

The effects of lead on the gaseous exchange and photosynthetic carbon metabolism of pea seedlings

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Abstract

Roots of whole 3 week-old pea seedlings (*Pisum sativum* L.) var. "Bordi" were immersed for 24 h in solutions of lead chloride at Pb concentrations of 200, 400, 800, 12000 mg dm⁻³. Accumulation of lead in roots was independent of the Pb concentration, whereas the accumulation of Pb in shoots was an almost linear function of the concentration of this element in the root medium. This treatment caused Pb concentration-dependent inhibition of apparent photosynthesis (APS), photorespiration (PR), ¹⁴CO₂ uptake, stomatal opening and transpiration of shoots and also germination of seeds. The most sensitive to Pb contamination was CO₂ exchange, then transpiration and to a lesser degree germination of seeds. Lead caused a considerable alteration of photosynthetic and photorespiratory carbon metabolism, restricted the ¹⁴C-labeling of: phosphoglycerate, ribose+ribulose, sucrose, glycolate and glycine+serine. Under conditions of CO₂ uptake limited by lead, an enhancement of the ¹⁴C-labeling of malate+citrate, alanine and glucose was observed.

Key words: lead, CO₂ exchange, carbon metabolism, germination, pea

INTRODUCTION

Lead is contaminant of soils and plants whose sources are automobile exhausts and emissions factories and industrial centers where Pb is used. This element is considered to be a serious phytotoxicant. Extensive reviews of the toxicity of heavy metals on the physiological and biochemical processes of plants were recently published (Woolhouse 1983, Clijsters and Van Assche 1985, Baszyński 1986). In the literature however, only a few papers described the effects of Pb on the gaseous exchange of plants. Earlier data have indicated that accumulation of lead in leaves of maize, sunflower and soybean causes a reduction in the rates of photosynthesis and transpiration (Bazzaz et al. 1974, 1975, Carlson et al. 1975).

To date, nothing is known about the effects of this element on photosynthetic carbon metabolism, although such knowledge is obviously important for the better understanding of the toxicity of this pollutant to plants. Therefore it seemed appropriate to study the processes of gaseous exchange and metabolism of photoassimilated carbon in plants treated with this toxicant. In the experiments with pea we examined the effects of various concentration of lead in the medium of roots on: accumulation of this element in roots and shoots, rates of apparent photosynthesis, photorespiration, ^{14}C -labeling of photosynthetic and photorespiratory intermediates, transpiration and stomatal opening. For comparative purposes, the toxicity of lead on the germination of seeds was also determined.

MATERIAL AND METHODS

Seedlings of pea (*Pisum sativum* L. var. Bordi) were used in the experiments. After germination of seeds, the seedlings were selected for uniformity and placed in 2 dm³ plastic pots containing a Knop solution that was adjusted to pH 6.0 with 1N KOH. Solutions in pots were aerated continuously and kept nearly constant. Seedlings were grown in a growth chamber with a 14 h photoperiod, 80% relative humidity and temperature of 26°C during the day and 18°C during the night. The photosynthetic photon flux density (PPFD 400–700 nm) was 15 W m⁻² provided by cool white fluorescent tubes. After 3 weeks of growth, similar sized seedlings were transferred for 24 h to solutions containing PbCl₂ dissolved in distilled water. The concentrations of Pb were (in mg dm⁻³): 0, 200, 400, 800 and 1200. In order to check any possible effect of Cl⁻, the seedlings were treated with solutions of the same concentrations of KCl. It was found that these seedlings exhibited essentially the same CO₂ exchange rates as the control plants.

Before the measurements of CO₂ exchange the roots were washed with distilled water, then immersed in distilled water in a test tube. The shoot was sealed into a plexiglass photosynthesis chamber and connected to a closed circuit system of an infra red CO₂ analyzer Beckman model 865. That volume of the system was 996 cm³ and the flow rate of air was 3 dm³ min⁻¹. Shoots were illuminated from both sides with four 500 Watt photoflood lamps PR 500. To reduce infra-red irradiation the light source was filtered through a screen with running water. The PPFD at the level of the leaves was 46 W m⁻². The temperature in the chamber was 28°C ± 1°C.

The rates of apparent photosynthesis were determined as the CO₂ concentration decreased from 320 to 280 cm³ m⁻³. When CO₂ concentration compensation was reached, the light was turned off and the system was opened. Following 30 min adaptive illumination, the measurements were

repeated three or four times. The rates of photorespiration were calculated as described (Poskuta and Nelson 1986). Detached shoots were used in experiments on $^{14}\text{CO}_2$ uptake and distribution of radioactivity among the photosynthetic products. They were placed with their cut ends in a bag with distilled water and sealed into a closed circuit photosynthetic chamber. The leaves were first illuminated for 30 min with PPFD 45 W m^{-2} , then $^{14}\text{CO}_2$ (185 KBq) was introduced into the system and photosynthesis was carried out for 2 min. The initial CO_2 concentration in the system was $400 \text{ cm}^3 \text{ m}^{-3}$. The radioactive plant material was treated according to the procedure described (Grishina et al. 1974). All compounds were assayed for ^{14}C content using a liquid scintillation technique and a Beckman scintillation counter.

Five separate plants per Pb concentration were measured and gave similar results. Data were averaged and standard errors calculated.

Rates of transpiration were determined by weighing pots with plants in the conditions of the growth chamber. The degree of stomatal opening was determined using the silicone rubber impression technique as described (Karpowiczowa and Poskuta 1971). The Pb content was determined by atomic absorption spectroscopy. Plant material was burned in a mixture of nitric and perchloric acids in a proportion of 4:1. The germination of seeds was tested in two manners:

1. The seeds were soaked for 24 hours in the appropriate solution of PbCl_2 and then placed on a sheet of filter paper with 10 cm^3 PbCl_2 of the appropriate concentration in Petri dishes.
2. The seeds were placed directly on sheet of filter paper with 10 cm^3 PbCl_2 solution in Petri dishes. The concentrations of Pb in the solutions were (in mg dm^{-3}): 0, 200, 400, 800, 1200 and 2400. Both treatments showed similar results. For each Pb concentration 500 seeds were tested.

RESULTS

ACCUMULATION OF Pb IN SEEDLINGS

The content of Pb in seedlings after 24 h exposition of the roots to solutions of lead chloride is shown on Fig. 1. It is seen that the Pb content in the roots reached about $300 \text{ mg kg}^{-1} \text{ DW}$ and was practically independent of the concentration of this element in the medium of the roots. In contrast, the content of Pb in shoots increased almost linearly with the increase of the Pb concentration in the root medium. As a consequence, the ratios shoot Pb: root Pb content followed a straight line

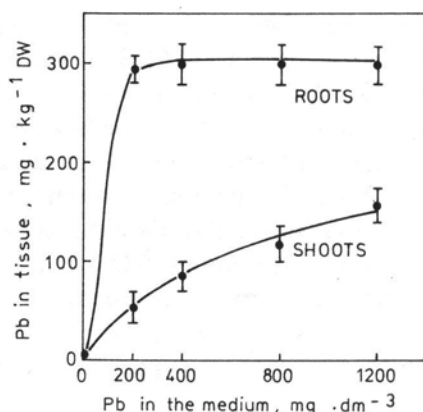


Fig. 1. The relationship between the amount of Pb accumulated by shoots and roots of pea seedlings and the concentration of Pb in the medium of roots. Bars represent SE of the means

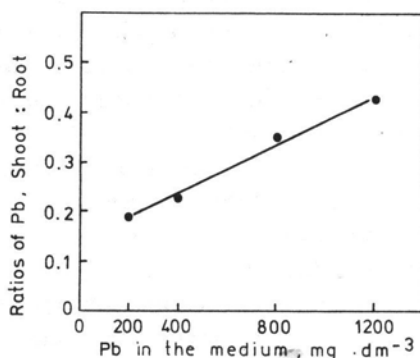


Fig. 2. The relationship between the shoot: root ratios of Pb content and the concentration of Pb in the medium of roots

relationship between the concentration of Pb in the medium and the accumulation of this element in whole seedlings (Fig. 2). It is also seen that roots are a much stronger sink for Pb than shoots.

THE EFFECTS OF Pb ON GASEOUS EXCHANGE OF SHOOTS AND ON GERMINATION OF SEEDS

Figure 3 depicts the effects of Pb accumulation in shoots on the rates of APS, PR, T and the Pb concentrations on the germination of seeds. It is seen that the most sensitive to Pb were APS and PR. These processes were inhibited practically in similar magnitudes. The inhibition followed an almost linear relationship between the concentration of Pb in the medium of roots and percentages of inhibition. The magnitudes

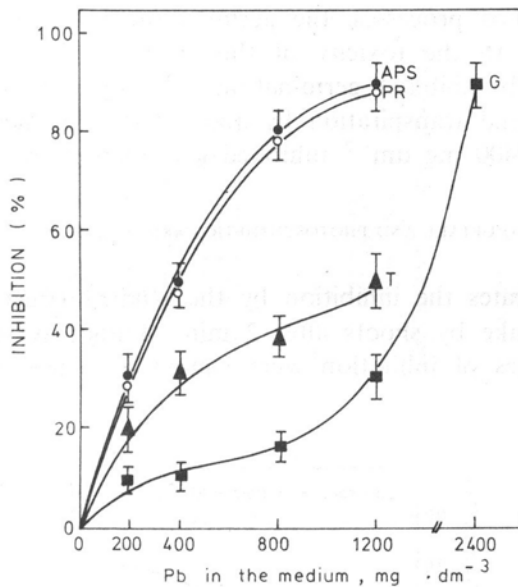


Fig. 3. The relationship between apparent photosynthesis (APS), photorespiration (PR), transpiration (T), germination (G) and the concentration of Pb in the medium of roots. The processes are expressed as a percentage of the control values: APS = $5.6 \text{ mg CO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$; PR = $1.4 \text{ mg CO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$; T = $140 \text{ mg H}_2\text{O g FW h}^{-1}$. Bars represent SE of the means

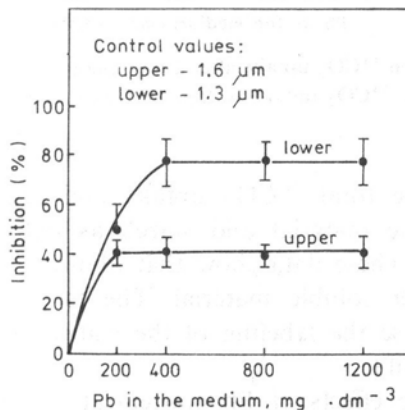


Fig. 4. The relationship between the stomatal openings on leaf lamina and the concentration of Pb in the medium of roots. The degree of stomatal opening is expressed as a percentage of the control values: lower epidermis $1.3 \text{ } \mu\text{m}$, upper epidermis $1.6 \text{ } \mu\text{m}$

of the inhibition of transpiration by the same concentrations of Pb were by about 30% lower as compared with those of APS and PR. The Pb also inhibited stomatal opening. Stronger inhibition was noticed for the lower as compared with the upper epidermis of the leaf lamina (Fig. 4).

Among the examined processes, the germination of seeds appeared to be the most tolerant to the toxicity of this element. The concentration of 1200 mg dm^{-3} Pb inhibited germination by only 28% whereas of APS and PR by 90% and transpiration by about 50%. An increase in the Pb concentration to 2400 mg dm^{-3} inhibited germination by about 90%.

$^{14}\text{CO}_2$ UPTAKE AND PHOTOSYNTHETIC CARBON METABOLISM

Figure 5 illustrates the inhibition by the studied concentrations of Pb of the $^{14}\text{CO}_2$ uptake by shoots after 2 min. of photosynthesis. It is seen that the percentages of inhibition were similar to those of the inhibition of APS and PR.

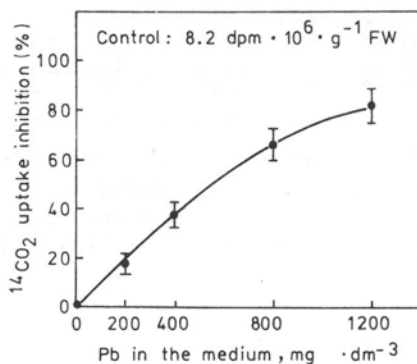


Fig. 5. The relationship between $^{14}\text{CO}_2$ uptake after 2 min. photosynthesis and the concentration of Pb in the medium of roots. $^{14}\text{CO}_2$ uptake is expressed as a percentage of the control value

Table 1 presents the total $^{14}\text{CO}_2$ uptake and the distribution of ^{14}C among the water-soluble material and starch as influenced by Pb at the concentrations applied. These data show that about 2/3 of the radioactivity was found in the water soluble material. The tendency of increasing Pb concentration to decrease the labeling of the water soluble material at the expense of starch is visible.

Figure 6 depicts the effects of Pb concentrations on the incorporation of ^{14}C into organic acid, amino acid and sugar fractions. It is seen from this Fig. that the largest stimulating effect of Pb was found with respect to the sugar fraction. The opposite is true for the fraction of organic acids. There was practically no effect of lead on the incorporation of ^{14}C into the fraction of amino acids.

Figure 7 presents the effects of Pb in the studied concentration range on the distribution of radioactivity among the individual compounds in the fractions of organic acids, amino acids and sugars. It is apparent

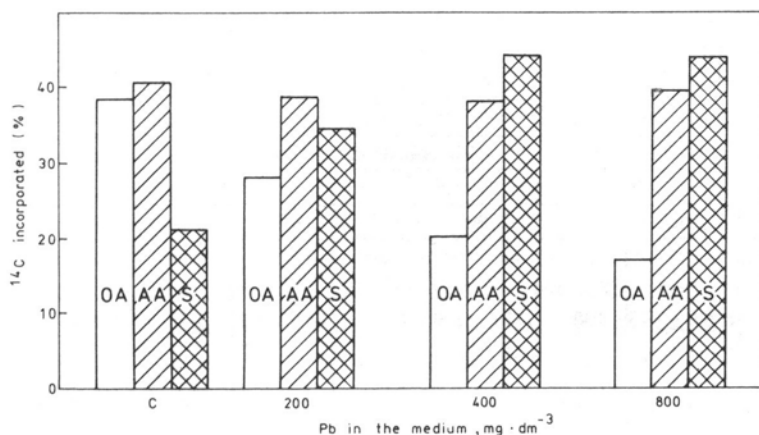


Fig. 6. The relationship between the distribution of ^{14}C among the fractions of water soluble material after 2 min. photosynthesis in $^{14}\text{CO}_2$ and the concentration of Pb in the medium of roots. ^{14}C is expressed as a percentage of the radioactivity of each fraction in water soluble material. C—control, OA—organic acids, AA—amino acids, S—sugars

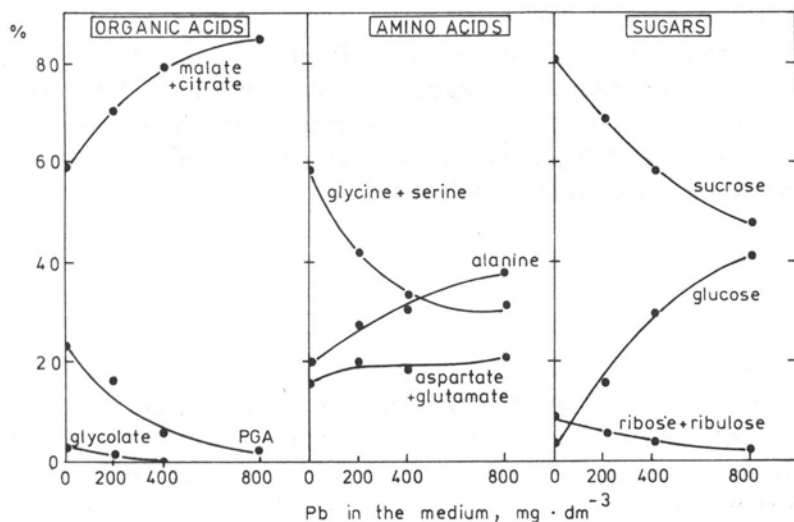


Fig. 7. The relationship between the distribution of ^{14}C among individual compounds of the fractions of organic acids, amino acids and sugars

that the bulk of radioactivity in the organic acid fraction was located in malate+citrate and that increased Pb concentration enhanced the incorporation of the label into these acids. In contrast, Pb greatly suppressed the incorporation of ^{14}C into PGA and glycolate. In the amino acids fraction the bulk of radioactivity was located in glycine+serine and there was a strong inhibitory effect of Pb on the flow of carbon into these

Table 1

The effects of Pb concentration on the distribution of ^{14}C among the products of 2 min. photosynthesis of pea shoots

	Pb concentration, mg dm^{-3}							
	0		200		400		800	
	KBq	%	KBq	%	KBq	%	KBq	%
Total ^{14}C incorporated	136.6 \pm 13.3	100	112.0 \pm 8.3	100	84.7 \pm 8.3	100	45.0 \pm 3.3	100
Water soluble material	95.0 \pm 6.6	69.5	75.0 \pm 5.0	66.9	53.0 \pm 3.3	62.6	27.7 \pm 2.3	61.6
Insoluble material (starch)	46.6 \pm 3.3	30.4	36.6 \pm 1.6	32.7	31.7 \pm 1.7	37.4	17.7 \pm 1.1	39.3

acids. At the same time there was considerable stimulation by this factor of the incorporation of ^{14}C into alanine. The labeling of aspartate and glutamate however, was little influenced by Pb in the concentrations applied.

In the sugar fraction, the largest inhibitory effect of Pb was found on the incorporation of the label into sucrose and ribulose+ribose. The decrease of labeling of these sugars was accompanied by a strong increase of the incorporation of radioactivity into glucose.

DISCUSSION

Data from literature indicate that Pb exerts multilateral inhibitory effects on photosynthesis at structural and metabolic levels: it accumulates in chloroplasts and disorganizes their ultrastructure (Rebechini and Hanzley 1974), decreases the biosynthesis of chlorophyll (Hampp and Lendzian 1974, Burzyński 1985), inhibits PS II (Miles et al. 1972), inhibits photophosphorylation and the activities of several enzymes of the reductive pentose phosphate cycle (Hampp et al. 1973a, b).

The data of the present study further contribute to our knowledge about the toxicity of lead on plant metabolism. The results show that the Pb content of pea shoots increased almost linearly with the increase of the Pb concentration in the medium of roots. At the same time the content of this element in roots already reached a maximal value at the lowest concentration in the medium of roots and exceeded by several times the content of Pb in shoots as the concentration of the pollutant in the medium increased (Fig. 1). Because the shoot:root ratios of Pb

content were a linear function of Pb concentration in the medium of roots (Fig. 2), we conclude that the root presents a strong sink for Pb. It is possible to assume that this pollutant is transported via the transpiration stream to the shoots, hence, the increasing of the concentration of Pb in the medium of roots is reflected by an increased content of this element in shoots. As a consequence of the accumulation of this toxicant in shoots, the rates of APS, PR and $^{14}\text{CO}_2$ uptake were severely inhibited. The magnitudes of inhibition of these processes were similar (Figs. 3, 4, Table 1). The inhibition by Pb of the fixation of CO_2 by shoots was associated with a change in the flow of assimilated carbon into photosynthetic and photorespiratory intermediates (Figs. 5, 6, Table 1). The labeling of PGA and ribose+ribulose was largely restricted. This indicates that Pb limited not only the formation of the recent photosynthetic intermediates but also a regeneration of the precursors of the CO_2 acceptor-RuBP. The labeling of the photorespiratory intermediates glycolate and glycine+serine was greatly diminished. This indicates that the flow of carbon into the photorespiratory cycle was also restricted. Under the conditions when both photosynthetic as well as photorespiratory pathways were greatly reduced by Pb, the stimulatory effects of this element on the incorporation of the label into malate+citrate and alanine and glucose occurred (Fig. 6). In these circumstances, one can assume that Pb increased the β -carboxylation, although the flow of carbon to the whole fraction of organic acids was strongly limited. The relative increase of the labeling of the sugar fraction and starch may reflect an enhancement of the outflow of photosynthetic intermediates from the chloroplasts to the cytoplasm where the synthesis of oligosaccharides occurs and accounts for the increase of labeling of the sugar fraction and also of starch at the expense of the organic acids fraction (Fig. 5, Table 1). This may imply the involvement of Pb in the alteration of the properties of chloroplast membranes which resulted in increasing their permeability.

With respect to the inhibition of photosynthesis and transpiration by Pb our data qualitatively confirmed the earlier observations of Bazzaz et al. (1974). In contrast to the results of these authors however, we noticed a higher inhibition by Pb of the rates of both apparent photosynthesis and photorespiration as compared with transpiration.

At comparable Pb concentrations in the medium of roots, the percentages of inhibition of APS and PR were always higher than of transpiration, indicating a greater sensitivity of CO_2 exchange in light to Pb of whole pea seedlings. Further, we observed that Pb reduced differentially the degree of stomatal openings (Fig. 4) and it obviously contributed to the limitation of gaseous exchange of leaves. At present we have no data to quantitatively assess the contribution of stomatal resistance to the reduction of gaseous exchange of these seedlings. The discrepancy in the

sensitivity to Pb of the mentioned processes in experiments of Bazzaz et al. (1974) and of the present study can be a result of species specificity and of differences in the Pb content in leaves of whole seedlings with roots immersed in Pb solutions (our experiments) and that of detached leaves placed in Pb solutions with their cut ends. The relatively low toxicity of the Pb concentrations observed in the present study on the process of germination is of interest, but the mechanism of this finding remains to be elucidated.

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Wpływ ołowiu na wymianę gazową i metabolizm węgla w siewkach grochu

Streszczenie

Korzenie całych siewek grochu (*Pisum sativum* L.) odm. Bordi, zanurzano na 24 godz. w roztworach chlorku ołowiu w stężeniach Pb: 200, 400, 800, 1200 mg dm⁻³. Gromadzenie się ołowiu w korzeniach nie zależało od stężenia tego pierwiastka w roztworze zewnętrznym, natomiast gromadzenie się ołowiu w pędach było prawie liniową funkcją jego stężenia w roztworze w którym zanurzone były korzenie. Zabieg ten spowodował: inhibicję fotosyntezy netto (APS) zależną od stężenia Pb, fotooddychania (PR), asymilacji ¹⁴CO₂, rozwarcia szparek na liściach, transpiracji pędów (T) oraz kiełkowania nasion. Najbardziej czułym procesem na zanieczyszczenie przez Pb okazała się wymiana CO₂, następnie transpiracja a najmniej kiełkowanie nasion. Akumulacja ołowiu w pędach spowodowała także zmiany w metabolizmie węgla w fotosyntezie i fotooddychaniu; ograniczała znakowanie: ¹⁴C kwasu fosfoglicerynowego, rybozy + rybulozy, sacharozy, kwasu glikolowego, seryny + glicyny. W warunkach ograniczonej przez Pb asymilacji CO₂, zwiększone było znakowanie ¹⁴C jabłczanu + cytrynianu, alaniny i glikozy.