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Compound contamination and secondary ecological effects of Cd and As in soil-alfalfa ecosystems

Zhou Qixing*, Gao Zhengmin

Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, China

Abstract—Cd and As both have harmful effects on the growth, development and seed germination of alfalfa, especially in such a condition as the coexistence of Cd and As in soil environment. The research using the pot-culture imitative method first found that if soil was simultaneously polluted by Cd and As, function of alfalfa absorbing Cd from soil may be promoted because of the existence of As; in conversely, Cd may inhibit alfalfa plant from absorbing As. It was also found that secondary ecological effects were most likely to be brought out due to the coexistence of Cd and As. For example, alfalfa is passive to excessively absorb Cu and Pb. The harmful effects undoubtedly intensify the contamination of alfalfa. The results showed that the mechanism of the interaction among Cd, As, Pb and Cu in soil-alfalfa ecosystems is very complicated.

Keywords: compound contamination; ecological effects; Cd; As; soil.

1 Introduction

Cd or As has been regarded as one of the most harmful and toxic heavy metals and one of the most important contaminants from soil and in the food-chain. At present, environmental scientists take great interest in the accumulation of Cd and As in agricultural soils and assimilation of Cd and As in food plants. However, relevant researches are only confined to dose-effect relationships of single Cd or As. In fact, contamination occurring in environment and toxic effects on organisms usually result from interactions among several pollutants (Babich, 1986; Biesinger, 1986; Wallace, 1989). Our investigation showed that Cd and As usually occurred in ecosystems simultaneously and had some interactive influences on the food-chain. For example, the concentrations of Cd and As in soil of west Shenyang were both high because of long-term sewage irrigation; contents of Cd and As in soil of the Qingchengzi Lead-Zinc Mining area are both abnormal due to mining activities. Therefore, it is necessary to study on combined contamination and ecological effects of Cd and As in environment.

To elucidate the laws and mechanisms of combined harmful effects resulting from

* Present address of the author: The Postdoctoral Research Station, Zhejiang Agricultural University, Hangzhou 310029, China.

the interaction between Cd and As, soil-alfalfa ecosystems were selected as alfalfa is an important component of the food-chain, a green manure crop and one kind of feed for domestic animals in China. Some single-factor experiments showed that alfalfa is sensitive to arsenic but insensitive to cadmium, but the effects of coexistent Cd and As on alfalfa is still unclear.

2 Materials and methods

All the experiments were carried out with soil-alfalfa ecosystems under pot-culture conditions. The test materials in each pot consisted of 5.0 kg unpolluted surface brownsoil (0−20 cm) from the Shenyang Ecological Research Station, 4.0 g alfalfa seed, 1.0 g $(NH_4)_2SO_4$ and 0.7 g $K_2HPO_43H_2O$ as base fertilizers. On the basis of single-factor tests and field investigations, the concentrations of Cd and As were selected as follows:

Cd 0.5 1.0 2.0 3.0 20 mg/kg;

As 5.0 15 20 30 40 50 mg/kg.

Therefore, the experiment was designed (Table 1) according to statistical method.

Table 1 The concentrations designed for the combined pollution of Cd and As

Unit: mg/kg

Factor	Level							
	1	2	3	4	5	6	7	8
Cd	0.0	20	20	20	20	20	20	20
As	0.0	0.0	5.0	15	20	30	40	50
Cd	0.0	1.0	2.0	3.0	0.0	1.0	2.0	3.0
As	20	20	20	20	40	40	40	40
Cd	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0
As	0.0	20	30	40	50	0.0	15	30

The tests were operated according to the following procedures:

(1) Pot culture

Air-dried soil samples were mixed with the contaminator (Cd and As) and the base fertilizers according to the design and were put into the pots marked with numbers. The alfalfa seeds were sowed and water was spread to moisten soil in each pot.

(2) Management

Sprinkling water was a key procedure during the germination, growth and development of alfalfa. In the meantime, it is necessary to prevent waterlogging and insect pests without using insecticides.

(3) Harvest

For each treatment, root length and stem length at harvest were measured. Moreover, roots and tops of alfalfa were weighed at harvest, respectively.

(4) Elemental analysis

The dried samples of entire plant, or roots and tops were ground to fine powders. All plants in each treatment were used for elemental analysis. The dried samples were digested with concentrated HNO_3-$HClO_4$, arsenic was analyzed using colorimetric method, cadmium, copper and lead using atomic absorption spectrophotometry method.

3 Results and discussion

3.1 Poisonous influences on alfalfa

The experiments showed that seed germination, growth and development of alfalfa were obviously affected by Cd and As incorporated into the potted soils. The poisonous effects may depend on the biological characteristics of alfalfa and the complicated interaction between Cd and As in soils.

In general, good seeds of alfalfa would germinate without any problem under normal conditions. However, in our experiments, the germination rate of good alfalfa seeds was decreased. The reason is clear. Cadmium and arsenic in soils had a joint effect on the germination of the seeds. The germination of the seeds was undoubtedly inhibited in the soils with high concentrations of Cd regardless of the concentration of As in soils; however, the germination of the seeds may be promoted because of the interaction Cd and As in soils with lower concentrations of Cd (<0.5 mg/kg).

Maximum root length, maximum stem length and biomass for each treatment were measured at harvest. The results indicated that the interaction between Cd and As in the soils had some poisonous influences on the growth and development of alfalfa, especially on the root growth. At the same concentrations of Cd in soils, the root growth was jointly inhibited with the increase in the concentrations of As in the soils. At the same levels of As, it was similar with the increase in Cd. As for the stem growth, it was jointly inhibited when soil As <15mg/kg, but the inhibition of the stem growth was lessened when soil As >15mg/kg, indicating the antagonism between Cd and As. This may due to the interaction between Cd and As in plants which is different from that in soils.

It was indicated that the biomass of tops was related to the stem growth. Under the test conditions, when the concentrations of Cd added into the soils were low, the biomass of tops was decreased with the increase in As in the soils; when the contents of Cd added into the soils was up to 20 mg/kg, the relationship between the biomass of tops and As in the soils was indeterminate. But the results preliminarily showed that the biomass of tops was increased with the increase in As in the soils when alfalfa plant was exposed to 5－10 mg/kg As concentration of the soil, and the biomass of tops was decreased with the increase in the amount of As added to the soils when the concentration of As in the soils was lower than 5 mg/kg or higher than 10 mg/kg.

3.2 Combined contamination of Cd and As

The elemental analysis of alfalfa plant showed that the reaction of alfalfa plant to coexistent Cd and As differed from that to Cd or As occurring individually. On the one hand, As in soils may promote the function of alfalfa roots absorbing Cd, especially when the concentration of As in the soils was at lower levels (Table 2). On the other hand, Cd in soils may inhibit the absorption of As by alfalfa, especially when the concentration of Cd in soils was lower than 0.5 mg/kg (Table 2), showing that combined contamination of Cd and As in the soil-alfalfa ecosystems is complicated.

Table 2 Concentrations of Cd(I) and As(II) in roots of alfalfa*

Unit: mg/kg

Factor	Level							
	1	2	3	4	5	6	7	8
(I) (Cd-As)	0.07	8.63	49.21	31.56	24.17	20.60	15.75	22.93
(Cd-As)	0.85	3.36	3.83	4.08	0.70	2.93	3.20	3.78
(Cd-As)	0.78	2.68	2.11	1.58	1.30	0.93	3.01	3.14
(II) (Cd-As)	0.15	0.28	0.16	0.94	1.73	2.30	2.87	4.05
(Cd-As)	3.89	0.73	1.40	1.44	7.08	2.23	4.30	5.62
(Cd-As)	0.38	0.42	1.52	2.08	3.10	0.42	0.59	1.68

*It corresponds to Table 1

The interaction between Cd and As in soils may also affect the translocation capacity of Cd or As in tops. Under the pot-culture conditions, the concentration of Cd in tops was on the high sid, the concentration of As in tops was on the low side (Table 3). In other words, when the concentration of As in soils ranged from 0.1 to 20 mg/kg, the translocation capacity of Cd in tops obviously increased in the occurrence of As, with the exception of the high concentration of Cd (20 mg/kg). Conversely, the translocation capacity of As in tops decreased due to the occurrence of Cd, especially when the concentration of Cd in soils was at lower levels.

Table 3 Concentration Cd(I) and As(II) in tops of alfalfa*

Unit: mg/kg

Factor	Level							
	1	2	3	4	5	6	7	8
(I) (Cd-As)	0.09	10.83	23.47	20.09	17.38	16.40	14.76	18.18
(Cd-As)	0.76	2.50	3.61	3.74	0.90	1.97	2.87	3.10
(Cd-As)	0.91	1.85	1.57	1.23	1.12	1.15	2.15	2.38
(II) (Cd-As)	0.17	0.20	0.07	0.72	1.63	1.23	1.98	2.77
(Cd-As)	2.67	0.59	1.31	2.08	9.26	2.12	2.48	4.04
(Cd-As)	0.31	0.92	1.80	2.60	3.23	0.33	0.47	1.58

*It corresponds to Table 1

3.3 Secondary ecological effects

It was found that secondary ecological effects resulted from the interaction between Cd and As in soils include two aspects.

3.3.1 Promoting the absorption of Cu

Both excessive Cu and lacking Cu are harmful to plants, animals and human. For example, when the concentration of Cu in plants is lower than 2−4 mg/kg, the growth of plants may be adversely affected due to the lack of Cu; and plants may be poisoned, when it is higher than 20 mg/kg.

Alfalfa absorbed Cu in soils excessively, although the content of Cu in the experimental soil was normal as shown in Table 4. When the content of As in soils was unchanged, the concentration of Cu in roots or tops was gradually increased with the increase in the amount of Cd added to the soils. Moreover, when the concentration of Cd in soils was at the same level, the concentration of Cu in roots or tops was also related to the amount of As added into the soils. It is showed that Cu absorbed by alfalfa was abnormal due to the coexistence of Cd and As. It may be presumed that AsO_4^{3-} may induce Cd to substitute for Cu in clay minerals and available Cu in soils may increase:

$$Cd^{2+} + Cu - soil \xrightarrow{AsO_4^{3-}} Cu^{2+} + Cd - soil.$$

Table 4 Concentrations of Cu in roots (I), tops (II) and litter (III) of alfalfa*

Unit: mg/kg

Factor	Level 1	2	3	4	5	6	7	8
I	10.38	12.98	14.60	15.21	16.33	17.47	18.09	20.15
	11.64	12.52	13.86	13.92	13.18	15.08	15.92	16.50
	11.03	11.56	12.96	14.12	15.55	11.93	12.18	13.24
II	10.45	10.64	10.77	10.79	11.24	11.76	12.54	13.18
	11.02	9.88	10.61	11.31	10.07	10.94	11.38	12.09
	9.27	9.20	9.56	10.71	11.79	9.32	9.80	10.08
III	7.51	5.42	6.04	6.19	6.74	6.96	7.34	7.87
	7.76	7.54	7.28	7.21	7.50	7.84	7.49	7.37
	8.18	9.42	9.04	8.92	9.64	7.94	8.39	7.39

*It corresponds to Table 1

3.3.2 Aggravating Pb pollution

In general, the concentration of Pb in plants ranges from 0.1 to 10 mg/kg. The concentration of Pb in the roots of the Cd-As treatments was all higher than that of the controls as shown in Table 5, although it was not beyond the normal range of Pb in plants. The concentration of Pb in some tops grown in the experimental soils treated by Cd and As went beyond the normal concentration range of Pb in plants, thereby reflecting the possibility of Pb pollution. The mechanism is presumed that Pb in the tops may be the summation of Pb from the roots and Pb from the air, which

Table 5 Concentrations of Pb in roots (I), tops (II) and litter (III) of alfalfa*

Unit: mg/kg

Factor	Level 1	2	3	4	5	6	7	8
I	0.60	2.21	2.84	2.92	3.07	3.19	3.23	3.51
	0.83	1.01	1.74	1.91	1.25	1.24	2.30	3.08
	0.80	0.92	0.96	1.12	1.23	0.83	0.91	1.16
II	4.32	8.74	9.98	10.19	10.83	11.31	13.87	15.69
	5.47	6.11	6.38	6.52	6.00	6.52	7.13	8.01
	5.40	5.88	6.17	6.40	6.82	5.52	6.00	6.37
III	10.42	21.48	25.41	27.12	27.54	28.39	28.41	30.17
	14.11	17.13	18.60	18.77	16.28	20.70	19.88	21.21
	13.39	15.48	16.00	18.47	20.37	14.12	16.09	19.08

*It corresponds to Table 1

was absorbed by leaves due to the interaction between Cd and As in plants. The concentration of Pb in the litter was noticeably higher than the normal value of Pb in plants. Pb pollution in the soil-alfalfa ecosystems is aggravated due to the coexistence of Cd and Pb.

4 Conclusion

Compound contamination of Cd and As obviously affected the seed germination, growth and development of alfalfa. This has been indicated that maximum root length, maximum stem length and biomass of alfalfa were related to the concentration of Cd and As added into the soil in each pot. Concentrations of Cd in alfalfa were increased due to the occurrence of As in the soil; conversely, cadmium added into the soil may inhibit the absorption of As by alfalfa. It is thus clear that the interaction between Cd and As in plants is a complicated biological process.

In the soil-alfalfa ecosystems with the coexistence of Cd and As, alfalfa absorbed Cu and Pb abnormally.

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