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BERYLLIUM PHYTOTOXICITY IN SOYBEANS

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(Received 10 September, 1993; accepted in final form 30 June, 1994)

Abstract. A greenhouse study was conducted to assess the effects of soil-applied beryllium (Be) on the growth and Be content of soybeans [*Glycine max* (L.) Merr.], grown on acid southeastern soils under limed and unlimed conditions. This study was conducted using a factorial design, with two soil types varying in clay content (Blanton sand, a loamy, siliceous, thermic Grossarenic Paleudult; and Orangeburg loamy sand, a loamy, siliceous, thermic Typic Paleudult), two soil treatments (limed and unlimed) and five Be concentrations (0, 25, 50, 100, and 150 mg Be kg^{-1} soil). Addition of Be to unlimed Blanton soil had the most toxic effects of all treatment combinations; at the 150 mg Be kg^{-1} treatment plant biomass was reduced as much as 90% and plant Be concentration was as high as 226 mg Be kg^{-1}. Beryllium concentrations were greater in plants grown in a soil low in clay (Blanton soil). Liming of soils treated with Be resulted in lowered tissue Be concentrations in plants grown on either soil type.

1. Introduction

Beryllium (Be) is a phytotoxic trace metal which is released into the environment primarily through the combustion of fossil fuels (Peterson and Girling, 1981; Horovitz and Petrecu, 1984; Adriano, 1986). In the U.S., approximately 1,250 t of Be (Zubovic, 1969) is emitted into the environment annually through the combustion of fossil fuels. Dusts and mists containing Be are a major health threat to certain mammals including humans causing delayed pneumonitis-berylliosis (Everest, 1964). Due to the highly toxic nature of this metal and its accumulation in the natural ecosystem, the quantitative understanding of this metal in a soil-plant systems is essential.

Beryllium is a trace element commonly found in nature. It has been reported (Kabata-Pendias and Pendias, 1984) that Be occurs in soil as BeO_2^{2-}, $Be_2O_3^{2-}$, BeO_4^{6-}, or Be_2O^{2+} and in calcareous soils as $Be(OH)CO_3^-$ and $Be(CO_3)_2^{2-}$; however, it is most commonly found as a divalent cation. The inorganic salts, $BeCl_2$, and $BeSO_4$, are soluble and readily available for plant uptake. On the other hand, Be readily complexes with organic compounds, accumulating in the surface layer of the soil profile and is unavailable for plant uptake (Korte *et al.*, 1976).

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Water, Air and Soil Pollution **86:** 117–124, 1996.
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TABLE I

Selected properties of the Ap horizons of the Blanton and Orangeburg soils

Soil	% Sand[a]	% Silt	% Clay	OM[b]	Texture	CEC[c]	pH	
							Unlimed	Limed
Blanton	91.9	6.2	1.9	11.00	s[†]	3.25	4.9	6.5
Orangeburg	87.4	7.9	4.7	18.50	ls[‡]	3.93	5.5	6.0

[a] Sand, silt, and clay determined by the Bouyoucos hydrometer method. [†]s and [‡]ls are sand and loamy sand, respectively.
[b] Organic Matter (OM) related in cmol (+) kg^{-1}, Walkley-Black Method (Council of Soil Testing and Plant Analysis, 1980).
[c] Cation Exchange Capacity (CEC) related in cmol kg^{-1}, Exchangeable, 0.05 *M* HCl in 0.05 *M* H_2SO_4 extract (Council of Soil Testing and Plant Analysis, 1980).

The phytotoxic action of Be largely depends on soil pH. The addition of 2 mg Be kg^{-1} to acid soils was toxic to mustard (*Sinapis alba* L.) while the same addition to calcareous soils was not toxic (Williams and Le Riche, 1968). Additionally, soil concentrations of Ca, Mg, and P, as well as cation exchange capacity, suppressed Be toxicity in several crop plants (Romney and Childress, 1965).

Recently, fly ash has been proposed as an amendment for agricultural soils (Carlson and Adriano, 1993). However, this could result in substantial addition of Be, since it has been reported that fly ash may contain as much as 100 mg kg^{-1} Be (Adriano, 1986). The phytoavailability of Be is poorly understood and our knowledge of its environmental biogeochemistry is limited. Additionally, little research has been conducted on Be toxicity on southeastern U.S.A. sandy loam soils. Therefore, investigations of the potential effects of such additions on the Be status of plants and soils are needed. The objective of this study was to assess the effects of soil-applied Be on the growth and Be content of soybean plants grown in two sandy loam soil types under limed and unlimed conditions.

2. Materials and Methods

The Ap horizons of a Blanton sand (Loamy, siliceous thermic Grossarenic Paleudult and an Orangeburg loamy sand (Fine-loamy, siliceous, thermic Typic Paleudult) were collected from both limed and unlimed sites on the Savannah River Site, Aiken, SC. The soils were air-dried and passed through a 2-mm sieve to remove gravel and coarse debris. Some chemical and physical characteristics of the two soils were determined (Table I). Treatments were arranged in a factorial design with 2 soil types (Blanton and Orangeburg), 2 soil treatments (limed and unlimed) and 5 Be concentration (0, 25, 50, 100 and 150 mg Be kg^{-1} soil). These relatively high levels of Be were selected to assess the effects of Be on soybeans after

ɔng-term application of fly ash to soils. Each treatment was replicated 4 times, for total of 80 pots. Beryllium was added to the soils by first dissolving $BeSO_4$ in ɛionized distilled water, then spraying the solution onto each pot containing six nd one-half kg air dried soil. The following amounts of nutrients were added (on per hectare basis): 67.2 kg N, 49 kg P, 93 kg K, 6.7 kg Mn, 11.2 kg Zn, and 1.1 g B. The soil was then thoroughly blended in a V-shaped blender. The bottom of ach 7-kg polyethylene pot was lined with gravel; the pots were then filled with ix and one-half kg of treated soil. The soils were permitted to equilibrate for 21 lays prior to planting. Soybean [*Glycine max* (L.) Merr.] seeds were planted at ı rate of 9 seeds per pot. After 2 weeks the plants were thinned to 5 plants per ɔot. The day and night temperatures of the greenhouse were maintained at 30 ± 3 °C and 25±3^{0}C, respectively. No artificial lighting was used since natural day engths, approximately 15 hr, during the course of the study were adequate. The ıbove ground portions of the plants were harvested 60 d after planting.

The harvested tissues from each pot were carefully rinsed in four successive deionized distilled water baths, oven-dried at 70^{0}C to constant weight, weighed to the nearest 0.01 g, and ground in a wiley mill to pass a 22 mesh (841 um) sieve. The dried samples were wet-ashed in a nitric-perchloric acid mixture (Council on Testing and Plant Analysis, 1980) and analyzed by atomic absorption spectrophotometry for Be (detection limit of <1.00 mg Be kg^{-1}) and inductively coupled plasma for Ca, Mg and P. Any Be values <1.00 mg Be kg^{-1} were corrected by dividing the detection limit by two (Helsel, 1990). This is a technique designed to report conservative values when the raw data is at or near the detection limit. National Bureau of Standard test material (tomato leaves, NBS No. 1573) was analyzed for quality control. Soil samples collected at the time of harvesting from each pot were air-dried, sieved to pass a 2-mm screen, and extracted with a double-acid method (Council on Soil Testing and Plant Analysis, 1980). The soil extracts were analyzed for Be and other cations similar to those done in the plant analyses. The statistics of the experimental design was conducted by using the General Linear Model procedure of the SAS Statistical package (SAS Institute, 1985).

3. Results and Discussion

3.1. Toxicity Symptoms

Within ten days of germination, plants growing in soils treated with various concentrations of Be displayed stunted growth with dark-green leaves and deep purple stems characteristic of Be toxicity (Romney and Childress, 1965). By the time plants were harvested these symptoms became more severe in plants growing in both unlimed soils treated with all four concentrations of Be. The most severe toxicity symptoms, however, were observed in plants grown on the Blanton unlimed soil.

TABLE II

Effect of soil-applied Be on soybean plant biomass and elemental concentrations in the limed and unlimed soils+

Soil	Treat-ment	Be applied mg Be kg^{-1}	Biomass g pot^{-1}	Tissue Be mg Be kg^{-1}	Tissue Ca g Ca kg^{-1}	Tissue Mg g Mg kg^{-1}	Tissue P g kg P^{-}
Blanton[a]							
	limed	0	10.59***	0.25***	10.35***	5.90NS	1.72NS
		25	7.66	1.25	9.40	5.80	1.55
		50	4.70	3.75	8.82	6.62	1.37
		100	2.95	12.50	7.37	6.60	1.22
		150	1.19	30.00	6.55	6.20	1.60
	unlimed	0	5.01***	0.60**	8.17NS	3.62NS	1.20***
		25	2.55	31.33	9.82	3.92	3.20
		50	1.15	51.40	9.15	3.12	3.50
		100	0.80	166.80	7.60	3.15	6.05
		150	0.51	226.97	5.50	3.12	5.37
Orangeburg[b]							
	limed	0	11.63***	0.25***	11.92NS	5.85NS	0.90NS
		25	8.01	15.75	11.37	3.90	0.90
		50	5.05	25.00	12.22	4.17	0.75
		100	3.19	53.50	6.70	4.90	0.75
		150	1.73	72.50	9.52	5.27	0.77
	unlimed	0	7.22***	0.48***	10.35NS	4.12NS	1.22***
		25	3.93	10.72	7.85	4.35	1.32
		50	2.37	34.68	8.67	3.70	2.85
		100	1.15	122.10	8.50	3.35	5.20
		150	0.83	153.28	7.55	4.57	5.27

*Signifance based on a one–way analysis of variance on Soil Be level: ** $P < 0.01$; *** $P < 0.001$ NS not significant.
[a]Blanton soil pH averaged 4.9 for unlimed and 6.5 for limed.
[b]Orangeburg soil pH averaged 5.5 for unlimed and 6.0 for limed.

3.2. BIOMASS

Additions of Be to soils resulted in substantial reductions in plant dry weights on either soil, limed or unlimed (Table II, Figure 1), with biomass reduced by as much as 90% at the highest Be treatment on Blanton unlimed soil. Similar reductions in biomass were observed in the Orangeburg unlimed soil (88.5%). Additions of Be to either type of limed soil also reduced aboveground biomass by 88.8% and 85.0% in the Blanton and Orangeburg soils, respectively. The significant linear correlation between soil Be concentrations (data not reported) and biomass and between plant

TABLE III

Correlation coefficients for added soil Be, soybean plant biomass, and soybean plant tissue Be concentrations

Soil	Treatment	Correlation coefficients		
		Soil Be × biomass	Soil Be × plant Be	Biomass × plant Be
Blanton	Limed	−0.87	0.90	−0.96
	Unlimed	−0.87	0.92	−0.88
Orangeburg	Limed	−0.87	0.97	−0.84
	Unlimed	−0.84	0.94	−0.91

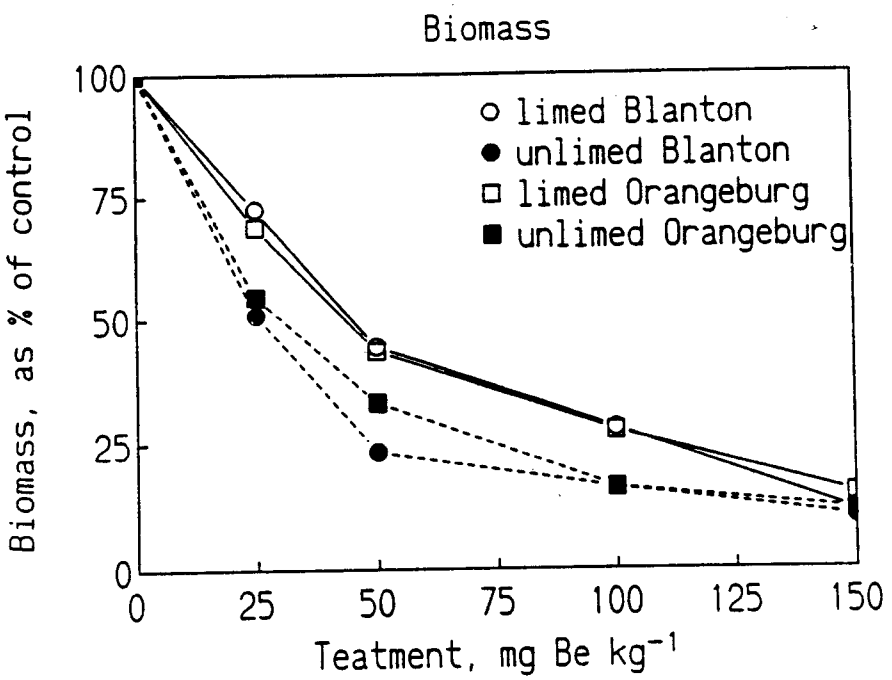

Fig. 1. Effects of soil-applied Be on the biomass of soybean plants grown on the limed and unlimed Blanton and Orangeburg soils. The values given represent means.

tissue Be and biomass further substantiate our findings (Table III). Similar reduction in plant biomass grown in soil amended with various concentrations of Be have been reported by Romney and Childress (1965). Reduced plant growth of collards in an acid soil at amendment rates greater than 150 mg Be kg^{-1} was also found by Kaplan *et al.* (1990). Our results indicate that reductions in biomass were similar in plants grown on either limed or unlimed Blanton or Orangeburg soils.

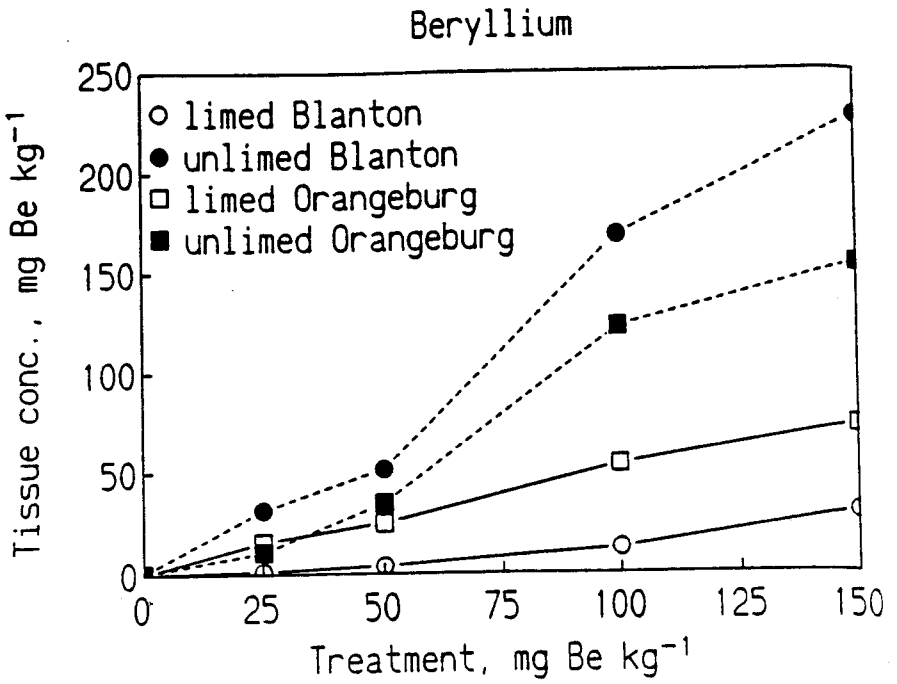

Fig. 2. Effects of soil-applied Be on the Be concentrations in aboveground tissues of soybean plan grown on limed and unlimed Blanton and Orangeburg soils. The values given represent means.

3.3. Tissue Be concentrations

Concentrations of Be in aboveground plant tissue increased with increasing E concentrations in the soil (Table II, Figure 2), with tissue concentrations as high 226 mg Be kg^{-1} observed in the plants grown on the Blanton unlimed soil. Tiss Be concentrations were lower in plants growing on the Orangeburg unlimed so which had the higher pH and clay content. The lower Be concentration in the plar grown on unlimed Orangeburg soil could be in response to unavailability of E due to the adsorption of Be onto the clays. This phenomenon has been reported f selenium in the same soils (Carlson *et al.*, 1991). Although the liming treatme resulted in lower tissue Be concentrations for plants grown in either soil treat with Be, the reduction was more pronounced in Blanton soil. In all soil treatme combinations we calculated significant positive linear correlation between soil E and plant Be concentrations (Table III).

In limed soils Ca, Mg, and P were unaffected by increased tissue Be except (in Blanton soil decreased with increasing tissue Be. Perhaps Be competes with (for absorption sites on the roots or possibly normal Ca absorption is interrupt masking root-absorption sites (Romney and Childress, 1965).

In unlimed soils, Ca and Mg were unaffected by increasing tissue Be. However, P increased significantly with increased Be tissue concentrations in both limed and unlimed Blanton and Orangeburg soils. This agrees with Romney and Childress, 1965 whereby increased concentrations of P found in tissues of Be treated plants was related to accumulation of inorganic P by a feed-back mechanism that would compensate for the inhibition of enzyme systems involved in phosphate metabolism by Be. However these possibilities need further testing.

4. Conclusion

Additions of Be to two acid soils significantly reduced aboveground plant biomass and increased plant Be concentrations. Addition of Be to both unlimed soils had a greater toxic effect on the plants, reducing plant biomass as much as 90% in Blanton unlimed and 88.5% in Orangeburg unlimed soils. Tissue Be concentrations were as high as 226 mg Be kg^{-1} for the Blanton unlimed and 153 mg Be kg^{-1} for Orangeburg unlimed soils. Liming resulted in substantially lower Be tissue concentrations in both soils. These results indicate that contamination of acid, sandy loam soils with Be can result in toxic effects on plants and can increase plant Be concentrations. Furthermore, our results indicate that Be accumulation in plants may be ameliorated by increasing soil pH through liming. This supports the findings of Williams and Le Riche (1968) regarding the effect of liming on Be mobility. It has been reported elsewhere (Adriano, *et al.*, 1982; Adriano, 1986; and Sajwan *et al.*, 1993) that alkaline coal fly ash can be used to raise soil pH, and is especially valuable when used as a companion amendment with sewage sludge (Adriano, *et al.*, 1982) that raises the soil nutrient status and at the same time decreases the bioavailability of heavy metals.

Acknowledgements

This research was supported by contract DE-AC09-76SR00-819 between the U.S. Department of Energy and the University of Georgia's Savannah River Ecology Laboratory. The authors express their gratitude to Dr. D.C. Adriano for his constant encouragement, helpful suggestions and help to carry out this research. Laboratory assistance rendered by Adam Burton, Brett Burton, and Greg Dubose is also greatly appreciated.

References

Adriano, D. C.: 1986, *Trace Elements in the Terrestrial Environment*, Spinger-Verlag, Inc, NY.
Adriano, D. C., Page, A. L., Elseewi, A. A. and Chang, A.C.: 1982, *J. Environ. Qual.* **11**, 197.
Carlson, C. L. and Adriano, D. C.: 1993, *J. Environ. Qual.* **22**, 227.

Carlson, C. L., Adriano, D.C. and Dixon, P.M.: 1991, *J. Environ. Qual.* **20**, 363.
Council on Soil Testing and Plant Analysis: 1980, *Handbook on Reference Methods for Soil Testing*, University of Georgia, Athens, GA.
Everest, D. A.: 1964, *The Chemistry of Beryllium*, Elsevier Publ. Co. NY.
Helsel, D. R.: 1990, *Environ. Sci. Tech.* **24**, 1766.
Horovitz, C. T. and Petrecu, O.: 1984, Beryllium, in *Trans 8th Intl. Cong. Soil Sci.* **4**, 1205.
Kabata-Pendias, A. and Pendias, H.: 1984, *Trace Elements in Soils and Plants*, CRC Press Inc., Buca Raton, FL.
Kaplan, D. I., Sajwan, K. S., Adriano, D. C. and Gettier, S.: 1990, *Water, Air, and Soil Pollut.* **53**, 203.
Korte, N. E., Skopp, J., Fuller, W. K., Niebla, E. E. and Alsii, B. A.: 1976, *Soil Sci.* **122**, 350.
Peterson P. J. and Girling, C. A.: 1981, 'Other Trace Metals', in N. W. Lepp (ed.), *Effects of Heavy Metal Pollution on Plants. Vol.1: Effects of Trace Metals on Plant Function*, Applied Science Publ., London, p. 212.
Romney, E. M. and Childress, J. D.: 1965, *Soil Sci.* **100**, 210.
Sajwan, K. S., Ornes, W. H. and Youngblood, T. V.: 1993, *J. Environ. Sci. Health.* (in press)
SAS Institute, Inc.: 1985, *SAS Users Guide*, (Statistics, version 5. SAS Institute, Inc., Cary, NC).
Williams, R. J. B. and Le Riche, H. H.: 1968, *Plant Soil* **29**, 317.
Zubovic, P.: 1969, Coal Emissions of Beryllium, in *Proc. of Beryllium Conference*, MIT, Cambridge, MA.