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Environmental Contamination and Toxicology

Effect of Cadmium on Root Exudes of Wheat (*Triticum aestivm* L.) Under Different Cultures Media

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The issue of pollution in the terrestrial ecosystem caused by heavy metals is getting more and more attention recently, especially Cadmium. Though Cd exists in the nature commonly, it doesn't take part in the structures and mechanism activities of organisms; it is fatally harmful if too much Cd was accumulated in the body of organisms (Leapo 1987). In many cases, if Cd is transferred and accumulated in food chain it will be harmful to human beings.

Many researches have been focused on effects of Cd on above-ground of plants, i.e. inhibiting photosynthesis and transpiration, destroying metabolize, decreasing quality and quantity of plant fruits, accelerating decrepitude of plants (Bazzaz et al. 1974; Lee et al. 1976; Lakshamn et al. 1992; Qin et al. 1998). However, root exudation is always affected by heavy metals firstly, because it is at the soil-root interface where ions exchange and heavy metals absorption take place. Yang et al (2001) report that exudation of organic compounds by roots may influence ion solubility and uptake through their indirect effects on microbial activity, rhizosphere physical properties and root growth dynamics and directly though acidification, chelation, precipitation and oxidation-reduction reactions in rhizosphere (Uren, 1988). Of these compounds, low-molecular-weight organic acids are of particular importance due to their metal chelation proportions for mobilization of heavy metals (Mench, 1991; Cieslinski, 1998).

The aim of this investigation was to study the ecological effects of Cd on Wheat roots exudates under two different cultures media in laboratory.

MATERIALS AND METHODS

Wheat (*Triticum aestivm* L.) seeds without any heavy metal pollution were bought from Kaiyuan City, Yunnan Province of China. Before geminated, wheat seeds had been sterilized by 75% ethanol for 10 minutes and washed by sterilized water for 20minutes, and then seedlings were planted in a growth chamber (LRH-250-G, 40 W) with the fluorescent lamps, photo flux density nearly 60 $\mu E\ m^{-2}\ s^{-1}$ for 12/12 hr photoperiod and control temperature $25/15 \pm 1$℃(day/night).

After 2 days preculture, seedlings (100 seedlings in each container) were transferred to two series of 1000 mL containers. Half of containers were filled

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with autoclaved Hoagland's nutrient solution (Hoagland and Aron, 1938) (water-culture, W-culture), the others filled with nutrient solution, vermiculite and sand (vermiculite-culture, V-culture).

The Cd treatment concentrations were 0 (control), 0.5, 5, 15 and 50 $mg \cdot L^{-1}$. During the treatment period (9 days), 20 mL nutrient solution contained target concentration of Cd as above was added to each container under both W- and V-culture everyday. Each treatment was replicated three times.

After 9 days, all of the wheat root in each container was marinated in 100 ml deionized water for 1 h, then rinsed by another 100 mL deionized water for 10 minutes. The V-cultures were treated as the same (Weerasuriya et al. 1993; Anna et al. 1995; Boeuf-Tremblay et al. 1995). 200 mL root exudates solution (RES) was collected altogether.

Electric conductibility of RES (EC_1) was measured immediately by electric conductance apparatus (DDs-11A type, made in China). After that, wheat roots was put in the RES and seethed for 20min, then electric conductibility (EC_2) of the solution was measured again. The exosmosis rate of electrolyte (ERE) of wheat roots was determined by the following equation: $ERE=EC_1/EC_2$ (Hu et al. 1995). Content of soluble sugar and reducing sugar ($\mu g \cdot g^{-1}$ dry weight of roots) was measured according to Zhang (1997). Amino acids were measured by PICO-TAG hydrolyzed analysis methods.

Secondary metabolism products (SMPs) were measured by GC-MS analysis as follows: RES was freeze dried and dissolved in 2mL NaOH (pH 11.73). Added acidic carbinol solution (40 mL carbinol+60 mL 37 % HCl) was added into the samples and methylated for 30min at 60℃. After the solution cooled, it was extracted with 200 mL chloroform and extracted solution was concentrated to 2ml, then centrifuged at 10000 rpm for 10 min. 1 mL supernatant of sample was transferred to GC-MS for analyzing. Injector temperature, 70 ℃; detector temperature, 260℃; column temperature was stabilized for 4 min at 70℃; temperature was programmed to 210℃ at 10℃/min; Then this temperature was kept for 25 min. Lauric acid was added as inside standard.

RESULTS AND DISCUSSION

The electric conductibilities of EREs were plotted in Fig 1 and 2, each curve representing the average value of three replicates. It was evident that the penetrability of root increased according to the increase of Cd treatment levels at two culture conditions. Therefore, EC_1 of RES increased too, especially at W-culture, but there was no significant correlative relationship between EC_2 and Cd levels. Higher EC_1 of RES at Cd stress resulted in the increased ERE, and the EREs were 14.5%, 30.3%, 38.7%, 44.3% and 57.2% (under W-culture) and were 16.3%, 18.1%, 27.0%, 32.2% and 42.2% (under V-culture) at the 0, 0.5, 5, 15 and 50 $mg \cdot L^{-1}$, respectively.

The pH of RES was increased with the exposure at low ($0.5mg \cdot L^{-1}$) concentration

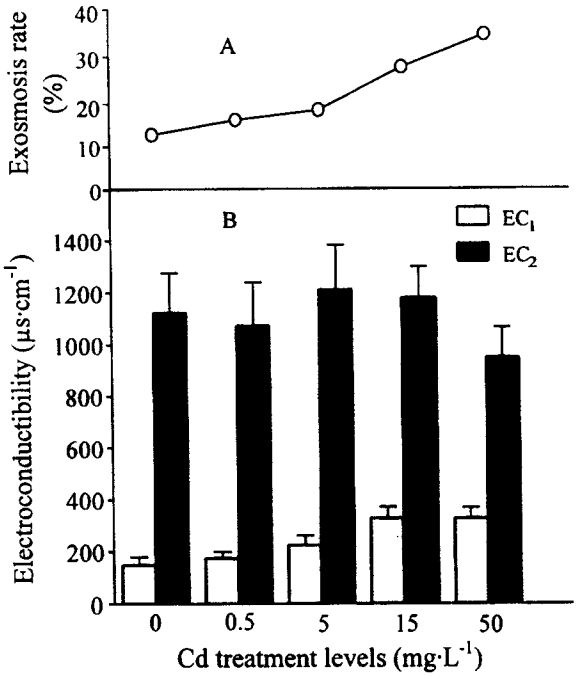

Figure 1. Effects of Cd on EC of RES (B) of wheat (*Triticum aestivm*) and ERE (A) exposed to various levels of Cd for 9 days at W-culture conditions. Treatmen means (± SE) were compared using Tukey test at $p<0.05$ (n=3).

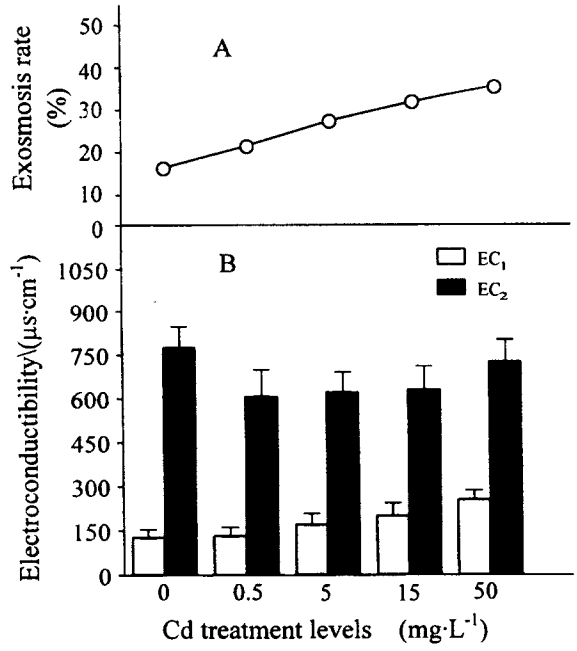

Figure 2. Effects of Cd on EC of RES (B) of wheat (*Triticum aestivm*) and ERE (A) exposed to various levels of Cd for 9 days at V- culture conditions. Treatment means (± SE) were compared using Tukey test at $p<0.05$ (n=3).

followed by decline at medium (5 mg·L^{-1}, 15 mg·L^{-1}) and the highest (50 mg·L^{-1}) concentration. However, all of the pH of RES with Cd was higher than that of the control (Fig 3). In W-culture, the pH of RES was increased by 9.5%、7.4%、6.9% and 5.4% at 0.5, 5, 15 and 50 mg·L^{-1} Cd , and in V-culture, that of RES was increased by 9.7%、3.9%、1.0% and 0.7%, respectively.

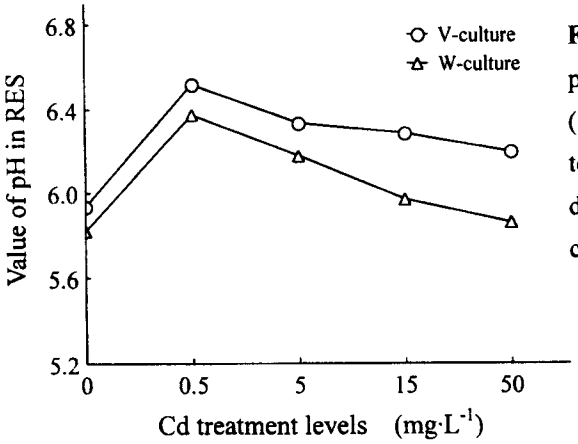

Figure 3. Effects of Cd o pH of RES of whea (*Triticum aestivm*) expose to various levels of Cd for days at two different cultur conditions.

The content of soluble sugar in RES of W-culture was always higher than that of V-culture at all treatments levels. However, there was no significant difference in the content of reducing sugar at the control and the highest (50 mg·L^{-1}) concentration (Fig 4). Both the content of soluble and that of reducing sugar found in RES exposed to 0.5 mg·L^{-1} were significantly increased under two culture conditions. At the high (15 mg·L^{-1}) and the highest (50 mg·L^{-1}) applied Cd concentrations, however, the contents of soluble and reducing sugar declined except for reducing sugar at 15 mg·L^{-1} at V-culture. All soluble sugar contents in RESes exposed to different Cd levels were significantly ($p < 0.05$) higher than the control by 41.9 ~86.8 % (W-culture) and by 12.4~51.0 % (V-culture), and those of reducing sugar were higher by 8.1~37.4 % (W-culture) and by 1.6 ~16.9 %

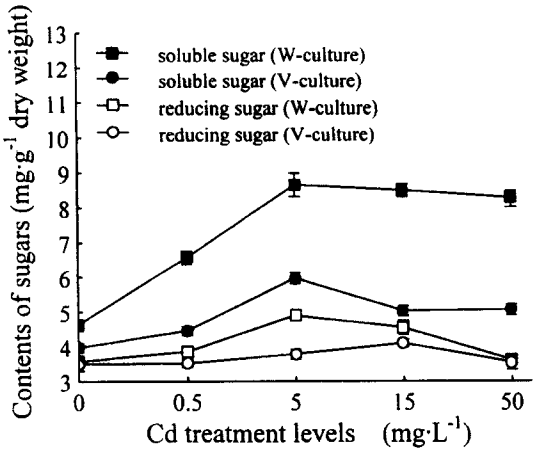

Figure 4. Effects of Cd on sugars in RES of wheat (*Triticum aestivm*) exposed to various levels of Cd for 9 days at two different culture conditon s. Treatment means (± SE) were compared using Tukey test at $p<0.05$ (n=3).

expect the highest Cd level (50 $mg \cdot L^{-1}$). It was clear that two kinds of sugars contents in RES of wheat exposed to different Cd levels (0.5, 5, 15 and 50 $mg \cdot L^{-1}$) were higher by 65.9 %, 44.6 %, 69.1 % and 63.8 % (soluble sugar) and 9.3 %, 29.7 %, 11.0 % and 2.0 % (reducing sugar) under W-culture as compared with V-culture.

Table 1 showed clearly that the number of amino acid hadn't significantly affected by Cd stress expect Asp and Glu disappeared at the highest (50 $mg \cdot L^{-1}$) level under two cultures media and Cys (in 0.5, 5, 15 and 50 $mg \cdot L^{-1}$ Cd) and His (in 50 $mg \cdot L^{-1}$ Cd) appeared at Cd treatment samples. However, the contents of many amino acids in RES of wheat changed significantly under different Cd treatment levels. In two culturing modes, low concentration of Cd (0.5mg/L) stimulated wheat root for secreting amino acid. About 82% (W-culture) and 59% (V-culture)

Table 1. Effect of Cd on 17 kinds of amino acids' contents in RES ($\mu mol \cdot ml^{-1}$) after 9 days under two culture conditions. Probability values are shown in bold where effects are significant at $p \leq 0.05$ level and arrows indicate the direction of the response to Cd stress.

	W-culture					V-culture				
Cd ($mg \cdot L^{-1}$)	0	0.5	5	15	50	0	0.5	4	15	50
Asp	2.5	**4.1**↑	2.9	2.6	N.D↓	0.6	**3.5**↑	**2.1**↑	**1.0**↑	N.D↓
Glu	7.9	**13.3**↑	**5.1**↓	**3.2**↓	N.D↓	1.0	**6.5**↑	**4.3**↑	1.1	N.D↓
Ser	10.5	**17.1**↑	**7.2**↓	8.7	**2.0**↓	3.5	**6.7**↑	**2.4**↓	**4.7**↑	**4.8**↑
Gly	16.3	**33.1**↑	12.8	13.6	**2.0**↓	3.3	**9.3**↑	**2.3**↓	3.0	**7.1**↑
Arg	4.0	**11.8**↑	**2.2**↓	**1.6**↓	**1.5**↓	0.4	**6.5**↑	**3.2**↑	**2.0**↑	**1.1**↑
Thr	10.5	**18.2**↑	10.2	8.0	**2.2**↓	4.6	**6.6**↑	**3.0**↓	**1.8**↓	**0.7**↓
Ala	19.9	**37.8**↑	22.1	**13.1**↓	**2.2**↓	7.8	9.3	**5.4**↓	**3.6**↓	**5.2**↓
Pro	5.6	**14.6**↑	**14.0**↑	**7.6**↑	**11.7**↑	1.6	**2.3**↑	2.0	**0.8**↓	1.7
Tyr	5.4	**10.6**↑	**9.3**↑	4.5	5.3	2.1	**3.8**↑	**3.1**↑	2.2	2.2
Val	12.8	**26.9**↑	**22.2**↑	10.3	15.2	6.7	**3.7**	5.7	**3.9**	5.9
Met	5.3	6.7	5.1	**2.2**↓	**8.2**↑	2.3	**4.3**↑	**1.4**↓	**3.4**↑	2.4
Cys	N.D	**2.4**↑	**3.4**↑	**3.3**↑	**3.4**↑	N.D	N.D	N.D	N.D	N.D
Ile	6.7	**14.8**↑	**14.4**↑	5.7	7.4	3.2	2.8	**2.1**↓	**2.0**↓	2.8
Leu	10.6	**22.6**↑	**20.6**↑	8.3	10.2	5.3	5.0	**3.3**↓	**3.4**↓	4.9
Phe	5.9	**11.5**↑	**9.5**↑	4.7	6.9	2.8	2.3	2.0	2.0	2.6
Lys	4.3	**10.3**↑	5.0	5.0	**5.9**↑	0.2	**1.0**↑	**1.2**↑	**2.7**↑	**2.9**↑
His	N.D	N.D	N.D	N.D	**1.8**↑	N.D	N.D	N.D	N.D	N.D

The values are arithmetic means (n=3) in each column; N.D: no detected

of amino acids' contents increased significantly ($p < 0.05$). In different culture conditions, Cd induced different affects different kinds of amino acid. Between W- and V- culture, at 5, 15 and 50 $mg \cdot L^{-1}$ Cd levels, respectively, there were 14, 12 and 7 kinds of amino acids' contents exhibited different change tendency. Among these amino acids, 4, 3 and 3 kinds of that changed completely oppositely. And yet, from table 1, it was found that all kinds of amino acids' contents in RES of wheat under W-culture were much more than those under V-culture.

In two culturing modes, secondary metabolism products (SMPs) in RES were significantly different when wheat was stressed by Cd (Table 2). The most kinds

Table 2. Effect of Cd on secondary metabolism products excreted by wheat root after 9 days under two culture conditions.

Cd ($mg \cdot L^{-1}$)	Secondary metabolism products excreted by wheat root	
	W-culture	V-culture
0	cyclohexyl alcohol, cyclohexanone,4-ethyl-1,2-bimethylbenzene, 1-ethyl-3,5-bimethylbenzene, 2-ethyl-1,4-bimethylbenzene, 1-ethyl-2,4-bimethylbenzene, 1,2,3,5-tretrtamethylbenzene, 1-methyl-2,3-indolinyl, naphthalin, 1-methylnaphthalin, 2-metnylnaphthalin, n-dodecaldehyde , hexadecenyl-1-alcohol, n-tetradecaldehyde, n-octadecanoicaldehyde, 1,13-tridecandioicdiol-diacetate	Cyclohexyl alcohol, cyclohexanone , 2-ethyl-1,3-bimethylbenzene , 1-methyl-4-benzene, 1,2,3,4-tretrtamethylbenzene, naphthalin 6-methyl-2-methyl-bicyclo-heptane, 1-methylnaphthalin, 2,6-bitert-butyl-phenol, β-methyl, 10-methyl-undecate, duotricemary-alkane
0.5	Cyclohexyl alcohol, cyclohexanone , 4-ethyl-1,2-bimethylbenzene , 2-ethyl-1,4-bimethylbenzene, 1-ethyl-2,3-bimethylbenzene , 1-methyl-ethyl-benzene, 1,2,3,5-tretrtamethylbenzene, 1-methyl-4-(1-methyl,ethyl)-benzene, naphthalin, citric acid ,	Cyclohexayl alcohol, cyclohexanone, 1-hexyl-3,5-bimethylbenzene, 4-ethyl-1,2-bimethylbenzene , 1,2,3,5-tretrtamethylbenzene, 3-methyl-furandione-(2,5), ethane diacid, N,N'-ethylbiglycine
5	Cyclohexyl alcohol, cyclohexanone, 1-ethyl-2,4-bimethylbenzene , 1-methyl-4-(1-methyl,ethyl)-benzene, 1,2,3,4-tretrtamethylbenzene, 1,2,3,5-tretrtamethylbenzene , naphthalin , citric acid, ethyl zctadecanoic acid , thirty-six-alkane	1,4-pentadiene, ethane diacid, 3-methyl-furandione-(2,5) , 3-nitro-pyrromonazole , 5-nitro-pyrromonazole , cyclobutanol, 6-methylaminoheptane-2 , pentanal
15	N-methylaminoethane	3-methyl-furandione-(2,5) , ethane diacid
50	Cyclohexyl alcohol, cyclohexanone, N-methyl-phenethylamine	Cyclohexyl alcohol, cyclohexanone , ethane diacid, 2-nitro-1-decene-4-alkyne

of SMPs in RES were detected in the control. With the concentrations of Cd increased, the numbers of SMPs in RES reduced. The number of SMPs in RES under V-culture (12, 8 and 8 kinds) was less than that under W-culture (16, 10 and 10 kinds) below the medium Cd levels (0, 0.5, and 5 $mg \cdot L^{-1}$), respectively. On the contrary, the number of SMPs in RES under V-culture (2 and 4 kinds) was a litter more than that under W-culture (1 and 3 kinds) at 15 $mg \cdot L^{-1}$ and 50 $mg \cdot L^{-1}$ Cd levels. And the structure and type of SMPs were significantly different too. There were not only many kinds of benzene and hydroxybenzene but also some kinds of toxicant, e.g. cyclohexyl alcohol, cyclohexanone, toluene and oxalic acid.

Nutrient solution and sterile vermiculite and sand are two cultures media that are normally used in researches. Using different cultures media will affect the results in controlled experiments. So it is important to consider the culturing modes carefully (Beijing Agriculture University, 1991). In our study, response of root exudes to Cd stress on wheat were significantly different under these culturing modes.

When plants are growing their roots absorb nutrient and water from environments and excrete protons, ions and lots of organic matters. TAhese root exudates are important biomarkers for plant to reflect soil habitats and it also plays an important role in the reciprocity between the plant and the environment. Any environmental stress factor would affect the physical and chemical characteristics of root exudates. Cd affected wheat root excreting, but the changes of excreting activity were complex because those are correlative to the selective penetrability of plant roots' cell membrane (Gao and Zhang 1998). If the integrality and selectivity of cell membrane are destroyed, the penetrability is increased under heavy metals stress, e.g. Cd, therefore, lots of organic matters are excreted by plant roots (Prikryl 1980; Jiang et al. 1994). In this study, so many kinds of important organic matter, such as sugar, amino acid, organic acid and other SMPs excreted by wheat root were affected by Cd stress.

The mechanisms and rules of roots excreting under the environmental stress need further investigation, therefore, we will know more about the reciprocity of plant to soil environment and soil microoganism.

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