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TOXIC EFFECT OF ORGANIC ARSENIC ON THE GROWTH OF SOYBEAN AND ECOLOGICAL RISK ASSESSMENT OF COMBINED ARSENIC CONTAMINATED FARMLANDS

T. Zhou[a,*], L. J. You[a], X. F. Wang[a], G. Rao[a], C. Wu[b], C. Guo[a] and J. X. Chen[a]

[a]Institute of Chemical Defense, Beijing, 102205, People's Republic of China;
[b]Research Institute of Chemical Defense, Beijing, 102205, People's Republic of China
Corresponding author E-mail: zhting_12@sina.com

ABSTRACT

The risk of arsenic (As) contamination in soybean farmlands in Jilin province was assessed and the effects of triphenylarsine(TPA) and phenylarsonic acid (PPA) on soybean germination, height of seedling, and organs such as root and hypocotyl were studied. Taking soybean as the assessed receptor, the ecological risk of the farmlands in this area was assessed. The results showed that TPA and PPA have toxic effects on soybean germination and soybean organs. There were significant logarithmic relationships between As-concentrations and germination rate, relative lengths of organs and corresponding inhibition ratio. With the increase of As-concentrations, the germination rate and the relative lengths reduced and the inhibition ratio increased. There were significant logarithmic or linear relationships between As-concentrations in organs of soybean seedling and the concentrations of TPA and PPA. Taking EC_{25} of germination inhibition as the assessment criterion for the farmlands in this area, only 1.8% of the area was determined as unacceptable for soybean growth. However the safety of ripe soybean crops as food still needs further assessment.

Keywords: High risk As-containing explosives, TPA, PPA, soybean, toxicity.

INTRODUCTION

About 60 years ago, in order to treat a large quantity of highly hazardous shells filled with diphenylcyanoarsine (DC), diphenylchloroarisine (DA) etc., simple treatment through artificial ammunition ignition was carried out and lead to soil contaminations. Through the survey from 2004 to 2006, about 26.0 hectares of soils were found to be contaminated. Further analysis showed that due to scattering during explosion and later long-term burial process, triphenylarsine (TPA), phenylarsonicacid (PPA), diphenylarsonic acid (DPAA), degradation products of DA and DC, and inorganic arsenic compounds were found in leaked shells and surrounding soils. However, this area was taken as a seed base of quality soybean.

In this paper, we used soybean as the toxic effect receptor to assess the toxicity of organic arsenic represented by TPA, PPA on the growth of soybean seedling. The stress effect, inhibition effect of organic arsenic on soybean growth and absorption and the accumulation of arsenic in soybean were also investigated. By extrapolating the 25% inhibition effect of soybean acquired by experiments to spots, the ecological risk in this area is estimated.

MATERIALS AND METHODS

Materials: The testing soybean variety was selected from the local planting variety. Clean white sand with a particle size of 0.5 – 1 mm was used as the growth carrier. All experiments were conducted in greenhouse.

Preparation of As compounds: Since TPA is insoluble in water, it was mixed into white sands. Different concentrations of PPA were added into water and mixed them in white sand.

Seed treatment: Soybean seeds were sterilized with 0.1% $HgCl_2$ for ten minutes and repeatedly washed with deionized water. Each germination box were placed with 40-soybean seeds. Each treatment were repeated 3 times.

Determination of germination rate: 3 days after soybean seed germination, the number of germinated seeds were counted and divided by the number of total tested seeds (Shan *et al.,* 2010).

Length measurement of different organs of soybean seedlings: 7 days after soybean germination, seedlings were took out of the sand culture, repeatedly washed with distilled water to ensure that the seedlings did not carry any white sands. The height, hypocotyl and root length of each seedling were measured respectively.

Determination of total arsenic: Harvested soybeans were separated into the root, hypocotyl and spire, marked and dried for 8 hours at 70°C, and then to make samples

(Qu *et al.*, 2013).Total arsenic of the sample was determined using atomic fluorescence spectrophotometer (GB/T 5009.11-2003).

RESULTS AND DISCUSSION

The effect on germination of soybean seed: The germination rates of soybean seeds cultured in sand beds with different concentrations of TPA and PPA are given in Table 1 and Table 2, respectively.

Table 1. Effects of TPA on germination rates of soybean seeds

C_{TPA}(mg/kg)	Germination Rate (%)
0	100
12.5	89.16
25	71.67
50	52.50
100	39.17
200	24.16
400	10.80

C_{TPA}: Concentration of TPA

Table 2. Effects of PPA on germination rates of soybean seeds

C_{PPA}(mg/kg)	Germination rate(%)
0	96.67
0.27	62.50
0.54	46.88
1.08	34.38
2.16	21.87
4.32	12.50
8.64	3.13

C_{PPA}: Concentration of PPA

As shown in Table 1 and Table 2, TPA and PPA both had a significant effect on the germination of soybean seeds. The germination rate decreased gradually with the increase of the concentration of TPA and PPA.

Statistics of effect on the growth of soybean organs: The effects of TPA and PPA on the height, hypocotyls and the root length of soybean seedlings are summarized in Table 3 and Table 4. Based on the data in Table 3 and Table 4, we calculated the relative ratio of the experimental group and the control, and plotted the fitted curve of the ratios to compare the effect of TPA and PPA on the organs of soybean, as shown in Fig.1 and Fig.2.

As shown in Fig.1, the ratio of height, hypocotyl and root length of soybean between the treatment and the control showed logarithmic relationships. The fitted curve of height, hypocotyl and root length respectively was *Eq*. (1), *Eq*.(2), *Eq*.(3),:

These results indicated that the organs of soybean seedling were affected by TPA; with the increase of the concentration of TPA, the effect increased in logarithmic form, i.e. the relative ratio reduced.

Table 3. Effects of TPA on the growth of organs of soybean seedlings

C_{TPA} (mg/kg)	Height (cm)	Hypocotyls (cm)	Root (cm)
0	16.19	6.37	12.20
12.5	14.53	5.48	10.59
25	11.69	4.86	9.39
50	10.18	4.65	7.56
100	8.15	4.43	6.94
200	6.07	3.62	4.86
400	3.02	3.33	2.34

C_{TPA}: Concentration of TPA

Table 4. Effects of PPA on the growth of organs of soybean seedlings

C_{PPA} (mg/kg)	Height (cm)	Hypocotyls (cm)	Root (cm)
0	15.83	5.89	12.11
0.27	12.51	4.91	9.52
0.54	10.80	4.68	8.16
1.08	8.72	3.37	6.36
2.16	5.71	2.53	4.45
4.32	3.24	2.32	1.47
8.64	1.62	1.52	0.74

C_{PPA}: Concentration of PPA

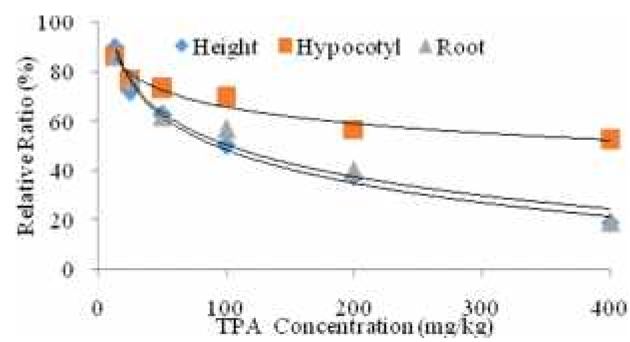

Fig. 1. The curve fitting result of the effect of TPA on organs of soybean

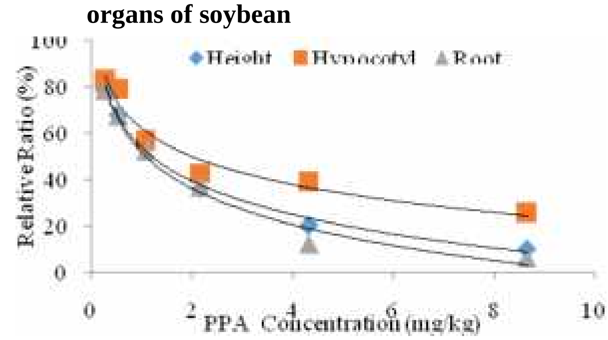

Fig. 2. The curve fitting result of the effect of PPA on organs of soybean

$$y_{ht} = -19.4\ln x_{ht} + 138.1, R^2 = 0.990 \quad Eq.(1)$$
$$y_{hl} = -9.50\ln x_{hl} + 109.4, R^2 = 0.965 \quad Eq.(2)$$
$$y_{rt} = -18.7\ln x_{rt} + 136.7, R^2 = 0.972 \quad Eq.(3)$$

As shown in Fig.2, the toxic effect of PPA on soybean seedling was similar to that of TPA. The fitted logarithmic of height, hypocotyl and root length respectively was *Eq*.(4), *Eq*.(5), *Eq*.(6):

$$y_{ht} = -20.8\ln x_{ht} + 53.69, R^2 = 0.992 \quad Eq.(4)$$
$$y_{hl} = -17.4\ln x_{hl} + 62.06, R^2 = 0.961 \quad Eq.(5)$$
$$y_{rt} = -22.4\ln x_{rt} + 51.74, R^2 = 0.982 \quad Eq.(6)$$

The comparison of effects on inhibition ratios of germination of soybean seed, growths of seedling and root: Regression analysis of the effects of TPA and PPA on germination of soybean seed and indexes of the growth of seedling were conducted, and the results are shown in Fig.3 and Fig.4. The results showed that the concentration of organic arsenic had significant correlation with indexes including the germination rate, height, hypocotyl length and root length ($P<0.01$). Their relationships can be represented as natural logarithmic curves. For TPA respectively was(from *Eq*. (7) to *Eq*.(10)):

$$y_{ger} = 22.57\ln x_{ger} - 44.04, R^2 = 0.995 \quad Eq.(7)$$
$$y_{ht} = 19.46\ln x_{ht} - 38.09, R^2 = 0.990 \quad Eq.(8)$$
$$y_{hl} = 9.505\ln x_{hl} - 9.476, R^2 = 0.965 \quad Eq.(9)$$
$$y_{rt} = 18.73\ln x_{rt} - 36.73, R^2 = 0.972 \quad Eq.(10)$$

For PPA respectively was(from *Eq*. (11) to *Eq*. (14)):

$$y_{ger} = 17.58\ln x_{ger} + 61.30, R^2 = 0.990 \quad Eq.(11)$$
$$y_{ht} = 20.86\ln x_{ht} + 46.31, R^2 = 0.992 \quad Eq.(12)$$
$$y_{hl} = 17.40\ln x_{hl} + 37.93, R^2 = 0.961 \quad Eq.(13)$$
$$y_{rt} = 22.42\ln x_{rt} + 48.25, R^2 = 0.982 \quad Eq.(14)$$

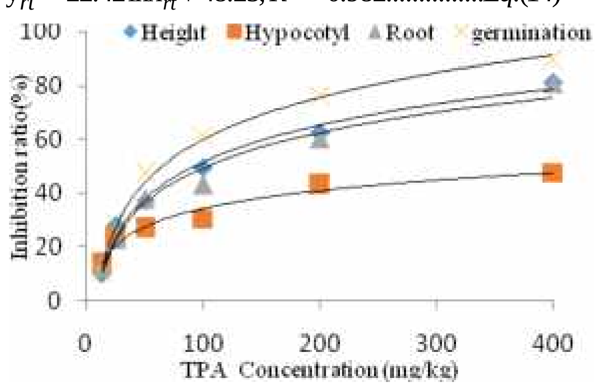

Fig. 3. Curve fittings of TPA concentrations vs. inhibition ratios of germination of soybean seed and the organs of seedling

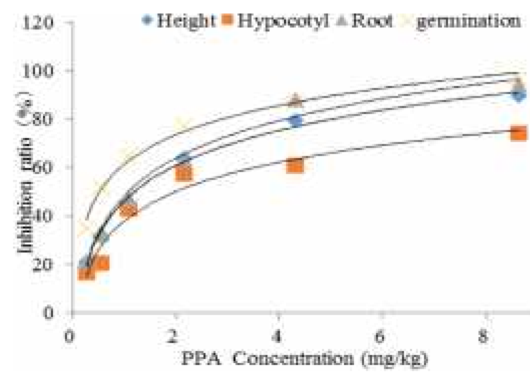

Fig. 4. Curve fittings of PPA concentrations vs. inhibition ratios of germination of soybean seed and the organs of seedling

As shown in Fig.3 and Fig.4, the concentrations of TPA and PPA had a significant positive correlation with germination of seed and indexes of seedling growth. These relationships can be fitted as logarithmic curves. Based on the regression equation, we calculated the corresponding EC_{25} when the inhibition ratio reaches 25 %. The $EC_{25}$of TPA and PPA were 21.30 mg/kg and 0.13mg/kg respectively.

Absorption and accumulation effect of organic arsenic of soybean seedling: Under different TPA treatment on centrations, the total arsenic concentrations in root, hypocotyl and the spire of soybean can be fitted as logarithmic curves (Fig.5), the fitted curves were (from *Eq*. (15) to *Eq*.(17)):

$$y_{hl} = 0.190\ln x_{hl} - 0.374, R^2 = 0.978 \quad Eq.(15)$$
$$y_{rt} = 0.403\ln x_{rt} - 0.770, R^2 = 0.983 \quad Eq.(16)$$
$$y_{se} = 0.129\ln x_{se} - 0.004, R^2 = 0.922 \quad Eq.(17)$$

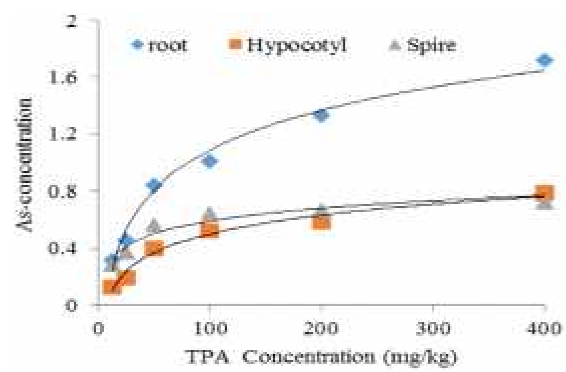

Fig. 5. Curve fittings of TPA concentrations of TPA vs. As-concentration in organs of soybean seedling

We also determined the total arsenic concentration in root, hypocotyl and spire under different PPA treatment concentrations, as shown in Fig. 6. The concentration of PPA vs. As-accumulation in root fitted better using logarithmic curve, while hypocotyl and spire were better fitted using linear fit; the fitting equations are (from *Eq*.(18) to *Eq*.(20)):

$$y_{hl} = 2.325 \ln x_{rt} + 2.112, R^2 = 0.985 \ldots\ldots\ldots\ldots Eq.(18)$$
$$y_{rt} = 6.936 \ln x_{rt} + 11.35, R^2 = 0.954 \ldots\ldots\ldots\ldots Eq.(19)$$
$$y_{se} = 1.932 \ln x_{se} + 1.130, R^2 = 0.990 \ldots\ldots\ldots\ldots Eq.(20)$$

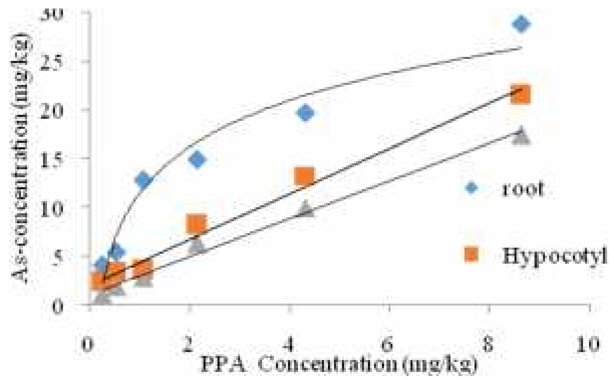

Fig. 6. The curve fitting result of concentration of PPA vs. As-concentration in organs of soybean seedling

The toxicity of inorganic As to soybean: Arsenic can inhibit the growth of crops, disturb photosynthesis and respiratory system and irritate secondary metabolism (Chang, *et al.*, 2005). Previous water culturing experiment showed that arsenic inhibits germination of soybean seed, development of seedling and early growth (Garg, *et al.*, 2011). Hu (1996) studied the effect of inorganic arsenic with different valence states on the germination of soybean seed. In laboratory, they used disodium hydrogen arsenate (Na_2HAsO_4) and sodium arsenite (Na_3AsO_3) to represent As^{5+} and As^{3+}, used the same steps as organic arsenic experiment in this paper to determine the inhibition ratio of germination of soybean, we used their results fitting the curves shown in Fig.7 and Fig. 8.

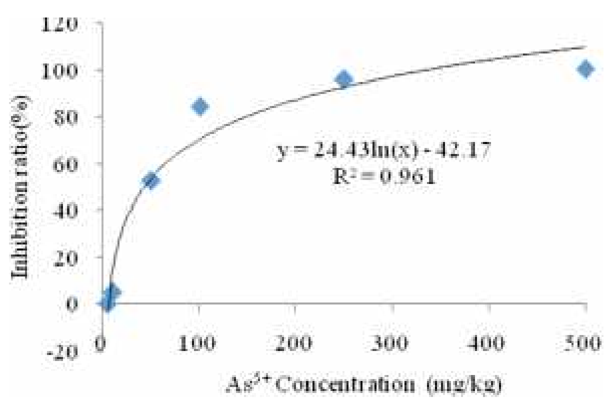

Fig. 7. The curve fitting result of As^{5+} concentration vs. inhibition ratio of germination of soybean

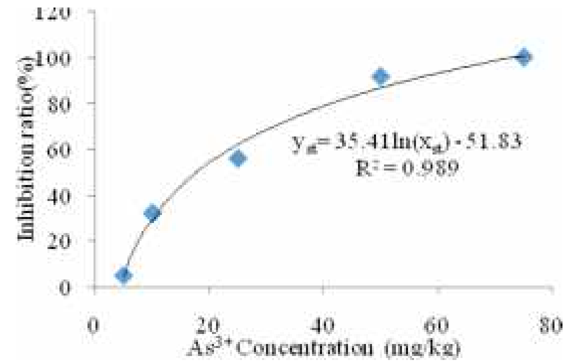

Fig. 8. The curve fitting result of As^{3+} concentration vs. inhibition ratio of germination of soybean

According to Fig.7 and Fig.8, the numerical calculation of EC_{25} for As^{5+} and As^{3+} were 15.64 mg/kg and 43.47 mg/kg respectively.

Ecological risk assessment of soil contaminated by combined arsenic

Receptor Assessment Criteria: Receptor, i.e. risk receiver, in risk assessment, means the constituent part in the ecological system which may suffer unfavorable actions from risk sources. As the main planted crop in the contaminated area, soybean is the typical representative of the field ecosystem. Combining the characteristics of the local ecosystem, we selected soybean as the risk receptor.

Assessment endpoint: Germination of seed is an important stage during the whole plant growing process, meanwhile is one of the most sensitive period to changes of the external environment (Chen, *et al.*, 2003). Therefore, we took the inhibition effect concentration of 25% germination of soybean seed as the endpoint of the toxic effect assessment.

Analysis of risk source: The analysis of risk source means identification and analysis of disturbances which may have adverse effects on the ecological system or its components. There were many kinds of highly hazardous arsenic containing chemicals with complicated structure, Currently, it is impossible to accurately calculate the concentration of arsenic chemicals in different forms in the sampling point. Therefore, we selected TPA to represent stable organic arsenic, PPA to represent active organic arsenic, and As^{5+} as the representative of inorganic arsenic for organic arsenic toxicity evaluation.

Determination the method for ecological risk characterization: Quotient method was used to calculate risk standard coefficient *Q* by Eq. (21):

$$Q_i = EEC_i / TOX_h \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.(21)$$

(Wherein: EEC_i-concentration of harmful substance at points; TOX_h- critical value causing hazard.)

In this assessment, when calculate Q_i, we took the actual data of total As-concentration at sampling points as *EEC*; and the As-concentration at which germination rate of soybean was 25% for TPA as TOX_h, PPA and As^{5+}. If $Q_i>1$, the risk was unacceptable, otherwise the risk was acceptable.

Assessment results: The EC_{25} of TPA germination inhibition ratios was used to quantize low toxic organic arsenic, and that of PPA germination inhibition ratio was used to quantize high toxic organic arsenic. As shown in the analysis of arsenic forms, the concentration of organic arsenic generally was below 10%. Therefore, select 10% as the assessment concentration, the EC_{25} of TPA on germination inhibition was 21.30mg/kg, accounting for 90% arsenic contribution of organic arsenic; the EC_{25} of PPA on germination inhibition was 0.13 mg/kg, accounting for 10% As-contribution of organic arsenic. Inorganic arsenic in soil accounted for 90% of total arsenic. The EC_{25} of As^{5+} on germination inhibition was 15.64mg/kg. Meanwhile, 1.69%, the maximum concentration of water soluble arsenic, is equivalent to the As-concentration under sand culturing conditions. Therefore Q_i was calculated by Eq. (22):

$$Q_i = EEC_i \times \left(\frac{1}{0.25 \times TOX_{TPA}} \times 0.09 + \frac{1}{0.37 \times TOX_{PPA}} \times 0.01 + \frac{1}{TOX_{As}} \times 0.09 \right) \times 0.0169 \quad \text{Eq.(22)}$$

Combining the distribution characteristics of arsenic space of the local area, Ordinary Kriging in Geostatistical extension module of Arcmap was used for spatial interpolation of assessment index of sampling points. Space distribution of contamination indexes according to the interpolation results are shown in Fig. 9.

As shown in Fig. 9, due to natural oxidation of up to a half century, a great amount of organic arsenic have been degraded into inorganic arsenic. Therefore, except for soil with high concentration of organic arsenic surrounding the artillery shells, the growth of soybean is hardly affected. The unacceptable area is only 0.48 hectares, accounting for 1.8% of the total area. And the unacceptable area is scattered, only the core area of shells reaches unacceptable level.

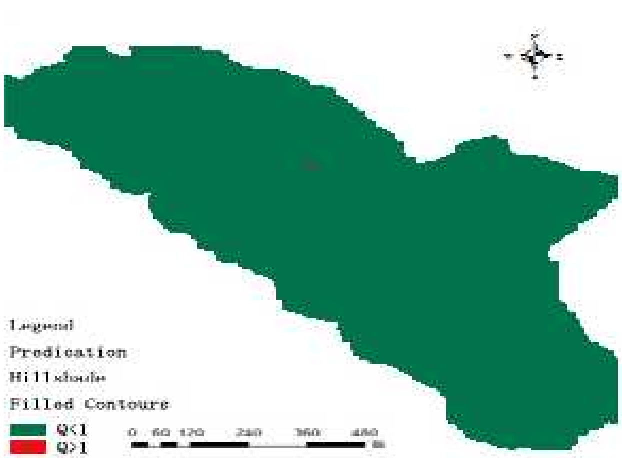

Fig. 9. Distribution of ecological risk of combined As contamination of the area

Conclusions: The contamination of farmland caused by highly hazardous arsenic containing chemicals is contributed by both organic and inorganic arsenic. The study of TPA and PPA showed the toxicity of highly hazardous arsenic containing chemicals DA, DC and their degradation products on soybean growth. Under lab test t, both TPA and PPA have inhibitive effect on soybean germination and seedling growth. Both TPA and PPA have significant toxic effect on soybean organs. Taking EC_{25} of germination inhibition as the assessment criterion for combined organic arsenic and inorganic arsenic contaminated farmlands in this area, when judged by quotient method, only 1.8% of the area was determined as unacceptable for soybean plantation. But for food safety, it still needs further assessment. Although our results were obtained from lab experiments and the sampled soil may not represent all possible soil contaminations in this area, this assessment can provide a good reference for future studies.

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