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EFFECTS OF BERYLLIUM IN PLANTS AND SOIL

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Industrial use of beryllium has increased markedly in recent years. Biologically this element is of interest because of its high toxicity and the industrial hazard it presents to those workers susceptible to beryllium effects. Clinical manifestations include bronchitis, dermatitis, conjunctivitis, rhinitis, and pneumonitis, terminating in death in some cases (7, 14, 19). Reports on the effects of beryllium in plant life are few but they include evidence of effects that are beneficial and effects that are detrimental (3, 6, 10, 12, 13, 17).

Increased use of beryllium in space research and atomic energy projects has increased the possibility of contaminating effluent streams, ground water, soil, and atmospheric surroundings with both stable and radioactive beryllium. More information is needed to evaluate hazards arising from beryllium dispersed into natural environments.

The purpose of the investigation reported herein was to study the effects of beryllium in crop plants and the reactions of beryllium in soil.

MATERIAL AND METHODS

Alfalfa, barley, lettuce, and pea plants were grown for periods of 54, 20, 28, and 24 days, respectively, on aerated nutrient solution treated with $BeCl_2$ at levels of 0, 2, 4, 8, and 16 ppm. Be. The nutrient solution contained 2.25×10^{-3} *M* KNO_3, KH_2PO_4, and $MgSO_4$; 1×10^{-3} *M* NH_4NO_3; and 3.75×10^{-3} *M* $(CaNO_3)_2$. Micronutrient levels were 0.3 ppm. B, 0.5 ppm. Zn, 0.01 ppm. Mo, and 1.0 ppm. Fe (EDDHA). Nutrient solution was renewed at 4-day intervals and the pH was maintained at 5.3 ± 0.2. Three replicates of each treatment were grown in controlled environment of 75°F. air temperature, 60 per cent relative humidity, and 1000 foot-candles light intensity on a 12-hour-light to 12-hour-dark cycle. Additional barley, bean, sunflower, and tomato plants were grown for 30 days on nutrient solution containing the Be7 isotope in order to study the distribution of Be among the different plant parts.

Bean, wheat, and ladino clover plants were grown on 4-kg. lots of Aiken, Hanford, and Vina soil treated with Be at levels equivalent to 0, 2, 4, 8, and 16 per cent of the soil cation-exchange capacity. Properties of these soils are listed in table 1. Beryllium treatments were applied by dispensing appropriate solutions of $BeSO_4$ onto the soil surface along with $(NH_4)_2HPO_4$ fertilizer solution equivalent to 200 pounds N and 221 pounds P an acre. Upon drying, the friable crust formed at the soil surface was pulverized and before potting the soil was mixed 30 minutes in a McLellan batch mixer. Each treatment had four replicates in which the moisture level was adjusted to field capacity 10 days before planting. Beans were harvested at 35 days growth, wheat at 75 days, and 3 cuttings of ladino clover were harvested at 105, 165, and 225 days after planting.

The harvested plant samples were dried at 70°C. and ground by a Wiley mill through a 40-mesh screen. One-gram samples were ashed at 530°C. and dissolved in 2 *N* HCl. Upon removing Si, the samples were passed through cation-exchange resin (Dowex 50W-X4) to separate cations from anions. Phosphorus was measured in the filtrate by photoelectric spectrophotometry; Ca, Mg, Na, and K cations were eluted from the resin and measured by flame spectrometry. Stable Be was measured by emission spectrograph and Be7 isotope was radioassayed by deep-well scintillation counting.

Equilibrium batch methods were used to study the reactions of Be7 in Hanford and Vina soil, kaolinite, and bentonite. Five-gram soil or 1.0-g. clay samples were placed in polyethylene centrifuge tubes and treated as follows:

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TABLE 1

Properties of experimental soils and clays

Soil or Clay	pH	Organic Matter	CEC	Exchangeable Cations				Texture*	Predominant Clay*
				Ca	Mg	K	Na		
		%	me./100 g.						
Hanford	6.72	1.95	5.40	4.42	0.56	0.11	0.01	s.l.	I
Vina	6.45	1.82	23.74	20.00	4.14	4.56	0.20	l.	MK
Aiken	5.65	0.76	13.15	5.33	1.46	2.60	0.08	s.c.l.	K
Bentonite	8.20	—	98.30	69.00	16.60	0.25	1.32	c.	M
Kaolinite	4.30	—	5.50	3.60	0.52	0.09	0.57	c.	K

* s.l. = sandy loam; l. = loam; s.c.l. = sandy clay loam; c. = clay; I = illite; M = montmorillonite; and K = kaolinite.

Replacement: A known amount of Be7 in 20 ml. of distilled water was added to dry soil or clay samples and the suspensions agitated frequently during a 24-hour equilibration period. Stable Be, Ba, Ca, or Mg treatments varying from 0.1 to 3.0 me. were added and the volume of suspension adjusted to 25 ml. Following another 24-hour agitation-equilibration period, the suspensions were centrifuged and portions of the supernatant liquid were radioassayed for Be7 content by deep-well scintillation counting.

Competition: Be7 was added to solutions containing stable Be, Ba, Ca, or Mg in amounts ranging from 0.1 to 3.0 me., which were subsequently added to samples of dry soil or clay. These suspensions were given the 24-hour agitation-equilibration treatment and then centrifuged and radioassayed.

Where Ba, Ca, or Mg was the replacing or competing cation, 0.1 me. of stable Be accompained the Be7 dose. Although tests showed that batch equilibrium was reached within 15 hours, for convenience of procedure all samples were equilibrated 24 hours.

RESULTS

Beryllium levels greater than 2 ppm. in nutrient solution significantly reduced the growth of higher crop plants (fig. 1). Levels of Be equivalent to 4 per cent of the cation-exchange capacity of soils significantly reduced the yield of beans, wheat, and the first cutting of ladino clover. The growth-inhibiting effects of Be became progressively more severe in the second and third cuttings of clover at Be levels greater than 2 per cent of the soil cation-exchange capacity.

Figure 2 illustrates the typical growth-inhibiting effects of Be in plants grown on nutrient solution and soil. Visual signs of damage occurred in root tissue within 1 week after the seedlings were transferred to nutrient solution containing 4 ppm. Be. The effects of Be were similar in each plant species. Roots turned brown and ceased normal elongation. Unusually large numbers of stubby rootlets emerged from pudgy, secondary roots, and the root tips developed a swollen appearance. Both roots and foliage became stunted, and the foliage turned a darker blue-green color than normal. No chlorosis nor mottling of foliage occurred in Be-treated plants; at the higher Be levels, however, the leaf size was conspicuously dwarfed. Increased stooling occurred in the small-grain plants, and earlier flowering and senescence was induced in the legume plants treated with Be.

Table 2 shows the relative distribution of Be7 isotope among parts of different plants grown on nutrient solution. Very little Be7 was translocated to the foliage of plants compared to that which was retained by roots. Some of the Be7 might have been adsorbed on the outer root surface even though the roots were washed in 0.1 *N* HCl and rinsed in distilled water when harvested. Leaves concentrated higher levels of Be7 than did stems. In beans and barley the Be7 content was lowest in fruit tissue: fruit was not produced from the other plant species during the period of treatment.

The influence of Be solubility on plant growth was studied in Vina soil treated with Be salts of varied solubility at levels equivalent to 0, 10, and 20 per cent of the soil cation-exchange capacity. Insoluble $BeCO_3$ and BeO did not

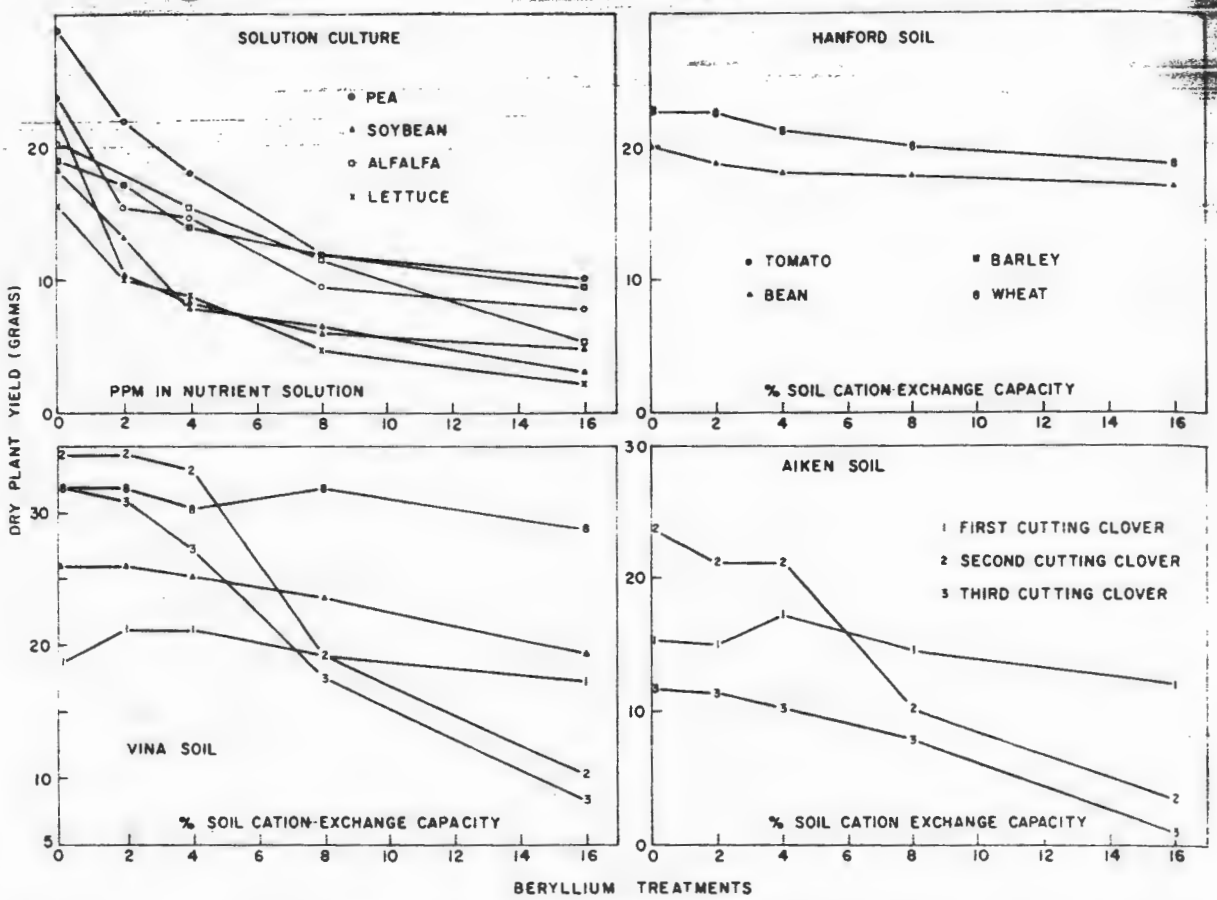

FIG. 1. Effects of Be on the dry yield of plants grown on nutrient solution and soil

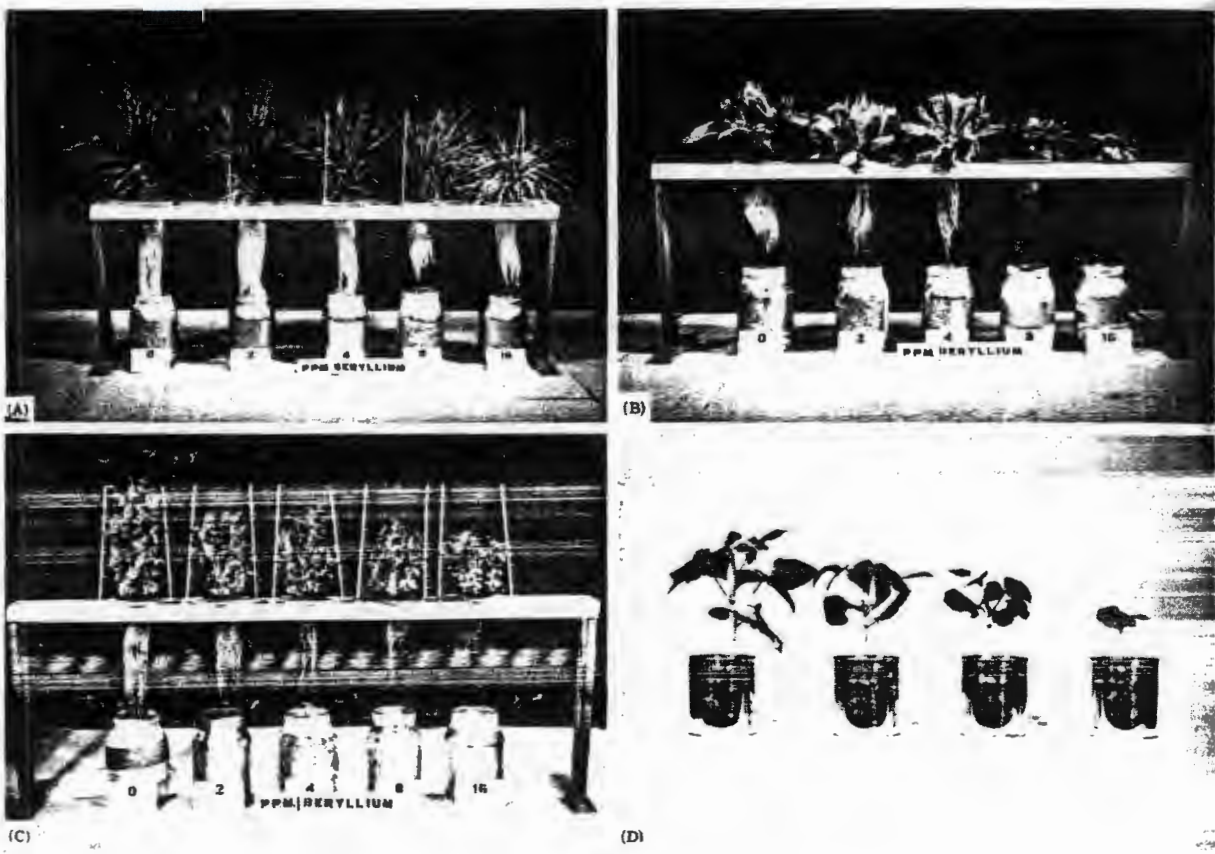

FIG. 2. Effects of Be on top and root growth of plants. Levels of Be in soil were 0, 4, 8, and 16 per cent of the cation-exchange capacity.

influence growth of beans; soluble $Be(NO_3)_2$ and $BeSO_4$, however, significantly reduced plant growth at each treatment level (table 3). These results indicate that soluble forms of Be must be present in soil in order for Be to inhibit plant growth.

TABLE 2

Distribution of Be7 in parts of plants grown on nutrient solution

Plant Part	Be7 Content* (cpm./g.)
Barley	
Root	66,968
Leaf and stem	476
Grain	212
Bean	
Root	43,670
Stem	566
Leaf	784
Fruit	146
Tomato	
Root	50,000
Stem	243
Leaf	458
Sunflower	
Root	29,717
Stem	363
Leaf	910

* Air-dry tissue from single plants.

TABLE 3

Yield of beans grown on Vina soil treated with Be salts differing in solubility

Be Applied to Soil	Solubility of Be Salt	Yield Dry Plant Tops†
% CEC	g./100 ml. cold water	g.
BeO	2.3×10^{-5}	
0		8.76
10		8.72
20		8.64
$(BeO_4)\cdot CO_2\cdot 5H_2O$	Insoluble	
0		8.68
10		8.36
20		8.30
$BeSO_4\cdot 4H_2O$	42.5	
0		8.81
10		7.03*
20		5.92**
$Be(NO_3)_2\cdot 3H_2O$	Very soluble	
0		8.31
10		6.09**
20		2.97**

† Mean of four replicates. Significant at P = 0.05 (*); Significant at P = 0.01 compared to 0-Be treatment (**).

TABLE 4

Effects of Be on plant uptake of Ca, K, Mg, Na, and P from nutrient solution

Be Level in Nutrient Solution†	Nutrient Element in Dry Plant Tissue‡					
	Be	Ca	K	Mg	Na	P
ppm.	µg./g.	mg./g.				
Alfalfa (l & s)						
0	0	9.5	27	2.1	0.21	3.4
4	5.3	7.2	23	2.1	0.21	4.0
8	21.8	5.5*	22	1.8	0.23	4.7*
16	27.6	4.8*	26	1.6*	0.22	5.2*
Barley (f)						
0	0	5.8	60	3.1	0.69	11.0
2	8.6	5.4	70	3.2	0.73	13.3*
4	11.3	5.8	65	3.5	0.79	14.8*
8	22.8	5.2*	57	3.5	0.84	15.0**
16	68.0	4.8*	52	3.2	0.76	20.3**
Barley (r)						
0	0	9.8	46	3.3	1.4	15.1
2	110	9.7	46	2.8	1.1	29.2*
4	775	4.3**	51	1.6*	0.9	36.2**
8	1130	3.4**	50	1.7*	1.1	37.0**
16	2030	1.8**	48	1.3**	1.1	49.0**
Pea (l & s)						
0	0	9.8	39	2.8	0.40	6.3
2	15.1	8.5	39	2.6	0.46	6.6
4	23.0	7.2*	40	2.5	0.48	6.8
8	31.4	6.2**	37	2.6	0.43	7.6*
16	75.3	6.4**	41	2.7	0.49	8.6*
Lettuce (f)						
0	0	15.6	55	5.0	0.60	9.1
2	23.7	12.2*	48	4.5	0.58	10.1
4	33.0	11.4**	53	4.6	0.64	10.7
8	37.0	11.8*	46	4.9	0.78	11.7*
16	55.0	11.2**	51	4.0*	0.63	13.4**

† l = leaves; s = stems; f = foliage; and r = roots.

‡ Mean of four replicates. Significant at P = 0.05 (*); significant at P = 0.01 compared to 0-Be treatment (**).

Typical results of Be treatments on plant uptake of some essential nutrients are shown in tables 4 and 5. Increased levels of Be in nutrient solution and soil progressively increased plant uptake of P and decreased plant uptake of Ca. These effects of Be on Ca and P uptake persisted over an extended cropping period, according to results from the successive cuttings of ladino clover (table 5). In some cases Mg uptake was reduced by high levels of Be; this effect, however, was more apparent in root tissue than in foliage. Plant uptake of Na, K, Fe, and Mn was not influenced significantly by Be treatments. Uptake of Be was greater from Aiken soil than from Vina or Hanford soils.

Results of batch equilibrium studies of Be

TABLE 5

Effects of Be on Ca, K, Mg, Na, and P uptake by ladino clover from Aiken and Vina soil

Be Applied to Soil	Nutrient Element in Dry Plant Tissue†					
	Be	Ca	K	Mg	Na	P
% CEC	μg./g.	mg./g.				
Aiken s.c.l.	*1st. cutting*					
0	0	17.3	28	3.7	0.29	1.5
2	1.3	16.2	27	3.6	0.33	1.4
4	1.7	18.2	31	4.2	0.29	1.6
8	8.9	13.1**	27	3.5	0.25	1.8*
16	18.7	12.5**	29	3.8	0.26	1.9*
	3rd. cutting					
0	0	13.8	11	4.6	0.36	0.6
2	1.6	13.3	10	4.2	0.41	0.6
4	2.1	13.5	13	4.1	0.41	0.9*
8	15.2	11.5*	11	4.1	0.42	1.0*
16	22.7	8.3**	12	4.8	0.38	1.6**
Vina l.	*1st. cutting*					
0	0	18.1	26	3.7	0.28	2.2
2	0.6	18.2	26	3.8	0.31	2.7*
4	1.0	18.1	29	3.9	0.31	2.7*
8	2.9	17.3	30	3.9	0.30	2.9**
16	6.8	15.8**	28	4.2	0.26	3.0**
	3rd. cutting					
0	0	19.8	19	4.3	0.58	2.6
2	0.8	14.4**	17	4.2	0.52	2.3
4	1.7	13.7**	16	4.0	0.46	2.5
8	2.4	13.5**	17	4.2	0.53	3.2
16	5.6	13.0**	2	4.5	0.46	4.5**

† Mean of four replicates. Significant at P = 0.05 (*); significant at P = 0.01 compared to 0-Be treatment (**).

reactions in soil and clay systems are shown in figure 3. Since interactions of Ba, Ca, and Mg cations with Be were similar, only data from the Mg tests are illustrated. The Be7 isotope was strongly adsorbed by Hanford and Vina soil and bentonite, but not by kaolinite. Magnesium, Ba, or Ca did not effectively replace the Be7 already adsorbed by soil or bentonite, even at high concentrations. On the other hand, these divalent cations effectively competed with Be7 for the sorption sites in soil, but not in bentonite, under batch-equilibrium conditions. In kaolinite clay these divalent cations competed with Be7 for sorption sites and also replaced the adsorbed Be7. Stable Be replaced Be7 and competed with Be7 in each of the exchange systems tested, as should be expected of this stable element and its isotope.

Additional tests on 300-g. lots of Be-treated soil in leaching columns showed that virtually no Be was extracted from Hanford or Vina soil by 600 ml. of distilled water and 0.1 *M* $BaCl_2$, $CaCl_2$, or $MgCl_2$. Five successive leachings with 300 ml. increments of 0.1 *N* HCl were required to extract significant amounts of Be from the treated soil. Equilibration-extraction tests showed that Be effectively displaced Ca, Mg, and Sr from sorption sites; Fe and P adsorbed by Hanford and Vina soil, however, were not influenced by Be treatments (table 6).

DISCUSSION

Research of the literature disclosed some areas in which results from this study support the findings of other investigators. Gerola and Gilardi (3) observed that the apical roots of pea plants increased in weight and absorbed more P when grown in Knop's solution containing 10^{-3} *M* $Be(NO_3)_2$. We found that Be induced stubby root growth and swelling of root tips in each plant tested. Mazé and Mazé reported that small amounts of $BeCl_2$ had a slight beneficial action on growth of corn in liquid media. On several different occasions we detected what appeared to be growth stimulation in bean seedlings from low levels of Be in soil and nutrient solution; this effect, however, disappeared as the plants developed. Haas (4) reported that Be induced mottling in leaves of lemon trees, but we observed no mottling or chlorosis caused by Be treatments. Hoagland (5) found that Be inhibited the growth of tomato plants in nutrient solution containing more than 2 ppm. Be at pH levels below 9.0. The affected plants had dark green leaves and deep purple stems, which were characteristic of the effects we observed.

In studies involving lower forms of plant life, Feardon (2) noted that Be was a frequent constituent but not necessarily an essential micronutrient of the lichens *Parmelia saratilis* and *Xantharia paretina*. In tests with *Aspergillus niger*, Steinberg (17) found that Be was the only element periodically related to Mg that could stimulate growth of these fungi. Lebedeva (10) found that Be inhibited growth of an aquatic plant (*Lemma minor*) at concentrations above 8 ppm. in the nutrient media.

Several workers have investigated biochemical effects of Be in plants and report that Be in-

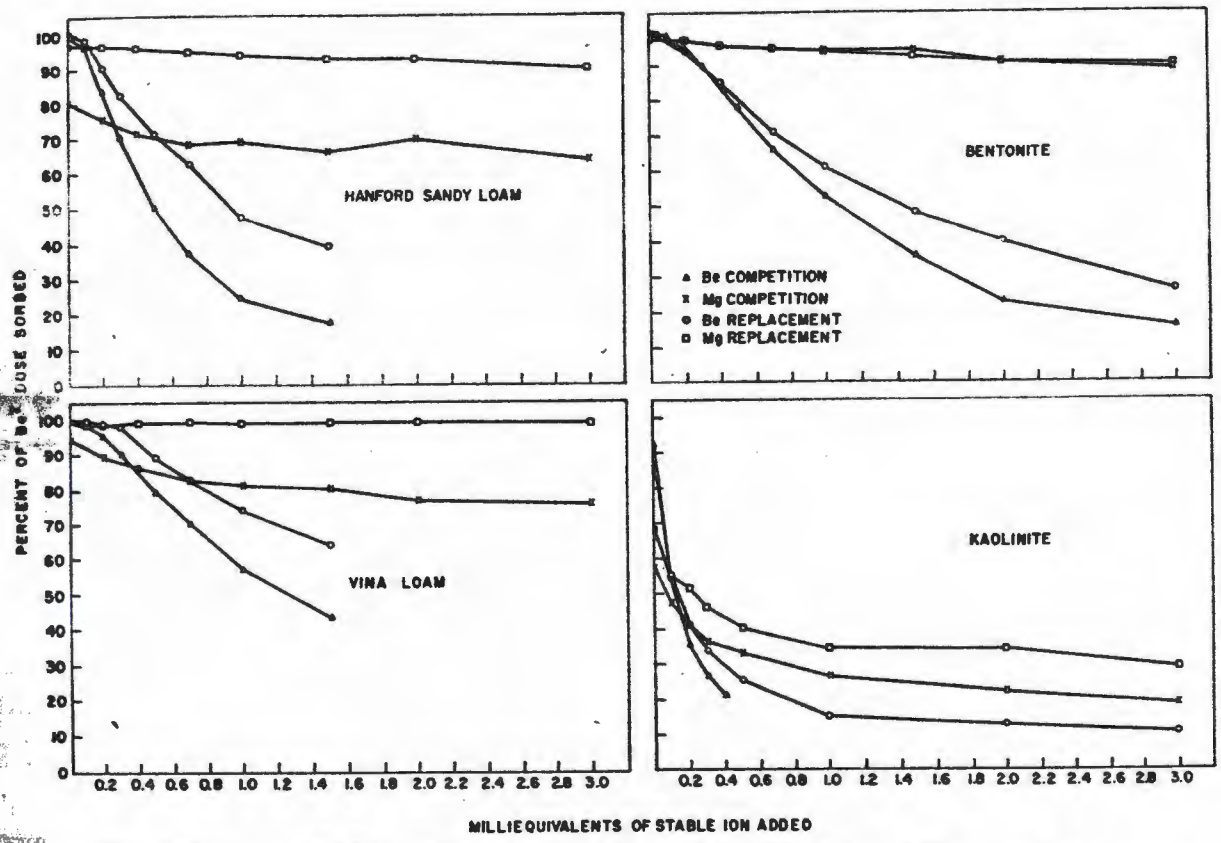

Fig. 3. Sorption of Be7 by soil and clay as influenced by stable Be and Mg applied as competing or replacing cations.

hibited the normal activity of certain enzyme systems, including urease and several of the phosphatases involved in metabolic utilization of P (6, 8, 11, 15). In some separate tests, we observed that Be inhibited the R5P carboxylation sequence and also the PEP carboxylase reaction when more than 1 μM of $Be(NO_3)$ was present in the reaction mixture.

Since the reactions of Be in nutrient solution and soil were strongly influenced by pH, some indirect factors had to be considered in experiments with this element aside from its health hazard. The small, divalent Be cation had a strong tendency to form complexes and hydrolyze to form colloidal aggregates when (1) the pH was raised above 5.5. This occurred with phosphates in our nutrient-solution experiments, making it necessary to hold the pH close to 5.3 and to renew the nutrient solution frequently. We observed that the growth-inhibiting effects induced by a given level of Be became less severe as the solution pH was raised above 6.0 because of the precipitation of Be-phosphate. Hoagland (5) reported evidence of Be-stimulated growth in green algae (*Chlorella pyrenoidosa*) cultured for 70 hours on nutrient solution containing low concentrations of Mg at pH levels above 11.2. We believe this apparent Be-stimulation at high pH probably resulted from the precipitation of Be and also some essential nutrient elements, thereby creating a more favorable mineral balance in the nutrient media.

Our findings show that Be must be soluble in order to inhibit the growth of higher plants, and that direct contact of plant roots with insoluble Be salts is not a significant mechanism involved in growth inhibition. The reduced content of Ca and Mg in tissues of Be-treated plants indicates that Be either competes with those cations for root-absorption sites, or in some way disrupts the normal influx of Ca and Mg by damaging or clogging the absorption sites. The increased concentrations of P found in tissues of Be-treated plants might be evidence of inorganic P accumulation through feed-back mechanisms seeking to overcome the inhibitive effects of Be on enzyme systems involved in phosphate metabolism. Additional work is needed to elucidate the severe morphological damage that was induced by Be in roots of higher plants.

TABLE 6

Ca, Fe, Mg, P, and Sr in water extracts from Be-treated Aiken and Vina soil

Be Applied to Soil	Nutrient Element in Soil Extract*				
	Ca	Fe	Mg	P	Sr
% CEC	*ppm.*				
Aiken sandy clay loam					
0	15	1.6	4.2	3.0	0.3
2	16	2.1	5.1	3.7	0.3
4	26	1.7	6.0	3.0	0.5
8	36	1.1	6.2	3.6	0.9
16	57	1.9	7.3	3.5	1.6
Vina loam					
0	60	1.0	20	12	1.0
2	78	0.7	28	9	1.3
4	120	0.5	34	11	2.0
8	162	0.6	46	10	2.8
16	252	1.1	76	11	4.2

* Extract from a suspension of 5 g. soil in 25 ml. distilled water allowed to stand 48 hours with frequent shaking.

From the practical viewpoint, Be could become injurious to higher plants if high levels of Be were dispersed onto the soil or into ground and irrigation water where roots would come into contact with soluble Be. Sources of such contamination are those industrial facilities and research installations which produce waste effluents containing Be. Aside from creating radiation safety problems, any radioactive Be7 that might be dispersed into the natural environment probably would contain only insignificant concentrations of stable Be. The levels of Be which naturally occur in most cultivated soils would not be expected to damage crop plants because of the physical and chemical nature of this element. The principal Be-bearing primary mineral is beryl, which contains only about 5 per cent Be. Inasmuch as Be has a small ionic radius, a low coordination number, and is strongly electropositive, very few crystal structures will permit high concentrations of Be. Most sedimentary rocks contain only small quantities of Be, which are subsequently dissipated by the processes of weathering. Estimates of the Be content of the earth's lithosphere range from 0.0006 to 0.002 per cent (9, 16, 18). Beryllium shows a strong chemical resemblance to Al, which enables it to undergo isomorphic substitution in secondary clay minerals in addition to cation-exchange reactions. Beryllium is strongly fixed in soils and clays and may displace other divalent cations that share common sorption sites in the exchange complex.

SUMMARY

The effects of soluble Be were investigated in plants and soil. Beryllium inhibited the growth of several different crop plants at levels above 2 ppm. in nutrient solution and 4 per cent of the cation-exchange capacity in soil. The visible effects of Be in plant tissues are described. Although roots concentrated high levels of Be, this element was not readily translocated to other plant parts. Leaves accumulated more Be than did stems or fruit tissue. The uptake of P was increased and Ca and Mg uptake was decreased in Be-treated plants. Insoluble forms of Be did not inhibit plant growth.

Reactions of Be in nutrient solution and soil were influenced by pH. Beryllium was strongly adsorbed by soils and bentonite but not by kaolinite. Previously adsorbed Be was not effectively replaced by Ba, Ca, or Mg; these cations, however, competed with Be for sorption sites under batch-equilibrium conditions. Applications of Be to soil displaced some adsorbed divalent cations, including Ba, Ca, Mg, and Sr.

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