synthesis in roots, which reduces soil Cd bioavailability [52-53]. Although Fe nutrition mitigated Cd toxicity because of the competition for membrane transporters, the root Cd concentration of pennisetum was positively correlated with Fe concentration in the present study, this may due to the plants forming an iron plaque to fix and sequester Cd on the root surface to inhibit heavy metal absorption [54]. Plants have homeostatic mechanisms to maintain appropriate concentrations of Cu in different environmental conditions in order to precisely deliver it to specific compartments and to target it to metalloproteins while avoiding its toxic effects [4]. In pennisetum, the Mg, P, and Zn concentrations in the roots were negatively correlated with Cu, which was similar to results from the study reported by Alva, who found that increased external Cu decreased the uptake of Zn, Fe, and Mn in citrus rootstocks [55]. Negative correlations were detected in shoots of these grasses because Cu and Zn are involved in many of the same metabolic activities. For example, they are components of metalloenzymes, which are involved in oxidation-reduction processes and in protein and lignin synthesis [21].

## Conclusions

Although the energy grass pennisetum had less total Cd/Cu accumulation, the extracted Cd in the shoots and total Cu amounts were higher than those in tall fescue, suggesting that pennisetum could be a promising plant material for phytoremediation in Cd/Cu-polluted areas. Although our experiment was conducted in the seedling stage, the Cd/Cu concentration in solution was higher than that in real-life soil conditions. Hence, the results provide convincing evidence for pennisetum as a promising candidate species for the phytoextraction of Cd and Cu from soils and for tall fescue as a potential Cd-phytostabilizer and Cu-indicator plant in polluted areas. When the solution Cd/Cu reached the highest concentration, the plants could not grow normally and the K/Na values were dramatically decreased in the roots of pennisetum and tall fescue, except for tall fescue under Cd stress. Hence, heavy metals could lead to osmotic stress by interrupting the balance between K and Na in the roots; moreover, the value of K/Na could be considered a reference for measuring the adjustment capacity of plants to heavy metals. Contrary correlations were observed with elements between Cd and Cu in pennisetum, indicating that plants use different strategies to regulate Cd/Cu absorption by mineral elements. A non-invasive micro-test technique is required to further clarify the relationship between heavy metals and elements.

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## Conflict of Interest

The authors declare no conflict of interest.

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