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Source DD 311
0246

COMMUN. IN SOIL SCIENCE AND PLANT ANALYSIS, 8(9), 757-764 (1977)

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NICKEL PHYTOTOXICITY IN RELATIONSHIP TO SOIL pH MANIPULATION AND CHELATING AGENTS

KEY WORDS: Nickel, heavy metal toxicity, chelating agents, soil pH iron chlorosis, spectrographic analysis, plant analysis

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ABSTRACT

The main objective of this study was to ascertain effects of some edaphic factors on the uptake and influence of Ni on plant growth since Ni is a common trace element contaminant as well as an important component of serpentine soils. Corn (Zea mays L. inbred Ys_1/Ys_1) was grown in Yolo loam soil amended to give soil pH values of 4.2, 5.6, 7.5, and 8.2. A level of 100 μg Ni/g soil was not toxic to the corn. Shoot concentrations of Ni increased as soil pH decreased for both application rates of Ni. A level of 250 μg Ni/g soil decreased yields more at soil pH below 7 than above 7. Iron, Zn, and Mn levels in shoots did not appear to be directly related to the Ni applications although Fe levels tended to increase as a result of smaller plant size. PI54619-5-1 soybeans (Glycine max L.) were grown in soil at two different pH values (with and without $CaCO_3$) and with and without a level of 1000 μg Ni/g added as the sulfate and thoroughly mixed with the soil and equilibrated for 1 month prior to transplanting the soybeans with and without application of a chelating agent, DTPA (diethylene triamine pentaacetic acid), commonly used to correct Fe deficiency in plants. Plants were killed in the soil of pH 6.2 when the 1000 μg Ni/g soil was added. The pH 7.2 soil decreased the toxicity of Ni. The DTPA had little effect on yields, but increased the amount of Ni in plants. Nickel decreased the Fe, Zn, Cu, and Mn concentrations of the plants. Stems contained less Ni than did leaves. In another experiment, EDTA (ethylenediamine tetraacetate) greatly increased Ni concentrations in bush beans (Phaseolus vulgaris L. C.V. Improved Tendergreen) and in barley (Hordeum vulgare L. C.V. Atlas 57) grown in Yolo loam soil, and simultaneously increased Fe concentrations. Lime ($CaCO_3$ or $MgCO_3$) decreased toxicity of Ni in bush beans. DTPA increased Ni transport in bush beans and increased the ratio of Ni in leaves to that in stems at soil pH 7.5 and 8.2, but not at pH 4.0 and 5.8.

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INTRODUCTION

Nickel is one of the constituents of serpentine soils which causes depressed plant growth[5 10 11 12 13]. Nickel toxicity in plants has been known for some time[1 11]. Nickel accumulation in soil from manmade sources has caused adverse effects on plants[4]. Nickel and other heavy metals causing these problems are believed to be able to induce Fe chlorosis in plants[8 13 15 17 18]. It is well known that $CaCO_3$ or $NaHCO_3$ can decrease Ni uptake and toxicity[6 7 9]. The present investigations were made to explore interactions of relatively high levels of Ni on leaf concentrations of several essential micronutrients, including Fe at different soil pH values. Another purpose was to explore effects of synthetic chelating agents on the magnitude of Ni toxicity and Ni uptake by plants.

MATERIALS AND METHODS

The Fe inefficient Ys_1/Ys_1 corn inbred (Zea mays L.)[2 3 11] was grown for 19 days in Yolo loam soil amended to give different soil pH values (4.2 with 1/2% S, 5.6 for control, 7.5 with 2% $CaCO_3$, and 8.2 with 1% $MgCO_3$ + 1/2% $CaSO_4$). Prior to transplanting of previously germinated plants, $NiSO_4$ at rates of 0, 100, and 250 μg Ni/g soil, was blended with the soil. The soils received 200 μg N/g as NH_4NO_3 and were maintained at soil moisture near -1/3 bar. After 14 days, the plants were separated into upper shoot and lower shoot, washed in 1/10 N HCl and deionized water, dried, and prepared for analysis by emission spectrography[18].

Iron-inefficient PI54619-5-1 soybeans (Glycine max L.) were grown for 12 days in Yolo loam soil (pH 5.8) and in the same soil with 2% $CaCO_3$ (pH 7.2) with and without the chelating agent DTPA (diethylene triamine pentaacetate), 50 μg/g soil of the acid form, but as the Na salt. Also applied uniformly was 1000 μg Ni/g soil as $NiSO_4$ before mixing and the soil was then equilibrated at -1/3 bar soil moisture for 1 month with eventual leaching before transplanting plants. Control soils were set up without Ni. Nitrogen at 200 μg N/g soil as NH_4NO_3 was added to each pot. Pots were 500 g soil, 2 plants were in each pot, and there were four replicates. Germinated seedlings were transferred to each pot 4 days before addition of DTPA. Leaves and stems were washed in 1/10 N HCl and deionized water, dried, weighed, and prepared for analysis by emission spectrography.

Bush beans (Phaseolus vulgaris L. C.V. Improved Tendergreen) and barley (Hordeum vulgare L. C.V. Atlas 57) were grown for 28 days in Yolo loam soil (500 g/pot) with different levels of Ni (0, 25, 100, and 400 μg/g soil) as $NiSO_4$ mixed throughout the soil, and of EDTA and $CaCO_3$. Plants were grown and handled as above.

Bush beans were grown for 16 days in Yolo loam soil which had been variously amended to give four different pH values (4.0 with 1/2% S, 5.8 control, 7.5 with 2% $CaCO_3$, 8.2 with 1/2% $MgCO_3$ + 1/4% $CaSO_4$). Nickel as $NiSO_4$ at 0, 100, and 250 μg/g soil was mixed uniformly into the soil before transplanting of previously germinated plants. Growth procedures were as in other experiments and the plants were harvested and handled as above.

The cultural methods used were such as to allow growth without macronutrient deficiencies.

RESULTS AND DISCUSSION

In corn the 100 μg Ni/g soil was not toxic, but at 250 μg Ni/g soil the lowest three soil pH values resulted in Ni toxicity (Table 1). The 250 μg Ni/g soil also decreased yields more at pH 4.2 (72%) and at pH 5.6 (81%) than at pH 7.5 (47%) and at pH 8.2 (4%). More Ni was present in the upper shoots than in the lower shoots and the concentration decreased with increasing pH in both upper and lower shoots. Nickel did not depress Fe concentrations in shoots in this Fe-inefficient corn inbred. Iron levels tended to increase probably partly as result of smaller plant sizes. The Fe-inefficiency of this corn inbred may be due to excess Zn and Mn uptake.

Soybean plants in soil pH 5.8 were killed by the 1000 μg Ni/g soil with or without DTPA (Table 2). The $CaCO_3$ (pH 7.2) greatly decreased the Ni toxicity to a mild chlorosis and mild yield depression. Although DTPA increased the level of Ni in both leaves and stems, it did not result in further

TABLE 1

Effect of Ni Additions at Four Different Soil pH Values on Ys_1/Ys_1 corn (dry weight basis).

Soil pH	Vegetative yield	Ni lower shoot	Ni	Fe	Mn	Zn
			upper shoot			
	g/shoot	μg/g	μg/g			
		No Ni added to soil				
4.2	0.99	0	2	42	187	32
5.6	1.18	0	2	32	82	39
7.5	1.21	0	2	28	44	32
8.2	0.48	0	2	72	91	69
		100 μg Ni/g soil				
4.2	0.96	24	58	41	159	50
5.6	1.24	6	24	38	85	50
7.5	1.16	3	4	40	45	39
8.2	0.44	4	5	148	85	45
		250 μg Ni/g soil				
4.2	0.26	106	272	99	150	86
5.6	0.24	71	300	71	97	62
7.5	0.61	7	30	80	49	57
8.2	0.46	4	10	96	80	78
LSD .05	0.22	6	12	24	20	10
LSD .01	0.29	9	15	32	26	13

TABLE 2

Leaf Yield and Microelement Concentrations in Leaves and Stems of Soybean Plants Grown in Soil of Different pH Values and with and without 1000 μg/g of Ni (dry weight basis).

Ni treatments	Soil pH	Yield	Fe	Zn	Cu	Mn	Ni
		mg/plant	μg/g				
		Leaves, without DTPA					
-	6.2	160	84	52	10.3	50	3.1
-	7.2	178	76	59	9.3	66	2.9
+	6.2	D	D	D	D	D	D
+	7.2	121	45	36	5.4	16	39.6
		Leaves, with DTPA					
-	6.2	173	91	74	9.4	58	3.3
-	7.2	157	52	72	10.7	74	4.2
+	6.2	D	D	D	D	D	D
+	7.2	129*	47	39	5.1	26	69.4
LSD .05		40	6.3	10.3	3.3	6.0	5.6
LSD .01		53	8.4	13.7	4.4	7.9	7.3
		Stems, without DTPA					
-	6.2	67	49	39	7.5	15	2.7
-	7.2	68	28	54	8.6	17	2.6
+	6.2	D	D	D	D	D	D
+	7.2	49	16	37	2.9	7	17.5
		Stems, with DTPA					
-	6.2	57	33	65	6.0	10	1.4
-	7.2	61	21	66	10.0	18	2.0
+	6.2	D	D	D	D	D	D
+	7.2	66	17	46	3.7	13	30.5
LSD .05		15	9	8	3.0	5	3.4
LSD .01		20	12	12	4.0	7	4.4

*Moderately chlorotic; D is dead

yield depression. A concentration of 40 μg Ni/g of leaves seemed necessary to cause the slight yield depression under the conditions of the experiment. Leaves contained about twice as much Ni as did stems.

The Ni depressed the Fe, Zn, Cu, and Mn concentrations in leaves and in stems of soybeans. This is somewhat different from the corn inbred also used in the study. It does appear that heavy metal-induced chlorosis differs among plant species. In other studies, Fe chelates did ameliorate Zn toxicity[15] but did not markedly ameliorate the toxicity of some other heavy metals[-9].

A level of 25 μg Ni/g soil caused toxicity in barley without chlorosis and the 100 μg/g soil caused chlorosis with and without EDTA (Table 3). The 400 μg Ni/g soil (with $CaCO_3$) caused chlorosis and yields were greatly decreased. The EDTA increased Ni concentrations in shoots. The Fe concentrations were not decreased by Ni in spite of chlorosis which was evident.

A level of 2% $CaCO_3$ partially protected bush beans from Ni toxicity and decreased Ni uptake (Table 3). The 100 μg Ni/g soil decreased yields of bush beans, but the 25 μg/g did not, in contrast to barley. With EDTA the leaf and stem concentrations of Ni were greatly increased. EDTA resulted in a large increase of Fe in leaves which might have decreased the ability of the high level of Ni to cause chlorosis.

TABLE 3

Effect of Ni on Vegetative Yield of Mineral Composition of Barley and Bush Beans (dry weight basis).

Ni treatments μg/g soil	Yield	Ni	Mn	Fe	K	Ca	P
	mg/plant	μg/g			%		
		Barley					
0	279	5.0	27	51	3.72	2.05	0.284
25	69	7.7	22	50	4.24	1.61	0.324
100	82	44.1	27	64	4.40	1.77	0.348
100 + 100 μg EDTA/g	69	549.0	33	160	2.65	1.56	0.250
400 + 2% $CaCO_3$	46	34.0	14	32	1.56	2.23	0.295
LSD .05	42	10.9	14	80	1.48	NS	NS
LSD .01	57	14.8	NS	109	2.01	NS	NS
		Bush bean leaves					
0	658	5.0	184	129	2.03	3.65	0.482
25	710	25.7	117	70	2.03	2.83	0.461
100	361	94.6	49	68	2.07	3.19	0.400
100 + 100 μg EDTA/g	293	500.0	43	250	1.70	2.90	0.429
400 + 2% $CaCO_3$	380	68.9	87	80	1.48	5.44	0.296
LSD .05	91	9.2	34	52	NS	0.88	0.080
LSD .01	123	12.5	46	71	NS	1.20	0.109
		Bush bean stems					
0	267	2.4	37	38	1.76	1.42	0.257
25	294	19.3	22	33	1.76	1.50	0.262
100	361	61.7	7	46	1.79	1.00	0.368
100 + 100 μg EDTA/g	192	202.8	5	80	1.70	1.20	0.352
400 + 2% $CaCO_3$	240	52.6	13	20	1.00	1.68	0.177
LSD .05	72	31.9	11	28	0.39	NS	0.065
LSD .01	97	43.4	15	39	0.53	NS	0.089

TABLE 4

Leaf Yield and Metal Concentrations in Leaves and Stems of Bush Bean Plants Grown with Ni and DTPA Applications in Yolo Loam Soil Amended to Give Different Soil pH Values (dry wt basis).

Ni treatments	Leaf color*	Yield	Leaf Ni	Stem Ni	Leaf/stem Ni ratio	Leaf Fe	Leaf Cu	Leaf Zn	Leaf Mn
μg/g soil		mg/plant	μg/g			μg/g			
Control (soil pH 5.8)									
0	G	728	2	1.0	2.0	109	6.4	28	104
0 + DTPA	G	731	15	3.5	4.3	174	8.5	29	163
100	Y	261	79	45	1.8	92	7.2	25	47
100 + DTPA	Y	272	110	51	2.2	107	8.0	34	52
250	T	155	206	400	0.5	118	12.9	47	55
250 + DTPA	T	135	248	420	0.6	140	14.7	37	53
Sulfur (soil pH 4.0)									
0	T	141	3	3.3	0.9	131	15.2	40	252
0 + DTPA	T	121	3	4.0	0.8	122	13.5	42	202
100	T	117	36	35	1.0	93	15.2	36	234
100 + DTPA	T	130 +	47	51	0.9	118	13.2	29	192
250	T	124	111	263	0.4	181	17.2	37	392
250 + DTPA	T	122	166	327	0.5	161	20.6	56	266
$CaCO_3$ (soil pH 7.5)									
0	G	527	2	2.3	0.9	78	7.3	27	65
0 + DTPA	G	627	17	1.3	13.1	85	13.3	42	72
100	G	633	20	17	1.2	82	6.3	20	63
100 + DTPA	G	597	55	25	2.2	76	7.0	23	49
250	SC	337	57	31	1.8	112	10.4	28	42
250 + DTPA	SC	269	131	34	3.9	134	9.9	32	39
$MgCO_3$ + $CaSO_4$ (soil pH 8.2)									
0	G	534	2	1.0	2.0	119	7.7	42	60
0 + DTPA	G	527	23	2.0	11.5	113	22.4	71	126
100	G	560	13	12	1.1	104	7.6	29	74
100 + DTPA	G	558	59	19	3.1	102	7.5	25	68
250	Lg	531	27	22	1.2	136	7.6	29	73
250 + DTPA	Lg	631	65	27	2.4	108	7.5	25	64
LSD .05		130	20	46	1.2	27	2.7	13	60
LSD .01		171	24	61	1.6	35	3.6	17	53

*Green, yellow, light green, toxicities, slightly chlorotic.

The $MgCO_3$ (soil pH 8.2) protected bush beans from Ni toxicity at 100 and 250 μg Ni/g soil more than did $CaCO_3$ (soil pH 7.5) (Table 4). The carbonates decreased the Ni uptake also which is probably the reason for the protection. The $MgCO_3$ produced slight Fe chlorosis patterns with the highest level of Ni which seemed to be independent of the DTPA application. Even though Ni increased Fe concentrations in leaves, the chlorosis was present.

The DTPA increased Ni transport to leaves of bush beans (Table 4), but not as much as did EDTA. The ratio of Ni in leaves to that in stems was pH dependent, and was increased by DTPA at pH 7.5 and 8.2 as would be expected from consideration of the stability constant of NiDTPA. The DTPA had no effect on distribution of Ni between leaves and stems at soil pH 4.0 and 5.8 where Ni had been added as it did at soil pH 7.5 and 8.2. Similar results have been obtained for ^{241}Am[14]. This is further evidence that DTPA can facilitate movement of metals through stems to leaves of plants[17], particularly at high soil pH.

Interactions of Ni with other elements in this experiment were not pronounced. At soil pH 6.5, the Mn concentration in leaves was decreased, however.

These experiments with four different plant species indicate somewhat differential response for species. Responses to Ni vary with soil pH because uptake is decreased as the pH is increased. Chelating agents modify the effect of Ni by enhancing uptake and transport to leaves, particularly at soil pH values over 7. Nickel had some interactions with other trace metals. It often increased Fe concentrations in leaves part of which can be explained as a concentration effect due to smaller plant sizes. The chlorosis caused by excess Ni did not seem related to low Fe levels in leaves. The levels of Ni which caused toxicity are helpful in explaining effects of serpentine soils where poor growth often occurs with lower Ni concentrations in plants than in these studies[5 8 9 11].

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COMMUN. IN SOIL SCIENCE AND PLANT ANALYSIS, 8(9), 765-772 (1977)

SOME INTERACTIONS OF CADMIUM WITH OTHER ELEMENTS IN PLANTS

KEY WORDS: Cadmium, phytotoxicity, manganese, iron

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ABSTRACT

Cadmium in solution culture at 10^{-4} M decreased Mn concentrations in bush beans (Phaseolus vulgaris L. C.V. Improved Tendergreen) at both low and high concentrations of Mn (noncompetitive inhibition). When Mn was decreased, the concentrations of Fe and several other ions were simultaneously increased, particularly in leaves and roots. Toxicity due to the 10^{-6} M Cd and the 10^{-4} M Mn was additive in the experiment. When barley (Hordeum vulgare L. Atlas 57) was grown in amended soil, 15 μg Fe as DTPA (diethylene triamine pentaacetic acid) per g soil resulted in increased uptake of Cd and in somewhat greater yield depression for soil pH of 3.9, 6.0, and 7.6. Acidification of soil without DTPA also increased Cd uptake to high levels with associated yield decrease. The Cd decreased the uptake of Mn and Cu most when $CaCO_3$ had also been added to the soil. When salts were added to soil with Cd before bush beans were grown, KCl (200 μg K/g soil), and equivalent KH_2PO_4 increased Cd concentrations of leaves while $CaSO_4$ and KCl did so for roots. In bush beans with different levels of Cd and Zn, there were no yield interactions, but some interactions of Cd on Zn concentrations in leaves, stems, and roots at the high Zn level.

INTRODUCTION

Although Cd is generally considered to be so much like Zn that Cd-Zn interactions in biology would be very pronounced[1 2 3], other interactions of Cd seem to be more important in plants[4 5 7]. Cadmium depressed the Mn uptake by plants[4 5 9]. Potassium fertilizer influenced Cd uptake; Cd causes a type of Fe deficiency in plants[5]. Selenium decreased Cd uptake[2]. Chelates influenced Cd uptake[2 9]. The purpose of this study was to further investigate such relationships. It was especially desired to determine if the ability of Cd to decrease Mn uptake by plants was also expressed at a high level of Mn.

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