EFFECTS OF DIFFERENT CATIONS ON THE TOXICITY AND ABSORPTION OF CD IN BARLEY ROOT

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ABSTRACT

Cadmium (Cd) as one of the widest distributions in the soils has caused significantly damage to crops. It also gave rise to agricultural products safety matter. This study aimed at investigating the effects of Cd toxicity and absorption to barley root elongation. Under the conditions of various cations such as Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Zn^{2+} , H^+ , toxicity and absorption of Cd were affected. It showed that the EC50 (median effective concentration) of free Cd^{2+} activity for barley root elongation was in the range of 1.12-5.12 $\mu\text{mol/L}$. Ca had a significantly impact on Cd metal toxicity. When the content of Cd in the solution was constant, median dose K^+ or Na^+ addition promoted Cd absorption rate of barley. Ca^{2+} or Mg^{2+} had greatly influenced on Cd absorption dose. An effective method is urgently needed to assess toxicity of barley root and provide environmental standards for crop growth.

1. INTRODUCTION

In recent years, soil heavy metal pollution has been paid attention to environment science field because of its toxic effects on crops grown on contaminated soils^[1]. Among the metals, Cadmium (Cd) is one of the widest distributions in the

soils worldwide^[2,6]. Cd is increasingly found in contaminated soils due to the excessive use of chemical fertilizers, sewage irrigation, discharge of sludge and atmospheric deposition^[4,5].

With regard to human health, concentration of Cd in agricultural production plays a particular important role in agricultural production. The entry of Cd into the food chain can cause health problems such as bone disease, lung edema, liver damage and so on^[6,7]. Due to this, Cd is one of a very small group of metals for which the Food and Agriculture Organization and World Health Organization (1978) had set a limit for the provisional daily intake by humans (70 $\mu\text{g Cd d}^{-1}$). In the 1980s, the U.S. adult was reported to receive about 20% of the World Health Organization (WHO) allowable daily intake of Cd from the consumption of grain and cereal products^[8]. Cadmium enters plants by the soil solution. In plants, Cd accumulation in the roots was increased with high rhizosphere Cd concentrations^[9]. Cadmium could decrease the transfer and absorption of essential substances by plants, including wheat and barley. Comparing with wheat under the same soil conditions, barley accumulated much lower levels of Cd in the crop^[10]. Besides, the bioaccumulation of Cd was mostly in roots. Cd-induced damage in the plant was closely associated with biological mechanisms and substance which also enhanced Cd-stress tolerance^[11].

To address the prediction of metal toxicity, major cations were considered as competitors for metal uptake and binding, which might offer protective effects against toxicity by metal-induced^[12]. Ca^{2+} and H^+ in solution interacted with other heavy metal cations. Barley root elongation in the test series was significantly higher at low Ca concentration than higher concentration. In addition, Ca^{2+} was also a well known ameliorant cation that had been observed for many times to relieve single metal toxicity (e.g., Cu^{2+} , Ni^{2+} , Cd^{2+} , etc.)^[13, 14]. This can probably be explained by a more optimal Ca/Mg/Na ratio for growth of the barley plants^[15]. The effect of pH on the adsorption of cadmium to lettuce was investigated. It's found that there was a growing binding of cadmium at a pH value of more than

three^[16]. Besides, zinc (Zn) would alleviate the toxicity of cobalt (Co). But there is no clear evidence of the effect of zinc on cadmium^[17].

This paper aimed at investigating the effects of Cd toxicity to barley root elongation. What's more, adding other cations, i.e., Zn^{2+} , Ca^{2+} , K^+ , Mg^{2+} , Na^+ and H^+ , we studied the uptake and toxicity of Cd to barley roots. Finally, the assessment of Cd toxicity to barley will be established for a wide range of concentration.

2. Methodology

2.1 Test species and reagent

Cd as a kind of heavy metal, its toxicity was assessed by barley root elongation. Barley (*Brassica chinensis* L) seeds were purchased from Pinggu Beijing Seed Company. Root elongation was proved to be sensitive to metal toxicity and has been widely used as toxic identification^[18,19,20]. Recently, the root growth as a sort of toxicity response index was used to assess metal toxicity in the present scientific study.

2.2 Experimental design

To determine the effect of different cations respectively on Cd toxicity, the concentration of each group was varied. During the experiment, other ions background concentration remained essentially constant^[21]. We set different six groups cations to test the effect on Cd toxicity, which was performed as Zn-set, Ca-set, K-set, Mg-set, Na-set, pH-set. (Table 1) Cd was arranged six concentrations adding to one treatment without added Cd as a control group. The concentration of six selected cations was refer to the ranges of the natural soil pore water^[22]. All treatments were repeated three times

Table 1. Composition of the test media in the various root elongation and the observed EC50{Cd²⁺} based on free Cd²⁺ activity for barley root with 95% confidence intervals.

Cation	Concentration or value of ions (mM ^a)	Other ions concentration value of background solutions	EC50{Cd ²⁺ } and 95% confidence intervals (μM)
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Ca	0.2	0.05mMMg,2.5mMNa,0.0	1.36(0.61-3.01)
	5.0	8mMK,	4.28(1.24-14.78)
	15.0	pH 6.0	5.12(0.24-15.54)
K	0.1	0.2mMNa,0.05mMMg,2.5	1.60(0.48-5.31)
	1.0	mMNa,	1.47(0.35-6.11)
	2.5	pH 6.0	1.85(0.53-6.44)
Mg	0.05	0.2mMNa,2.5mMNa,0.08	1.98(0.41-9.55)
	0.2	mMK,	1.12(0.23-5.54)
	1.0	pH 6.0	2.94(0.39-21.99)
Na	2.5	0.2mMNa,0.05mMMg,0.0	1.77(0.25-12.60)
	5.0	8mMK,	1.14(0.33-3.97)
	10.0	2.5mMNa, pH6.0	1.35(0.34-5.37)
Zn ^b	0.1	0.2mMNa,0.05mMMg,0.0	1.79(0.40-7.94)
	1.0	8mMK,	2.18(1.53-3.11)
	5.0	2.5mMNa, pH6.0	2.56(1.89-3.46)
pH	4.7	0.2mMNa,0.05mMMg,0.0	1.15(0.25-5.30)
	5.5	8mMK,	1.49(1.14-1.97)
	7.6	2.5mMNa,	1.91(1.31-2.79)

Note: ^a mM(mmol/L) is referred to concentration that most cations is tested about the toxicity effect on Cd, except one cation-Zn^b, which unit is $\mu\text{mol/L}$. Except for pH-set, pH value of other background solution experimental group is always equal to 6.0.

2.3 Experimental Preparation of the test solutions Test species and reagent

All chemical reagents are adopted by analytical reagent, guaranteed reagent or higher grade in the tests. Barley grows in the test by hydroponic culture. All test solution cultures were prepared by adding different volumes of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, KCl, NaCl, NaOH and $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ into deionized water. These solutions were buffered with 1mmol/L MES for pH < 7.0 treatments and with 3.6 mmol/L MOPS for pH $\geq$ 7.0 treatments^[23]. Ions concentration or value of background solutions were summarized in the Table 1. To test effect of different cations on Cd, every bioassay was set up a series of Cd concentration by adding different amounts of CdCl_2 prepared solution. The concentration of Cd was designed as 0 μM , 0.5 μM , 3 μM , 20 μM , 100 μM , 200 μM .

2.3 Toxicity assays

Barley (*Brassica chinensis* L.) root elongation inhibition test was conducted by ISO standard (11269-1)^[24]. Barley seeds were disinfected by 10% H_2O_2 for 15 minutes. Then they were rinsed with deionized water, put on the filter paper soaked in deionized water. The seeds germinated in the incubator under temperature of $20 \pm 2^\circ\text{C}$ without illumination. After 24h seeds germination, seeds of nearly 2 mm in length were selected to conduct root elongation inhibition water culture experiment. In the test, germinating seeds were exposed to fit for the test solution of 250ml plastic beaker. Each plastic beaker was placed with 6 seeds. Meanwhile each treatment was repeated three times. After that, all of the treatments were placed in incubator for 5 days. The solution in a breaker was replace for every 2 days to maintain the stability of the ion concentration and pH value. Finally, root elongation was measured and calculated percentage of root elongation (RE) with respect to the control, as in Equation (1)

$$\text{RE} = \frac{\text{RE}_t}{\text{RE}_c} \times 100. \quad (1)$$

Where RE_t (cm) represents root length in the tested solution and RE_c root length in the control.

2.4 Chemical measurements

Atomic absorption spectrophotometry(AAS)(WFX-100)was used to determine the concentrations of Cd, Ca, K, Mg, Na and Zn. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) was applied to measure the content of Cd in barley root. The value of pH was measured by pH Meter(Delta320, Mettler).

2.5 Statistical analysis

Speciation of Cd was calculated by WHAM 6.0(Windermere Humic Aqueous Model)^[25]. The model was input pH and concentration of each cation (Cd^{2+} , Ca^{2+} , K^+ , Mg^{2+} , Na^+ , Zn^{2+} , Cl^- and SO_4^{2-}). As a result of the test for the open system, the CO_2 of the sub pressure was set as 35Pa for WHAM calculation. EC50 was fitted based on Logistic curve, as in Equation (2).

$$\text{RE}(\%) = \frac{Y_0}{1 + e^{b(X-M)}} \times 100. \quad (2)$$

Where RE (%) means percentage of root elongation with respect to the

control, X ($\mu\text{mol/L}$) is concentration of Cd or free ion activity. Y_0 , M ($\log_{10}(\text{EC}_{50})$) and b are regression constants.

3. Results and discussion

3.1 The speciation of Cd: free ion activity of Cd ($\{\text{Cd}^{2+}\}$) and total Cd(Cd_T)

The results about toxicity and absorption of Cd were analyzed based on the concept of free ion activity. One of the calculations by WHAM 6.0(Windermere Humic Aqueous Model) was listed in Table 2. We compared the concentration of total Cd with free speciation Cd. Different sets of cation concentration were the minimum referring to natural soil pore water^[13]. The concentration was set respectively to Ca (0.2mM), K (0.1mM), Mg (0.05mM), Na (2.5mM), Zn (0.1 μM), pH (4.7). Table 2 shows comparing with concentration of total Cd, contents of free Cd ion speciation in the solution are less. The activity of free ion indicates the effective concentration in the chemical reaction. Because of the electrostatic interaction between kinds of ions of solution, free movement of ions and reactive activity are affected. Free Ion Activity Model was first proposed in 1983 in the book of Principles of aquatic chemistry^[2]. The mechanism of free ion activity model is that free metal ions can be combined with the physical active sites on the cell surface, and then can cross the cell membrane producing toxicity to the organisms. In the recent years, Cd in the free ion speciation ($\{\text{Cd}^{2+}\}$) was widely reported in soil studies to be the determinant for phytoavailability by plants^[11].

Table 2. This is the comparison between the concentration of total Cd and Free Ion Activity ($\{\text{Cd}^{2+}\}$) for the table. All of the units are $\mu\text{mol/L}$.

Total Cd (Cd_T) (μM)	Free Ion Activity($\{\text{Cd}^{2+}\}$) (μM) under the condition of minimum cation concentration					
	Ca	K	Mg	Na	Zn	pH
0.01	0.01	0.01	0.01	0.01	0.01	0.01
0.50	0.40	0.42	0.42	0.31	0.41	0.40
3.00	2.39	2.51	2.50	1.86	2.45	2.42
20.00	15.84	16.66	16.54	12.39	16.22	16.03
100.00	77.49	81.20	80.70	61.11	79.28	78.44
200.00	151.05	157.73	156.91	120.24	154.39	152.79

3.1 Effects of various cations on Cd toxicity

The EC50 (median effective concentration) for barley root elongation was used to indicate the toxicity based on the logistic curve (Equation (2)). EC50 expressed as free Cd^{2+} activity varied obviously under different cations conditions (Table 1). In total, the EC50 of Cd was in the range of 1.12-5.12 $\mu\text{mol/L}$, which was separately in the Mg 0.2mmol/L and Ca 15.00mmol/L solution. The value of EC50 is smaller, which represents that corresponding concentration of the 50% inhibition ratio is smaller. The relevant toxicity is also greater. With the increase of Ca concentration, value of EC50 ($\{\text{Cd}^{2+}\}$) became enlarged gradually, from 1.36-5.12 $\mu\text{mol/L}$. This change was not similar to K^+ , Mg^{2+} , and Na^+ in each solution, which was no apparent regular pattern. Comparing with Ca^{2+} , EC50 ($\{\text{Cd}^{2+}\}$) under different Zn^{2+} and H^+ was relatively modest. Both of their maximum value could reach respectively 2.56 and 1.91 $\mu\text{mol/L}$. Therefore, we can conclude that Ca had a significantly impact on Cd metal toxicity. A comparison of 0.2mmol/L Ca with 15mmol/L Ca would be obtained the result that their 50% inhibition radio corresponding Cd concentration had increased approximately fourfold. We can also compare the dose-response curves to judge the toxicity of Cd in a series of solutions (e.g. Fig.1).

Fig. 1. This is the dose-response curves of Cd about Zn^{2+} , which indicates the relationship of barley root elongation (RE) and Cd^{2+} activity. The symbols $\blacksquare$ $\circ$ $\triangle$ show respectively three different cations

concentration. R^2 represents fitting degree between fitting values and the measured values. Dash line means 50% root elongation.

3.2 Absorption of cadmium by different cations in barley among

ICP-OES was used for measuring the content of Cd in the whole barley plants. Comparing Cd concentration between kinds of established different solution (Ca^{2+} , Mg^{2+} , K^+ , Na^+ , Zn^{2+} , H^+) and barley, we could know about absorption of cadmium affected by different cations (e.g. Fig.2). Ca^{2+} and Mg^{2+} effects on Cd uptake were similar ((e.g. Fig.2 (a), (b)). It showed that different concentration Ca^{2+} or Mg^{2+} had greatly influenced on Cd absorption dose. It seemed that low-dose Ca^{2+} and Mg^{2+} enhanced Cd uptake by barley. Cd accumulation in barley was reduced with the increase of Ca^{2+} and Mg^{2+} concentration. While adding different concentrations of K^+ or Na^+ to Cd solution, the results was departing from the above-mentioned trend (e.g. Fig.2 (c), (d)). When the content of Cd in the solution was constant, median dose K^+ or Na^+ addition promoted Cd absorption rate of barley. In contrast, in low concentration Cd solution, Zn^{2+} and H^+ had little difference in the absorption of Cd ((e.g. Fig.2 (e), (f)). More-or-less Zn^{2+} and H^+ content to Cd absorption in barley almost kept unchanged, although higher Cd concentration lead to variation for Zn^{2+} or H^+ . Under hydrodynamic conditions, the bioavailability of Cd in the sediment increased^[26]. There are many factors influencing Cd uptake. Natural dissolved organic matter (DOM) can have contrasting effects on metal bioaccumulation in algae because of complexation reducing free metal ion concentrations^[27].

Fig. 2. These are comparisons between total Cd concentration and Cd accumulated in barley. The symbols $\blacksquare$ $\circ$ $\triangle$ show respectively three different cations concentration. $\mu\text{M/g}^a$ means Cd content in per gram grated barley.

4. Conclusions

In conclusion, free ion activity of Cd tended to express its toxicity to barley. It could incorporate with the physical active sites on the cell surface, and then cross the cell membrane producing toxicity to the organisms. Effects of different cations on Cd toxicity varied. With the concentration of Ca increasing, value of $\text{EC}_{50} \{\text{Cd}^{2+}\}$ became enlarge from 1.36-5.12 $\mu\text{mol/L}$. However, K^+ , Mg^{2+} , and Na^+ in each solution were no apparent regular pattern. Comparing with Ca^{2+} , $\text{EC}_{50} \{\text{Cd}^{2+}\}$ under different Zn^{2+} and H^+ relatively modest. Both of their maximum value could reach respectively 2.56 and 1.91 $\mu\text{mol/L}$. It showed that different concentration of Ca^{2+} or Mg^{2+} have greatly influenced on Cd absorption dose. The degree of influence on Cd uptake was the content of the study in the future. There will also be needed to put forward to a promising tool or approach for improving the ecological relevancy of risk assessment procedures for divalent metals in water and soil environment.

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