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Effects of Simultaneous Exposure to Atmospheric Sulfur Dioxide and Heavy Metals on the Yield and Metal Content of Soybean Grain (*Glycine max* [L.] Merr.)

X. Xiong,[1] G. Allinson,[2] F. Stagnitti,[2] F. Murray,[3] X. Wang,[1] R. Liang,[1] J. Peterson[4]

[1] Department of Pollution Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Post Office Box 417, Shenyang 110016, People's Republic of China
[2] School of Ecology and Environment, Deakin University, Post Office Box 423, Warrnambool, Victoria 3280, Australia
[3] School of Biological and Environmental Sciences, Murdoch University, Perth, WA 6150, Australia
[4] School of Geography and Environmental Science, Monash University, Victoria 3800, Australia

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In China, approximately 7.3% of the total irrigated area (3.62 million ha) is irrigated with sewage. Although the recycling of wastes is desirable for sustainable agricultural production and economic and ecological survival, treated municpal sewage often contains significant concentrations of potentially toxic contaminants. For instance, the Zhangshi Irrigation Are (2720 ha), Shenyang, north-western China, is heavily polluted by Cd, As, Pb, and Zn resulting from use of sewage irrigation (Yanai et al. 1998; Wu et al.1986). Since the 1980s, China's economy has been developing rapidly, and there are areas in northern and southern China that regularly experience high atmospheric concentrations of sulfur dioxide emitted by the power stations fuelling the economic growth. For instance, the urban areas of Guiyang, Taiyang, Beijing, and Urumchi experience atmospheric SO_2 concentrations as high as 100 ppb in winter (Hongfa et al., 1999), while in the highly industrial area of Shenyang, Lioaning Province, the average atmospheric SO_2 concentration during the summer is 38 ppb (Kato et al., 1999). The Chinese "Environmental Air Quality Standard" states the daily average SO_2 concentration should be < 135 ppb for natural conservation areas; < 405 ppb for residential and agricultural areas, and < 675 ppb for industrial areas, respectively. However, for agricultural crops in their growth phase the maximum daily average should be less than 56 ppb, the maximum concentration observed less than 185 ppb, and the mean concentration less than 18.5 ppb (State Standard GB9137-88).

China is the world's third largest producer of soybeans. This crop is grown on approximately 11.7 million ha (1998), with an annual production of approximately 14.73 million tons (1997 data; People's Republic of China Yearbook, 1999). Soybean plants are known to be sensitive to atmospheric SO_2 (Hongfa et al, 1999), with the yield of soybean decreased by 4% at SO_2 concentrations as low as 50 ppb. Soybean plants are also known to be sensitive to soil contamination by some metals (Borkert et al, 1998). There is thus justifiable concern that metal and SO_2 contamination of soil and atmosphere, respectively, may be acting synergistically to enhance the negative effects on soybean plant growth and productivity seen after individual exposure to these pollutants. This paper presents results of a reconnaissance survey of the impact of simultaneous exposure to atmospheric SO_2

Correspondence to: G. Allinson

and heavy metals in an artificial root growth medium on the yield and quality of soybean.

MATERIALS AND METHODS

Open-top chambers similar to those described by Heagle et al. (1973) were used to fumigate soybean plants with SO_2. The chambers were located at the Field Experiment Station of Murdoch University, Western Australia (31° 57' 2" S, 115° 51' 36" E), and were essentially cylinders 2.4 m high and 3 m in diameter. The chambers were made rigid with an aluminum frame, and a single layer of UV-treated PVC plastic film covered the upper half of the frame. The lower half, covered by a double layer of the same film, served as an air duct. The inner layer of the lower panel was perforated with 250 holes, 2.5 cm in diameter, through which SO_2 was pumped for 4 hours daily (10 am to 2 pm) for up to 55 days during the soybean growing seasons. Temperature and relative humidity within the chambers were similar to ambient conditions.

The concentration of SO_2 in the chambers was monitored every half-hour using a Thermo Electron Series 43 pulsed fluorescent ambient SO_2 analyzer, with data stored in a data logger before downloading to computer. The analyzer was calibrated with a Thermo Electron Model 145 calibrator, with NBS traceable certified permeation tubes. The SO_2 concentration in the chambers was adjusted manually after daily examination of the logged SO_2 concentration. The Low and High-SO_2 treatment chambers had mean SO_2 concentrations of 97.3 and 489 ppb, respectively. The control chamber had no SO_2 fumigation, so concentrations represented background SO_2 (1.19 ppb; Control-SO_2).

Soybean seeds were planted in 2L plastic pots filled with 2L of an artificial potting mix made from 2 : 18 : 8 fine sand : coarse sand : coconut fiber. This artificial growth medium lacks many of the components of native soils. There are no clay particles, for instance. The medium, therefore, represents a worst-case scenario making the metals more available to the plants, but at the same time making it easier to observe interactions between SO_2 fumigation and metals on the plants. Six grams of the granular fertilizer Osmocote (plus) Controlled Release Fertilizer, containing NPK macro-nutrients as well as necessary trace elements, was added to each pot. The pots were top dressed with KH_2PO_4 three times (7, 16 and 24 April, 1998; on each occasion, 2g pot $^{-1}$ dressing^{-1}).

Treatment potting mix was spiked with the chloride salts of cadmium (Cd; 0.5 mg L^{-1} of potting material), lead (Pb; 250 mg L^{-1}), copper (Cu; 100 mg L^{-1}) or zinc (Zn; 150 mg L^{-1}), respectively. These concentrations were chosen as being comparable with the "Environmental Quality Standard for Soils" of the People's Republic of China (GB 15618-1995; Cd, 0.3-0.6; Pb, 250-350; Cu, 50-100; and Zn, 100-200 mg kg^{-1} respectively). Although control potting mix was not deliberately contaminated with metals, the control pots' potting mix (Control-HM) contained fertilizer-borne metals. For instance, Cu and Zn at 1.5 and 0.45 mg L^{-1}, representing 1.5 % and 0.3% of added Cu and Zn, respectively.

After being soaked for one day in water, 4 seeds were sowed per pot. After emergence, the three most vigorous plants were retained in each pot. The plants were grown in a nursery for one month. Thereafter, the potted plants were put in the fumigation chambers. Each chamber housed a total of fifteen pots, with three replicates for each of the four heavy metal treatments, plus three uncontaminated pots. In one chamber, the soybean plants were exposed to 97.3 ppb SO_2 (the Low-SO_2 treatment), in a second chamber the plants were exposed to 489 ppb SO_2 (the High-SO_2 treatment), and in the third to background SO_2 concentrations in ambient air (1.19 ppb, Control-SO_2). The three chambers were separated from each other by about 10 meters. In the Control-SO_2 chamber, those pots with no added metals added were designated the Control-Both group.Therefore in the experiment there were three controls, namely Control-Both, Control-HM and Control- SO_2.

The soybean grains were harvested 110 days after sowing on 23 May, 1998. Each tissue sample was put into a brown envelope and then into ovens at 80°C for drying to constant weight. The dry samples were then prepared for elemental analysis. Inductively coupled plasma emission spectrometry (ICP-AES) was used to determine the concentration of Cd, Cu, Pb, and Zn after acid digestion of 2g dry material with 20 mL acid (HNO_3 : $HClO_4$ 3:1; Zhang Suchun, 1986). A Certified Reference Material, Peach Leaves (GBW 08501; State Standardization Bureau, China; Han, 2000), was used to assess the performance of the analytical methods. Reagent blanks were also processed, with nitric acid quantities adjusted to maintain similar treatment volumes in samples and blanks.

RESULTS AND DISCUSSION

In the Control-SO_2 chamber, the Zn and Cu treatments caused in the yield of soybean grain compared to the Control-HM (grain reductions: Zn treatment, 63 %; Cu treatment, 41 %; Table 1, see data in Control-SO_2 column). However there was no apparent change to grain yield in the Cd treatment. Addition of Pb increased the yield of grain. These latter results confirm those of Xiong et al (1997), who indicated that when 50-500 mg kg^{-1} Pb was added to soil, the yield of soybean grain increased by 2.56-7.69 %.

Plants can obtain sulphur by direct absorption of atmospheric SO_2. Stomata in the leaves and pod skins allow the entry of SO_2 into the soybean, and acute and chronic injuries arise from sulfate and sulfite accumulation in the leaves. The response of soybean to SO_2 is complex, depending as it does on the concentration of SO_2 and the nature of the exposure. Injury arises from disturbances in plant

Table 1. Simultaneous exposure to atmospheric SO_2 and metals on yield of soybean grain (g pot^{-1}).

Treatment	Atmospheric SO_2 Chamber		
	Control- SO_2	Low SO_2	High SO_2
Control-HM	27.6 (8)	26.5 (9)	20.9 (12)
Cd	27.3 (7)	25.4 (9)	22.0 (10)
Cu	16.3 (10)	13.7 (9)	9.8 (11)
Pb	30.6 (7)	23.4 (11)	19.9 (13)
Zn	10.2 (9)	7.0 (10)	5.1 (14)

Figures in parentheses indicate coefficient of variance (%).

Table 2. Summary of CRM metal concentrations, and concentrations determined in this study.

	CRM (Peach Leaves)		
Element	Certified value	Observed value	Recovery (%)
Cd	0.018 ± 0.004	0.016-0.019	89-106
Cu	10.4 ± 0.8	9.4-10.9	90-105
Pb	0.99 ± 0.04	0.84-1.07	85-108
Zn	22.8 ± 1.3	20.5-24.1	90-106

Values quoted on a dry weight basis (mg kg^{-1}).

Table 3. Metal concentrations in soybean grain (mg kg^{-1}). Figures in parentheses indicate coefficient of variance (%).

Treatment	Atmospheric SO_2 Chamber		
	Control-SO_2	Low-SO_2	High -SO_2
Control-HM	0.02 (25)	0.03 (18)	0.03 (11)
	6.15 (4)	9.09 (6)	10.0 (10)
	0.13 (39)	0.15 (25)	0.14 (18)
	36.2 (17)	38.0 (12)	38.6 (13)
Cd	0.61 (19)	0.74 (6)	0.86 (7)
Cu	15.5 (10)	18.8 (11)	26.7 (6)
Pb	0.68 (27)	0.75 (11)	0.83 (8)
Zn	76.5 (19)	80.3 (9)	88.9 (11)

metabolism, and may cause a decrease of yield without injury to leaves, injury to leaves without decrease of yield, or be injurious to leaves and cause decrease in yield (Verma et al. 1996). In this study, although there was no visible SO_2 injury, the Control-HM treatment plant grain yields in the High-SO_2 treatment (20.9 g pot^{-1} (coeficient of variance (c.v.) 12%)) was lower than the Control-HM yields in the Low-SO_2 and Control-SO_2 treatments (26.5 g pot^{-1} (c.v. 9%), and 27.6 g pot^{-1} (c.v. 8%), respectively. See Table 1). In general, these results are consistent with those reported by Hongfa et al (1999), who reported that the yield of soybean decreased by 4% at SO_2 concentrations as low as 50 ppb.

Simultaneous exposure to atmospheric SO_2 and metals in the root growth medium also caused changes in soybean grain yield (Table 1). However, the effects varied. For instance, exposing soybean plants to Cd+Low-SO_2 caused little decrease in grain yield compared to Cd exposure in the Control-SO_2 chamber (Cd+Control-SO_2). However, Cd+High-SO_2 reduced grain yield by 19.4 % relative to Cd+Control-SO_2. The Pb+Low-SO_2 and Pb+High-SO_2 treatments reduced grain yield relative to Pb+Control-SO_2 (23.5 % and 35 %, respectively). The Cu+Low-SO_2 and Cu+High-SO_2 treatments reduced grain yield by 50.4% and 64.5% relative to Control-Both, and Cu+High-SO_2 reduced grain yield relative to Cu+Control-SO_2 treatment. The Zn+Low-$SO_2$and Zn+High-SO_2 treatments reduced grain yield by 74.6% and 81.5% relative to Control-Both, and also reduced yield relative to Zn+Control-SO_2 treatment. A reduction in grain yield was found between Low-SO_2+Cu (or Zn) and High-SO_2+Cu (or Zn). Essentially, in exploring the combined effect of SO_2 and heavy metals in potting medium on grain yield, for Cd or Pb, the SO_2 appears to play a major role. On the other hand, for Cu and Zn it is the metals themselves that appear to have the more important roles in affecting grain yield.

In order to explore the combined effect of SO_2 and heavy metals in potting medium on the translocation of heavy metals to soybean grain, metal concentrations in the grain were determined. To check analytical accuracy and precision, the analysis of the certified reference material (Peach Leaves, GBW 08501) was undertaken. Certified element concentrations were found to be within 15% of expected values (85-108% recovery; Table 2). What this reconnaisance study suggests is that the effects of simultaneous exposure to metals in root growth medium and atmospheric SO_2 on grain metal concentrations are quite different for the four metals investigated (Table 3). In the Control-SO_2 chamber (without SO_2 fumigation), metal treatments produced increases in metals content in the grain relative to Control-HM plants. For instance, Cd concentrations were approximately 31 times that of Control-HM. However, for Pb, Cu, and Zn the concentrations were much lower at approximately 5.2, 2.5,and 2.1 times that of control-HM, respectively. Metal concentrations in the grain exceeded the Chinese "Tolerance Limit in Food" for Cd, Cu, Pb, and Zn of 0.2, 10.0, 0.4, and 50.0 mg kg^{-1}, respectively (Code:GB 15201-94 ; GB 14935-94; GB 15199-94; GB 13106-91, respectively). Exposure to SO_2 did not advance translocation of Pb or Zn into soybean grain, but did promote uptake of Cd and, particularly, Cu. For instance, when exposed to Low-SO_2 and High-SO_2, the Cu content of soybean grain increased by 18 and 72% respectively (Table 3).

In some regions of China, there is simultaneous pollution of soil by metals, and the atmosphere by SO_2.There is thus understandable concern that metal and SO_2

contamination of soil and atmosphere, respectively, may be acting synergistically to enhance the negative effects on soybean plant growth and productivity seen after individual exposure to these pollutants. This reconnaisance study has shown that metals and SO_2 can individually have an effect on soybeans, but further, controlled greenhouse and field studies are required to assess whether this is truly the case, and whether their effects will be increased on simultaneous, multiple exposure.

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