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Research Article

Effects of Sodium Humate on Cadmium Content and Physiological Indexes of Chinese Cabbage (*Brassica chinensis* l)

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Abstract: With Chinese cabbage *(Brassica chinensis L*) as the test material in pot experiments, the effects of sodium humate on Chinese cabbage biomass, cadmium uptake and accumulation as well as the soil available cadmium content were studied, to provide a reference for sodium humate anti-heavy metal stress research. The results showed that when a suitable amount of sodium humate was fertilized, even Cd polluted soil could promote Chinese cabbage root system growth and the fresh weight increased, as well as the stems and leaves biomass. In addition, as the soil available Cd content decreased, the absorption and accumulation in the stems and leaves was reduced. The optimum fertilization amount was 1.00 g/kg soil. Correlation analysis indicated that sodium humate was significantly or extremely significantly negatively correlated to soil available Cd. Therefore, sodium humate was the main factor that affected the soil available Cd content.

Keywords: Cadmium stress, Chinese cabbage (*Brassica chinensis* L), nutrition indexes, sodium humate, soil available Cd

INTRODUCTION

Heavy metal pollution, especially cadmium (Cd) pollution in farmland soil has become more and more serious and has been paid much more attention. Compared with other plants, leafy vegetables are more liable to accumulate heavy metals (Ben *et al*., 2005; Cheng, 2003; Moreno-Caselles *et al*., 2000; Moriarty, 1999; Xu *et al*., 2005). In recent years, soil pollution bioremediation technology has become a very active research topic. Compared with traditional physical and chemical remediation methods, ecology remediation is more effective and convenient, while also being simpler.

Humic acid is a natural organic weak acid that exits widely in weathered coal, lignite and peat. It has been reported that humic acid can promote plant growth and enhance plant resistance (Chen, 2000; Karakurt *et al*., 2009; Lee and Bartlett, 1976; Yuan *et al*., 2009), while also imparting soil environmental protection and a remediation function (Han, 1997; Li *et al*., 2001). In this study, we use sodium humate synthesized from natural humic acid as the test fertilizer and Chinese cabbage (*Brassica chinensis* L) as the test material to study the alleviation effect of Cd stress on Chinese cabbage via the application of sodium humate.

MATERIALS AND METHODS

Materials: Chinese cabbage was chosen as the test material. The sodium humate was a powder with 99% purity and humic acid content ≥60%.

In total two soils with different degrees of Cd pollution were chosen. The first type was the background soil (low Cd) that was obtained from the cinnamon soil of the greening forest region in Hebei Normal University of Science and Technology. Its fertility was at the moderate level with a pH value of 7.45, the total Cd content was 0.475 mg/kg, which exceeded the soil background value (0.14 mg/kg) for the Qinhuangdao area (Liu *et al*., 1997) and the available Cd content was 0.011 mg/kg. The second soil (high Cd) was obtained artificially (Xia, 1989) via a method in which a 6 mg/kg Cd^{2+} ($3CdSO_4 \cdot 8H_2O$) solution was uniformly mixed with the background soil and left to stand for 60 days. The soil's total and available Cd contents were 5.606 mg/kg and 0.468 mg/kg, respectively.

Plant culture and treatments: In this pot experiment, six sodium humate concentrations were used 0 g/kg (Ck), 0.25 g/kg, 0.50 g/kg, 1.00 g/kg, 2.00 g/kg and 5.00 g/kg. Accordingly, in the experimental design,

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firstly, sodium humate powder was mixed uniformly with the two kinds of soil. Then, the soil put into 15 cm×20 cm plastic pots, with each pot containing about 2500 g and each treatment was repeated three times. The pot experiment was performed in a greenhouse from April 22nd to May 25th, 2014. After the first true leaf grew out, 12 seedlings was final singling in each pot. The field moisture capacity was maintained at 75±5%. To avoid the influence of other nutrient elements, no other fertilizers were used.

Analysis methods: To measure the total Cd content in the soil, aqua regia-$HClO_4$ was used to acid digest the soil, after which the available Cd was extracted using the method (Ma, 1998). Chinese cabbage stems and leaves were digested by HNO_3-$HClO_4$ (3:1). Then the digested solution and extracted solution were measured using an Inductivity Coupled Plasma Optical Emission Spectrometer (Optima 2100DV, PerkinElmer Instruments Limited Company, American).

The values presented in the text are mean± standard deviation (SD). To determine significant differences among the control and treatments, the results were statistically analyzed using a one-way ANOVA followed by Duncan's multiple range test at the $p<0.05$ significance level.

RESULTS

Effects of sodium humate treatments on fresh and dry weight of root system: As shown in Fig. 1 to 4, Cd stress did not have a strong effect on Chinese cabbage root system growth and dry matter accumulation, but there was an apparent effect on the root system fresh weight, the ratio of fresh weight and dry weight increased. For both types of soil, the root system growth was promoted and fresh weight increased when sodium humate was fertilized (Fig. 1 and 2), with the effect being the greatest at 1.00 g/kg. In the low Cd soil the increases were 10.22 and 9.30%, while in the high Cd soil the increases were 8.36 and 38.71% ($p<0.01$) compared with the control, respectively. When the amount of fertilizer was greater than 1.00 g/kg, the root system growth and biomass of the Chinese cabbage decreased significantly.

Effects of sodium humate treatments on fresh and dry weight of stems and leaves: Cd stress inhibited the growth of Chinese cabbage stems and leaves and decreased the fresh and dry weight (Fig. 5 and 6); however, there was no change in the fresh weight to dry weight ratio (Fig. 7). When sodium humate was added to both types of soil, the fresh and dry weight of the stems and leaves of Chinese cabbage were increased. In the low Cd soil, the optimum fertilization amount was 1.00 g/kg and compared with the control the stems and leaves fresh and dry weight increased by 12.93 and 14.49%, respectively; in the high Cd soil, the optimum

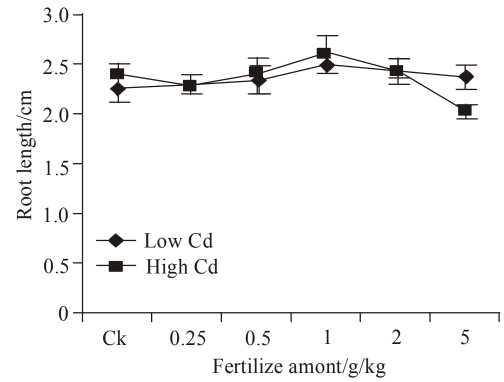

Fig. 1: The effects to cabbage root length

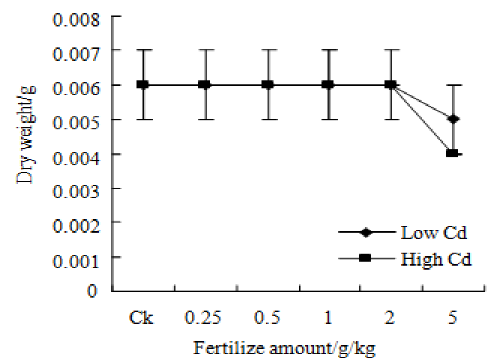

Fig. 2: The effects to cabbage root dry weight

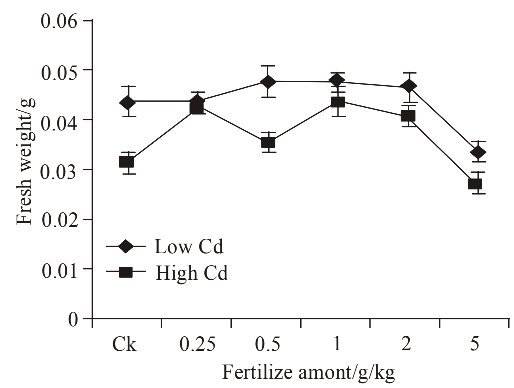

Fig. 3: The effects to cabbage root fresh weight

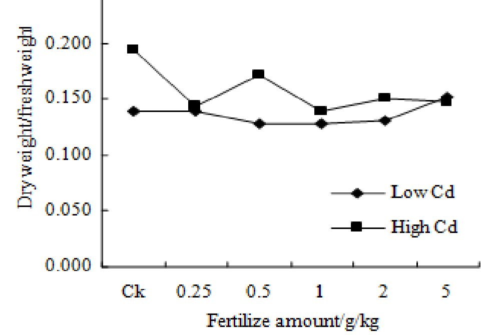

Fig. 4: The effects to cabbage root dry/fresh

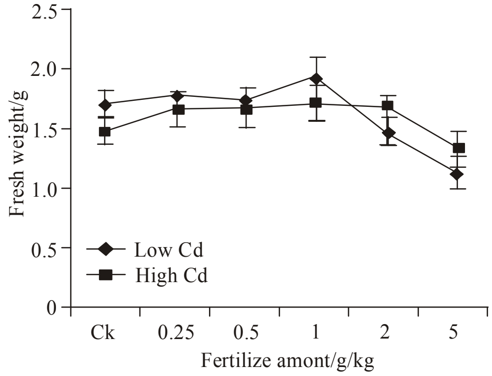

Fig. 5: The effects to cabbage stems and leaves fresh weight

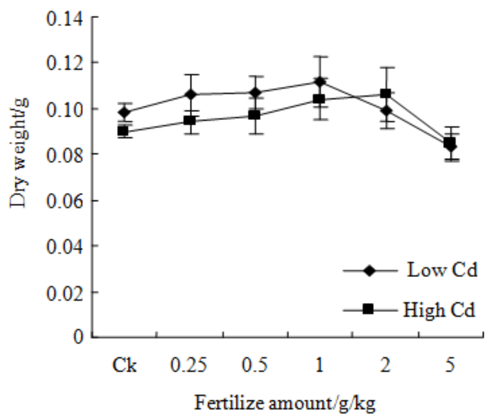

Fig. 6: The effects to cabbage stems and leaves dry weight

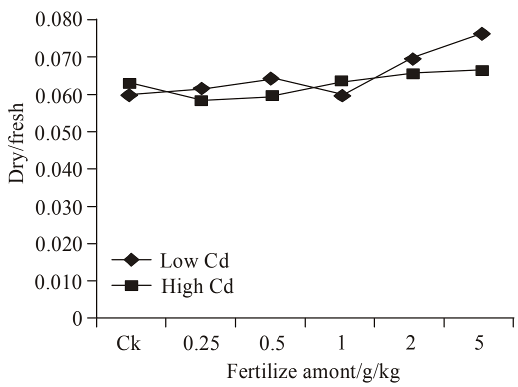

Fig. 7: The effects to cabbage stems and leaves dry/fresh

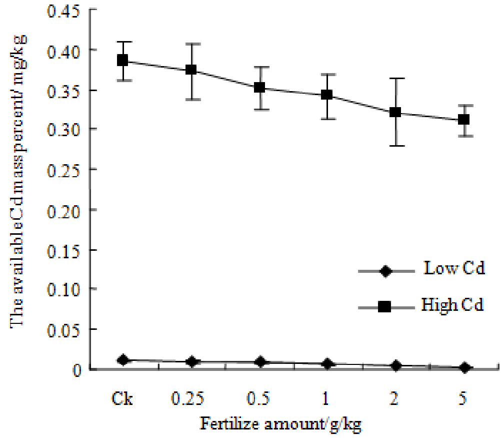

Fig. 8: The effects to soil available Cd content

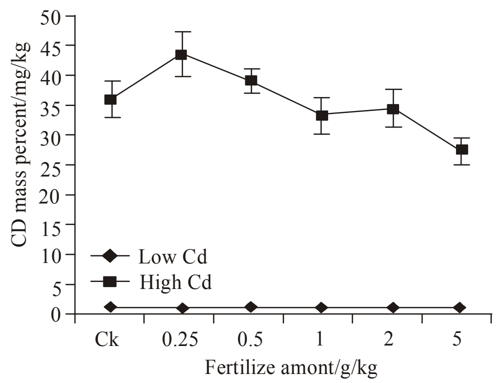

Fig. 9: The effects to Cd mass percent of cabbage stems and leaves

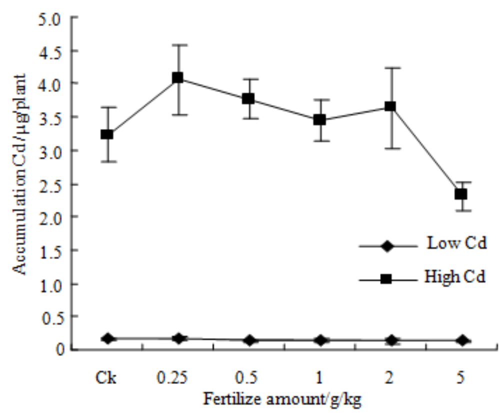

Fig. 10: The effects to Cd accumulation of cabbage stems and leaves

fertilization amount was 1-2 g/kg and the increases were 15.04 and 17.78% ($p<0.01$). When the sodium humate application exceeded 1-2 g/kg, the biomass of the Chinese cabbage stems and leaves decreased distinctly and the inhibition of the growth was obvious.

Effects of sodium humate treatments on soil available cd content and cd absorption of Chinese cabbage stems and leaves: Cd stress increased the soil available Cd content (Fig. 8) and the level of Cd accumulation in the Chinese cabbage stems and leaves (Fig. 9 and 10). The sodium humate treatments in both types of soil decreased the soil available Cd and the amount that accumulated in the stems and leaves of the Chinese cabbage and there was an obvious downward trend as the application level increased (Fig. 8). When the fertilization amount was 1.00 g/kg, in the low Cd soil, compared with the control, the soil available Cd content decreased 36.36% ($p<0.01$) and stems and leaves Cd

Table 1: Correlation analysis of sodium humate with soil, cabbage root system and stems leaves

	Root system				Stems and leaves					Soil
Soil types	Length	Fresh weight	Dry weight	Dry/fresh	Fresh weight	Dry weight	Dry/fresh	Total cd	Accumulation cd	Available cd
Low cd	0.986*	0.845	0.717	-0.845	0.907	0.935	-0.051	-0.885	-0.748	-1.000**
High cd	0.787	0.672	0.764	-0.681	0.804	0.999**	0.213	-0.496	-0.009	-0.964*

Data are means±standard deviation; *p<0.05; **p<0.01 (compared with the control group)

mass percent and accumulation decreased by 25.45 and 12.50%, respectively; while in the high Cd soil, the decreases were 11.43% ($p<0.01$), 7.70 and -6.50%. Under serious Cd stress, the alleviation effects of sodium humate on Chinese cabbage Cd absorption and accumulation were weaker.

Correlation analysis between sodium humate and Chinese cabbage nutrition indexes: The results show that when fertilized with 0-1.00 g/kg sodium humate, the sodium humate had positive biological effects on Chinese cabbage. Correlation analysis indicated that, in both types of soil, the amount of sodium humate fertilizer applied was positively correlated to root system growth as well as the fresh and dry weight of the roots, stems and leaves; some indexes were at the significant or extremely significant levels (Table 1). The amount of sodium humate fertilizer applied was negatively correlated with the available Cd content in the soil as well as the Cd mass percent and accumulation in the stem and leaves. With the available Cd in the soil, there was a significant or extremely significant negative correlation. Therefore, it has been shown that sodium humate was the main factor that affected the soil available Cd content.

DISCUSSION

Humic acid is a physiologically active substance with analogous auxin characteristics, which at suitable concentrations promotes plant growth, especially the plant root system, expands the nutrient absorption area then results in luxuriant plant growth (Adani *et al*., 1998; David *et al*., 1994; DeKock, 1955; Fagbenro and Agboola, 1993).

The results showed that under Cd stress, the growth and development of Chinese cabbage were significantly inhibited (Fig. 1), but when sodium humate fertilizer was applied to the basic soil and Cd polluted soil, the root system growth and root fresh weight were increased, thus increased biomass yield in the stems and leaves. The results indicated that sodium humate promoting the root system growth was the main reason that the biomass yield increased, which is consistent with previous work (Calvo *et al*., 2014; Cooper *et al*., 1998; Rauthan and Schnitzer, 1981).

Humic acid has buffering and purification effects on heavy metal polluted soil; by participating in ion exchange reactions or ion chelation reactions that take place in the soil it adsorbs or passivates heavy metal ions and reduces the biological effectiveness of heavy metal elements in the soil, thus preventing the heavy metal ions from entering into the plant systems, reducing the toxic effects of the heavy metals and promoting plant growth (Morard *et al*., 2011; Vaughan, 1974; Wang *et al*., 1996; Xu, 1986).

The results showed that humic acid can significantly alleviate the negative physiological effects caused by Cd stress and decrease the soil available Cd content; at the same time, it decreased the mass percent and accumulation in the cabbage stems and leaves. The sodium humate was significantly or extremely significantly negatively correlated with available Cd in the soil, which shows that sodium humate is the main factor that affects the available Cd content in the soil.

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