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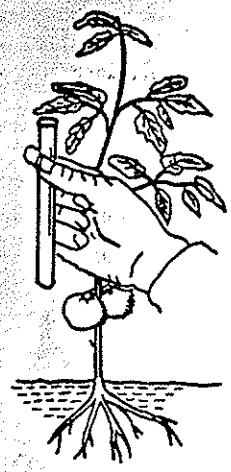
PLANT ANALYSIS AND FERTILIZER PROBLEMS



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PB-ABSORPTION AND EFFECT ON N-METABOLISM OF PLANTS

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Lead being an environmental chemical attained much interest in the last decade. Apart from lead contamination of plants by exhaustions of motor cars, the effect of lead compounds on plant growth and metabolism depends on their solubility and availability to roots.
In a number of pot and water culture experiments we investigated the uptake of Pb^{++} from $Pb(NO_3)_2$ and its effect on nitrogen metabolism of plants.

I. In pot experiments with sandy loam, 0 - 8 g Pb as $Pb(NO_3)_2$ were mixed to 15 kg soil.
Fertilization (g/pot): 2.5 g N as NH_4NO_3
2.0 g P_2O_5 as $CaHPO_4 \cdot 2\ H_2O$
2.5 g K_2O as KCl
(The total amount of N and K was splitted to 2 - 6 applications during vegetation period.)
0-0.5-1.0-2.0-4.0-6.0-8.0 g Pb as $Pb(NO_3)_2$/pot
The nitrate content of $Pb(NO_3)_2$ was compensated by KNO_3.
Methods: Determination of lead after acidic digestion and extraction of ashsolution with dithizone and measuring by atomic absorption spectrophotometry.
Results: Growth and Pb-uptake

Table 1 Dry matter production on sandy loam (g/pot)

soil and plant	Pb-level g/pot (15 kg soil)						
	0	0.5	1.0	2.0	4.0	6.0	8.0
a) pH 4.9							
perennial grass (5 cuts)	124	126	125	127	120	116	117
green oats	94	88	90	88	89	81	76
green rape	29	29	25	23	22	18	17
b) pH 7.0							
perennial grass (5 cuts)	130	124	122	124	120	125	129
bush beans	28	26	27	28	30	28	27

On pH 4.9 low amounts of $Pb(NO_3)_2$ did not influence the growth very much (Tab.1); high rates (6 - 8 g Pb) depressed the dry matter production of grass (5 cuts) only by 5 %, but of green oats by 20 % and of green rape up to 40 % finally.

On neutral/alkaline soil (pH 7.0) the effect of Pb-application was unsignificant both with grass and bush beans. Summarizing, the influence of lead on growth of different plants depends very much on Pb-level and soil pH.

The Pb-content of plants, grown on acid soil, was much higher than that from alkaline soil and increased with Pb-level in both cases. Roots contain 4-10 times more Pb than shoots or leaves; obviously there are some difficulties in lead transportation to stalks and leaves (Tab.2).

Table 2 Pb-content of plants from different Pb- and pH-levels (ppm i.dry weight)

soil and plant	Pb-level g/pot (15 kg soil)						
	0	0.5	1.0	2.0	4.0	6.0	8.0
a) pH 4.9							
grass, shoots (average from 5 cuts)	1	5	10	25	60	80	103
roots	7	39	43	118	144	250	405
green rape, shoots	1	2	4	6	10	14	17
roots	8	14	27	53	76	135	204
b) pH 7.0							
grass, shoots (average from 5 cuts)	2	4	4	5	11	17	23
roots	14	17	21	45	61	155	159
bush beans, shoots	3	3	3	3	9	6	7
roots	11	14	17	33	49	99	116

Against control there are marked differences in the Pb-uptake of shoots. It increased with Pb-level and reached maximum on the acid soil (Tab.3). But the Pb-uptake of shoots ranged only between 0.12 and 0.18 % of total Pb added; that means, that the upper most part of lead will

probably either be absorbed on organic matter and clay minerals of the soil or will be taken by plant roots. As it is nearly impossible to ascertain the roots production in pot experiments quantitatively, because of heavy losses during washing process, also the Pb-uptake by roots cannot be counted correctly either. The buffer effect of the soil complex may explain, why specific Pb-toxicity symptoms did not appear on the plants.

Table 3 Pb-uptake of plants from different Pb- and pH-levels (mg Pb/pot)

soil and plant	Pb-level g/pot (15 kg soil)						
	0	0.5	1.0	2.0	4.0	6.0	8.0
a) pH 4.9							
grass, shoots (total from 5 cuts)	0.18	0.63	1.27	3.59	7.32	10.59	11.82
green oats, shoots	0.19	0.35	0.63	1.23	2.48	3.55	4.66
green rape, shoots	0.03	0.06	0.10	0.14	0.24	0.25	0.28
b) pH 7.0							
grass, shoots (total from 5 cuts)	0.26	0.43	0.42	0.60	1.15	1.92	2.94
bush beans, shoots	0.05	0.08	0.08	0.08	0.36	0.17	0.19
clover, shoots (2 cuts)	0.08	0.08	0.18	0.13	0.25	0.31	0.27

II. In water culture experiments the Pb-concentration ranged between 0 and 10^{-3} M Pb as $Pb(NO_3)_2$.

Culture solution (per 1 water):

1.0 g KNO_3 — 0.74 g $Ca_5(PO_4)_3OH$
0.5 g $CaSO_4 \cdot 2\ H_2O$
0.5 g $MgSO_4 \cdot 7\ H_2O$
0.3 g Fe·EDTA
1 ppm Mn as $MnCl_2 \cdot 4\ H_2O$
0.35 ppm Cu as $CuSO_4 \cdot 5\ H_2O$
0.35 ppm Zn as $ZnSO_4 \cdot 7\ H_2O$
0.04 ppm Mo as $Na_2MoO_4 \cdot 2\ H_2O$
0.17 ppm B as H_3BO_3
5 plants/vessel (4 l)

48 resp.72 hours after Pb-application the plants were harvested and analysed.

Methods: Determination of lead as mentioned before. Protein-N after precipitation by trichloracetic acid and Kjeldahl determination.
Nitrate was determined as nitro-xylenol (Balks 1960), nitrate-reductase activity by measuring the formed NO_2 in an enzym assay after 15' (according to Hageman and Flesher 1960, modified). Amino acids were determinated in freez dryed material (Schaller and Wünsch, 1973) by column chromatography (aminoacidanaliezer BC 200, Biocal), the amides in a second run after hydrolysis of extract by H_2SO_4 (Oji and Izawa 1971).

Results: Growth and Pb-uptake
48 hours after Pb-application the plants dressed with 10^{-3} M Pb showed heavy wilting symptoms on leaves; the fresh weight of harvested shoots was slightly increased at 10^{-5} M Pb, but decreased by 1/10 - 1/3 at 10^{-4} and 10^{-3} M Pb respectively (Tab.4). The water content of stalks and leaves declined gradually by 1.5 to 3.5 %.

Table 4 Effect of Pb on growth and Pb-content of sun flower shoots

Pb-concentration (Mol) in culture solution	fresh shoots g/pot after		Pb i.stalks ppm i.dry m. after		Pb i.leaves ppm i.dry m. after	
	48 h	72 h	48 h	72 h	48 h	72 h
0	670	750	4	4	4	6
10^{-5}	781	832	2	10	6	6
10^{-4}	619	651	5	9	-	15
10^{-3}	482	444	14	8	12	13

The Pb-content of stalks and leaves was again unimportant and rised only a little according to Pb-level of culture solution. A rough calculation shows, that in all groupes the Pb-uptake of shoots (stalks + leaves) was less than 1 mg/pot more than in the control; so there is no influence of Pb concentration of culture solution on Pb transport to shoots, although no possibilities for fixation or sorption by other substances than plant

roots were given. It can be followed later on that the depression of shoot growth at 10^{-4} and 10^{-3} M Pb is not a consequence of Pb-toxicity of leaves or stalks, but must be caused by wilting process after a Pb attak on roots.
Also the root growth was markedly affected by Pb: the water content decreased by about 1 % and the dry matter production by 20 - 30 % (Tab.5).

Table 5 Effect of Pb on growth, Pb content and Pb-uptake of sunflower roots

Pb-concentration (Mol) in culture solution	dry weight g/pot after		ppm Pb i.dry m. after		Pb-uptake mg/pot after	
	48 h	72 h	48 h	72 h	48 h	72 h
0	1.53	2.00	61	423	0.1	0.9
10^{-5}	1.46	2.23	3484	3009	5.1	6.7
10^{-4}	1.41	1.77	25897	17953	36.5	31.8
10^{-3}	1.25	1.44	7976	8612	10.0	12.4

The Pb-content of roots arised enormously already 48 hours after Pb-application and reached 2.6 % Pb on dry matter basis (10^{-4} M) finally. The total Pb-uptake ranged between 5.1 and 37 mg/pot. 24 hours later Pb-content and Pb-uptake did not increase very much. That means, that Pb^{++} damages the roots very quickly and severely causing a denaturation of plasma protein of root cells followed by disturbing permeability and electrolyte balance and blocking enzyme systems (Bersin 1958). Consequently the plants are wilting gradually.
A comparison of Pb-concentration of culture solution with Pb-uptake of plants/pot (4 l) demonstrates after 48 resp.72 hours a Pb-utilization rate of 60 - 80 % at 10^{-5} M Pb, about 40 % with 10^{-4} M and about 1 % at 10^{-3} M Pb.
It should be emphasized here, that according to our experimental conditions it is not possible to decide between Pb-adsorption on root surface or Pb-absorption by roots. But in any case in nearly unbuffered solutions, where no other absorbing substances are present, a great

part of lead gets in a close contact with roots.

Nitrate uptake and nitrate reduction

With increasing Pb-concentrations the uptake of nitrate from culture solution is blocked resulting in a lower NO_3-content of roots and a strongly reduced nitrate uptake/pot, which is not higher than 1/4 (48 h) resp.1/6 (72 h) ultimately (Tab.6). This phaenomenon proves, that obviously root cells are injured by Pb so badly, that they are not able any more to take up water and nutrient ions regularily. This effect is mainly completed already within 48 hours. Also Ca-content of roots fell down to 50 %, as recent investigations have shown.

Table 6 NO_3-uptake of sunflower roots

Pb-concentration (M) in culture solution	NO_3-N uptake after 48 h		72 h	
	% i.dry m.	mg/pot	% i.dry m.	mg/pot
o Pb	2.79	43	2.13	43
10^{-5}	2.60	38	2.07	46
10^{-4}	1.83	26	1.44	25
10^{-3}	0.79	10	0.51	7

Under these circumstances it is not surprising that also nitrate-reductase activity in leaves and roots of maize plants dropped to about 1/4 in roots resp. to nearly 1/2 in leaves 48 hours after Pb application (Tab.7).

Table 7 Effect of Pb on nitrate reductase activity of maize (mg NO_2 formed after 15 min.)

Pb-concentration (M) in culture solution	roots	leaves
0	0.60	1.47
10^{-5}	-	-
10^{-4}	0.48	0.93
10^{-3}	0.15	0.81

Nitrogen metabolism

We have learned up to now, that Pb attack starts in the roots. Table 8 shows the influence of this heavy metal on

nitrogen fractions. The protein content of sunflower roots did not change very much within a period of 72 hours, but the soluble-N decreased to half and more. By denaturation of root plasma not only nitrate uptake but also amino acid synthesis is stopped. By this way the ratio $\frac{\text{protein-N}}{\text{soluble-N}}$ arised from 1.7 resp. 1.8 in the controls up to 4.4 resp. 4.9.

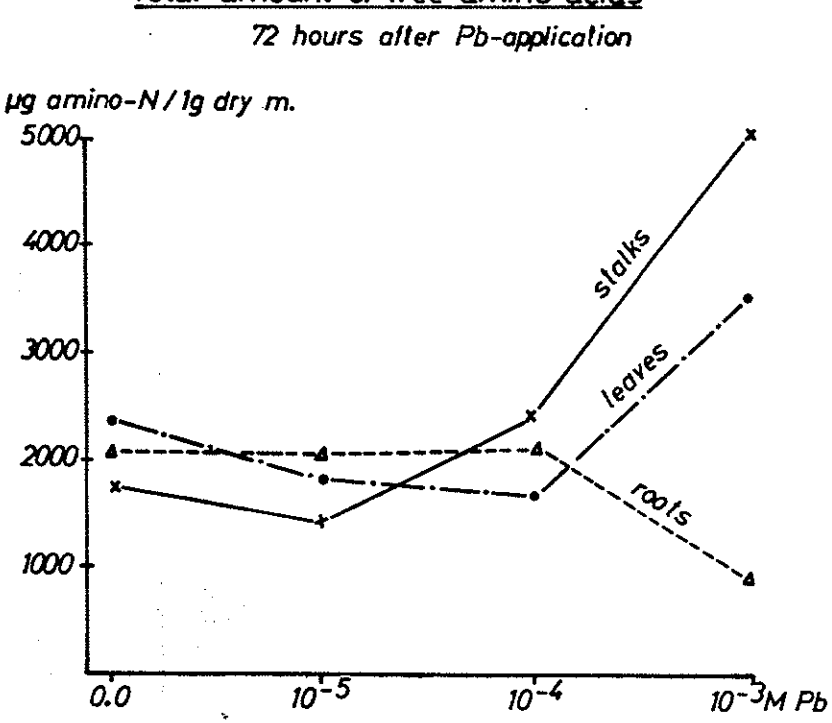
Table 8 Effect of Pb on nitrogen-fractions in sunflower (i.dry matter)

Pb-concentration (M) in culture solution	after 48			72 hours		
	prot.-N %	sol.-N %	prot.-N / sol.-N	prot.-N %	sol.-N %	prot.-N / sol.-N
a) roots						
0	2.83	1.72	1.7	2.89	1.63	1.8
10^{-5}	2.86	1.85	1.6	2.87	1.82	1.6
10^{-4}	2.99	1.32	2.3	2.68	1.48	1.8
10^{-3}	2.96	0.67	4.4	2.88	0.59	4.9
b) stalks						
0	1.28	1.77	0.7	1.31	1.49	0.9
10^{-5}	1.26	1.68	0.7	1.08	1.63	0.7
10^{-4}	1.17	1.61	0.7	1.04	1.44	0.7
10^{-3}	1.24	1.69	0.7	1.00	1.70	0.6
c) leaves						
0	5.43	1.04	5.2	4.61	1.25	3.7
10^{-5}	4.82	1.11	4.3	4.66	1.01	4.6
10^{-4}	4.77	0.80	6.0	4.55	0.80	5.7
10^{-3}	4.84	0.99	4.9	4.21	1.09	3.9

In the stalks protein-N declined not earlier than 72 hours after lead application by 1/3, whereas soluble-N showed an increasing tendency. Both results demonstrate a marked proteolysis, which does not end probably at free amino acids, but is persued to free amonia.
In the leaves both protein-N and soluble-N are decreasing continously by about 10 % combined with heavy wilting symptoms as a secondary effect of Pb-toxicity.

The total amino-N (as the sum of free amino acids determined by column chromatography) did not change very much in the roots between 0 and 10^{-4} M Pb after 72 hours, but decreased at higher rate by more than 50 %. In the leaves free amino-N was found one and a half, in stalks even three times more than in the control (Fig.1).

Figure 1



Among amides and free amino acids glutamic and aspartic acids together comprise about 15 % of total amino-N, that is just the same rate as γ-amino butyric acid alone; amides make up 40 % of total amino-N and more. 72 hours after Pb-exposition in roots amides, glutamic and aspartic acids decreased by nearly 2/3; arginin, γ-amino butyric acid-N and alanine by about 1/3 and more proving that denovo synthesis of amino acids is stopped, but proline increased by 30 % (Tab.9).

Table 9 Effect of Pb on amides and free amino acids in sunflower (µg amino-N/1 g dry matter)
72 hors after Pb-application

Pb-concentration (M) in culture solution	amides	arginine	γ-amino-butyric acid	glut.+ asp. acids	alanine	proline
a) roots						
0	974	83	194	301	171	29
10^{-5}	758	78	287	296	333	32
10^{-4}	1191	65	184	195	207	30
10^{-3}	315	50	126	108	73	42
b) stalks						
0	661	24	326	313	171	23
10^{-5}	562	17	242	239	109	21
10^{-4}	1543	37	246	195	108	29
10^{-3}	3176	119	183	239	174	239
c) leaves						
0	588	303	189	630	316	53
10^{-5}	291	198	254	462	312	43
10^{-4}	385	89	187	496	263	50
10^{-3}	1023	302	315	420	353	350

In stalks amides, arginine and proline arised enormously derivated from protein rich leaves and roots, whereas γ-amino butyric acid, glutamic and aspartic acids decreased by about 1/3.

In the leaves the primary amino acids glutamic and aspartic acids are markedly depressed, but amides, γ-amino butyric acid and proline increased highly as a consequence of proteolysis.

Discussion

Our experiments have shown, that Pb^{++} from $Pb(NO_3)_2$ will be adsorbed or taken up by plant roots in very high amounts but will scarcely be transported to shoots. Though Pb-content of roots is very high especially in water culture experiments where the soil absorption complex is missing. Lead acts as a typical root poison

similar arsenic and aluminium (Hurd-Karrer 1939; McLean and Gilbert 1927) resulting in stunting of roots, disturbing water balance and ion transport and causing severe wilting symptoms of shoots. By this secundary effect all other experimental data can by explained: nitrate uptake and amino acid synthesis (glutamic and aspartic acids) was blocked in roots and leaves and an increasing degradation of protein (Mothes 1928) in proteinrich leaves and roots took place producing prolin in excess, which is very typical for this process (Kemble and MacPherson 1954).

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Abstracts

In pot experiments with sandy loam 0 - 8 g Pb/15 kg soil were applied to grass, green oats, green rape, bush beans and clover. Low amounts of $Pb(NO_3)_2$ did not influence the growth very much; a high Pb level depressed the growth of shoots on acid soil (pH 4.9) by 8 %, under neutral/alkaline conditions the response was insignificant. Pb content of roots was 4 - 10 times higher than that of shoots. On behalf of buffering effect of soil complex the Pb uptake of shoots was less than 1 % (0.12 - 0.18). Toxicity symptoms on leaves have not been observed.

In water culture experiments Pb-application disturbed water balance of plant and blocked ion uptake. On behalf of wilting process nitrate absorption of roots was reduced highly, also nitrate reductase activity and amino-acid synthesis was inhibited. Protein breakdown and high amounts of free amino acids appeared in leaves and stalks with marked differences in the content of arginine, γ-amino butyric acid, alanine and serine.