ACTA SOCIETATIS
BOTANICORUM POLONIAE
vol. 52, nr 3-4: 231-239
1983

Influence of lead on auxin-induced cell elongation

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(Received: February 7, 1983. Revision accepted: March 2, 1983.)

Abstract

The influence of lead chloride on plant tissue growth is described. Lead reduced elongation of etiolated wheat coleoptile segments, green pea epicotyl fragments and etiolated and green sunflower hypocotyls. Green tissues were more susceptible to lead than etiolated ones. $PbCl_2$ in a 10^{-4} M concentration significantly reduced plastic and elastic extensibility of the wheat coleoptile cell walls and diminished the hydration of sunflower hypocotyl segments. Auxin (indolyl-3-acetic acid — IAA) applied in concentration optimal for growth of the particular tissues partly attenuated the inhibitory action of lead on elongation, plastic and elastic extensibility and water absorption. Auxin applied in supraoptimal concentrations did not abolish the inhibitory action of lead on tissue growth.

Key words: lead, auxin, elongation, extensibility.

INTRODUCTION

It is a known fact that lead taken up by plants may accumulate in various cell structures: nuclei (Hammett 1928, Skaar et al. 1973), mitochondria (Walton 1973, Bittel et al. 1974) or chloroplasts (Sabnis et al. 1969, Rebechini and Hanzely 1974). It seems, however, that this metal is mainly deposited in the cell walls (Malone et al. 1974). Lane et al. (1978) investigated lead accumulation in cell wall fractions of the wheat coleoptile. They found a high lead content in the pectic acid fraction, this suggesting its influence increasing the rigidity of the cell walls. It would seem, therefore, that binding of lead by the cell wall polysaccharides may be the main, though not the sole, cause of inhibition of cell elongation and growth. Lane et al. (1978) suggested that the presence of lead in cell wall structures may depress the extensibility of cell walls stimulated by auxin. Some reports (Muk-

herji and Maitra 1977) also suggest that the presence of Pb in plant cells affects the endogenous IAA level, modifyig the activity of the enzymes participating in auxin synthesis and disintegration. The results of the above mentioned studies do not, however, univocally elucidate the mechanism of the action of lead and are based on experiments performed on nonhomogeneous plant material (whole plants or coleoptiles with leaf primordium).

The aim in view in the present study was to investigate the influence of Pb^{++} ions on elongation of tissue segments and the interaction between Pb^{++} and IAA in this process. It was endeavoured to demonstrate whether the action of Pb^{++} is connected with changes in the extensibility of cell walls and water uptake. Various plant material was used such as wheat coleoptile segments and sunflower hypocotyls as well as pea epicotyls.

MATERIAL AND METHODS

ELONGATION OF ISOLATED TISSUES

Wheat (*Triticum vulgare* L., variety Luna) seeds were soaked in distilled water for one hour, then seeded in cuvettes lined with moist filter paper. They were left to germinate in a dark thermostat for 72 h. Equal coleoptiles were chosen and 10-mm segments were cut from them 2 mm below the tip. Glass needles were passed through these segments to press out the leaf primordia and then they were incubated on Petri dishes with 10 cm^3 of the tested solutions. Incubation was run in darkness for 24 h. All manipulations were performed under green light (540-620 nm).

The growth test with fragments of epicotyls was carried out on pea (*Pisum sativum* L.) according to Galston (1965). Pea seeds were germinated in darkness for two days and then the seedlings were planted on Hoagland's medium for 7 days. The 5-mm segments were cut out below the stipule of the youngest leaf and incubated under continuous light for 24 h.

Sunflower (*Helianthus annus* L.) seeds were soaked in water for 4 h and seeded in a cuvette with pure sand. Germination and growth of these seedlings was run under constant darkness or a photoperiod for 10 days. From equal seedlings 10-mm segments were cut out 5 mm below the cotyledons. Fragments of the hypocotyls were incubated in Erlenmayer flasks containing 50 cm^3 of the tested solutions. Etiolated and green segments were incubated for 24 h in darkness or under continuous illumination, respectively.

The temperature during germination and growth of the seedlings and incubation of the tissue fragments was maintained at 25°C. The pea

seedlings and green sunflower seedlings grew under a photoperiod of L:D = 18:6. As light source in all the experiments served fluorescent tubes of light intensity 5.83 $W \cdot m^{-2}$. In order to avoid geotropic curving of the tested sunflower hypocotyl segments and pea epicotyls, they were maintained in continuous movement by passing through the incubation solution an air flux. The length of the tested segments was measured in a graded binocular with an accuracy up to 0.1 mm.

All the solutions tested contained 1 mM KCl, various $PbCl_2$ concentrations and when needed suitable IAA solutions (as shown in Tables). The excess of chlorine ions introduced with Pb was compensated with NaCl. Phosphate buffer usually used in growth tests could not be applied because of the precipitation of phosphate ions with Pb^{++}. The control solution contained KCl and corresponding amounts of NaCl.

PLASTIC AND ELASTIC EXTENSIBILITY

Plastic and elastic extensibility of wheat coleoptiles was measured after C l e l a n d (1960). The plant material was reared as earlier described. After cutting of coleoptile fragments and removal of the first leaf one batch of the segments was immediately plasmolysed with a 1.5 M mannitol solution and after 1.5 h their length was measured (PLi). The remaining batch of coleoptiles was placed on Petri dishes with 10 cm^3 of the tested solutions. The dishes were transferred to a dark thermostat. After 20 h the length of the segments (Lf) was measured. The segments were plasmolysed and their length was measured once more (PLf). Plastic (P) and elastic (E) extensibility was determined with the use of the equations: P = PLf-PLi and E = Lf-PLf. All manipulations were performed under green light (540-620 nm). Each combination consisted of at least 20 segments. The experiments were replicated three times.

WATER UPTAKE

The influence of lead on water uptake was tested on etiolated sunflower (*Helianthus annus* L.) hypocotyls. The seedlings were cultured in darkness as described earlier. The 10-mm hypocotyl segments were washed with tap water for 1 h, then dried on filter paper, weighed and transferred to Erlenmayer flasks containing 50 cm^3 of the tested solutions. The segments were incubated in darkness for 20 h with an air flux passing through the solution. After incubation the segments were dried on filter paper and weighed once more. Each combination consisted of 15 segments, the experiments were replicated three times.

RESULTS

The influence of lead on the elongation of etiolated (wheat coleoptile test) and green (pea epicotyl test) tissues was tested in the presence and absence of auxin as shown in Fig. 1A and B. In the case of green pea tissues a lower lead concentration (10^{-5} M) had an inhibitory influence on elongation, whereas in the etiolated cylinders of wheat coleoptiles the inhibitory effect appeared at 5×10^{-5} M $PbCl_2$. Introduction of auxin in optimal concentration for wheat coleptile growth (10^{-5} M IAA) and pea epicotyls (10^{-4} M IAA) into lead solutions abolished the inhibitory effect of the latter only at lower concentratoins. When the lead concentration was high auxin only attenuated the toxic influence of Pb^{++}.

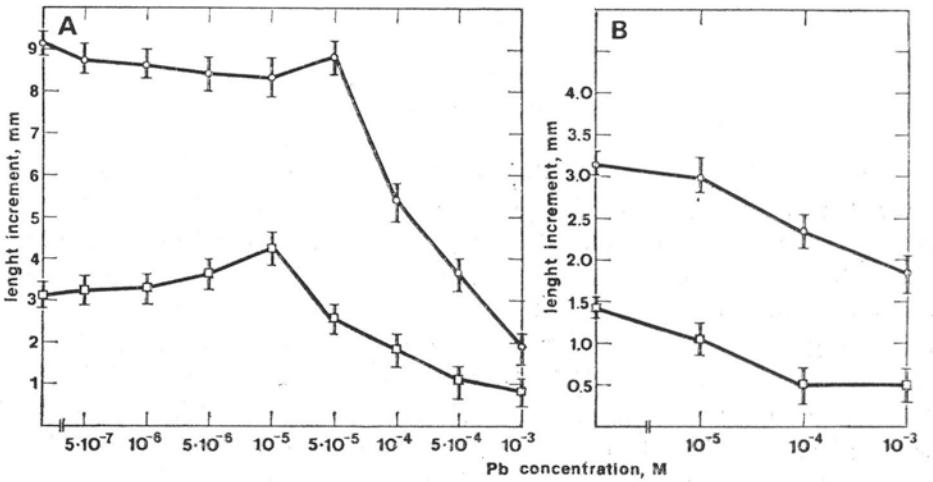

Fig. 1. Influence of lead chloride concentration on auxin-stimulated growth of fragments of etiolated cylinders from wheat coleoptiles (A) and green pea epicotyls (B). Circles — 10^{-5} M (A) and 10^{-4} M (B) IAA, squares — no IAA

For studying the IAA and Pb^{++} interaction experiments were performed with the use of various auxin concentrations in the presence of lead in concentrations which inhibit tissue elongation. The results are shown in Fig. 2A and B and they show that IAA concentrations optimal for the growth of both types of tissues depressed the inhibitory effect of lead. Superoptimal auxin concentrations did not give an additional effect depressing elongation growth inhibition by lead.

The different response of pea tissues and wheat coleoptiles to the lead concentration may have been due either to the difference between species or the different reaction of green and etiolated plants to lead. In this connection sunflower hypocotyls were used which could be tested both as etiolated and as green material. As seen from the results in Fig. 3A and B, elongation of green hypocotyls was inhibited by lead in

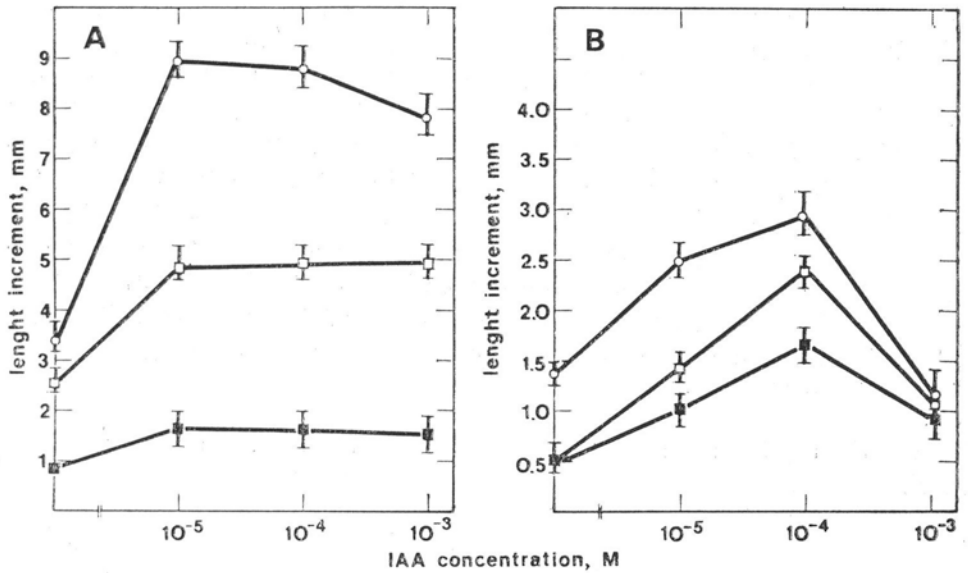

Fig. 2. Influence of increasing IAA concentrations on elongation growth of fragments of etiolated wheat coleoptile cylinders (A) and green pea epicotyls (B). Circles — no Pb, open squares — 10^{-4} M $PbCl_2$, solid squares — 10^{-3} M $PbCl_2$

a concentration of 10^{-5} M, whereas the etiolated tissues require a concentration of 10^{-4} M to show a noticeable inhibitory effect on elongation. It also appeared that the IAA concentration optimal for growth abolished the inhibitory influence of higher lead concentrations in etiolated tissues, whereas the same was not noted in green ones.

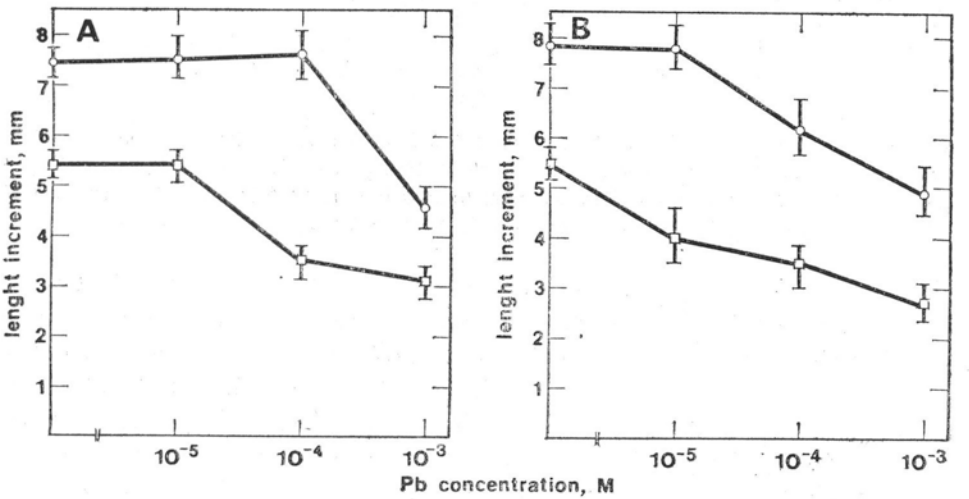

Fig. 3. Influence of various lead concentrations on auxin-induced elongation of etiolated (A) and green (B) sunflower hypocotyl fragments. Circles — in the presence of 10^{-4} M IAA, squares — no IAA

In order to establish the effectiveness of the action of lead throughout the incubation time, the growth kinetics of wheat coleoptiles was measured (Fig. 4A and B). The elongation growth of wheat coleoptiles treated with lead in a 10^{-4} M concentration was almost completely inhibited after 1 h of incubation. During the first two hours the curve of coleptile growth during incubation in a 10^{-4} M Pb^{++} solution with 10^{-4} M IAA (Fig. 4A) did not differ from the course of the growth curve for coleoptiles incubated in auxin alone. The influence of lead was noticeable as late as after 3 h of incubation. Tissue incubation in a 10^{-4} M $PbCl_2$ solution, however, for one or two hours (Fig. 4B) was sufficient to inhibit distinctly elongation, although the tissues were transferred from the lead solution to an IAA solution.

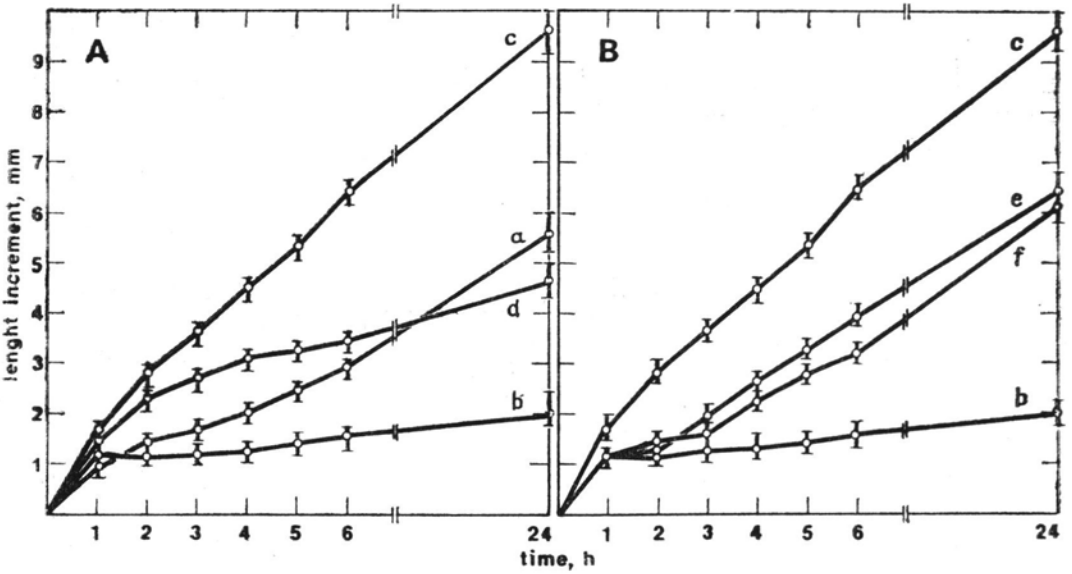

Fig. 4. Elongation growth kinetics of wheat coleoptiles in various incubation solutions. a — control (—IAA, —Pb), b — 10^{-4} M $PbCl_2$, c — 10^{-5} M IAA, d — 10^{-4} M $PbCl_2$ + 10^{-5} M IAA, e — first hour of incubation in 10^{-4} M and then in 10^{-5} M IAA, f — two first hours of incubation in 10^{-4} M $PbCl_2$ and then in 10^{-5} M IAA

It is indicated by the results presented in Table 1 that $PbCl_2$ in a 10^{-4} M concentration reduces by about 50 per cent both the plastic and elastic extensibility of wheat coleoptiles. IAA in an optimal for coleoptile elongation growth concentration enhanced both these kinds of extensibility. Lead chloride added to the auxin solution reduced by about 40 per cent the stimulating action of IAA on plastic extensibility and much less, but significantly, by 13 per cent the elastic extensibility. As a result of this the values of plastic and elastic extensibility in the combination with Pb + IAA attained the same values as in the control tissues (—Pb, —IAA).

Table 1

Influence of lead on elastic and plastic extensibility of wheat coleoptile segments

Combination	Elastic extensibility ⟨E⟩		Plastic extensibility ⟨P⟩	
	increment in length, mm	reduced by Pb^{++}, %	increment in length, mm	reduced by Pb^{++}, %
Control ⟨—Pb, —IAA⟩	1.1±0.17* a		4.4±0.63 a	
$PbCl_2$ 10^{-4} M	0.5±0.08 b	45	2.6±0.26 b	60
IAA 10^{-5} M	1.5±0.09 c		8.6±0.59 c	
IAA 10^{-5} M+$PbCl_2$ 10^{-4} M	1.3±0,11 a	87	5.2±0.46 a	60

* — mean±SE

Each value represents the mean from 20 replications. The mean denoted by different letters within each column differ significantly within 5 per cent of error.

Pb^{++} in a 10^{-4} M concentration reduced by about 60 per cent fresh mass increment and elongation of the etiolated hypocotyl segments of sunflower (Table 2). IAA in a concentration optimal for elongation growth of hypocotyls, when introduced together with $PbCl_2$ into the incubation solution abolished almost completely the inhibitory influence of Pb^{++} on elongation and markedly attenuated its inhibitory effect of fresh mass increment.

Table 2

Influence of lead on elongation growth and water uptake by etiolated sunflower hypocoltyls

Combination	Weight increment		Length increment	
	mg	reduced by Pb^{++}, %	mm	reduced by Pb^{++}, %
Control ⟨—Pb, —IAA⟩	17.9±1.3* a		4.4±0.4 a	
$PbCl_2$ 10^{-4} M	7.1±1.7 b	40	2.1±0.1 b	48
IAA 10^{-4} M	26.8±1.9 c		7.0±0.6 c	
IAA 10^{-4} M+$PbCl_2$ 10^{-4} M	19.5±2.7 a	73	6.2±0.9 c	89

* — mean±SE

Each value represents mean from 15 replications. The means within one column denoted by various letters differ significantly within 5 per cent of error.

DISCUSSION

The above described results indicate that lead chloride in concentrations exceeding 10^{-5} M distinctly inhibits elongation of all the tested tissues. Simultaneous introduction of auxin into the incubation solution in a concentration optimal for growth of the given tissue abolished the inhibitory action of lead only within certain limits of concentration of the latter. Such a relation has been observed earlier by L a n e et al.

(1978) in wheat coleoptiles. The results of the present study indicate that supraoptimal auxin concentrations do not increase its effectiveness in attenuation of the influence of lead. It would seem, therefore, that the mechanism of Pb^{++} action, at least in the present experiments, did not consist in a depression of the free auxin level. This mode of action has been suggested by Mukherji and Maitra (1977) when investigating its influence on the growth of rice seedlings. Measurement of growth kinetics demonstrated that lead significantly inhibits wheat coleoptile growth as late as after two hours of incubation in the Pb^{++}-containing solution. The presence of lead in the incubation solution, for one hour was, however, sufficient to cause a distinct inhibition of tissue growth. It may be concluded from these observations that lead penetrated into or was absorbed by the tissue relatively fast, but its effect on the growth process was delayed as compared with that of IAA.

Malone et al. (1974) demonstrated that lead taken up by maize roots accumulates in the dictyosomal vesicles. The latter transported lead beyond the plasmolemma simultaneously with materials for construction of the cell wall. Lane et al. (1978) found large amounts of lead in the pectic acids fraction isolated from the cell walls. It would seem, therefore, that the decrease in plastic and elastic extensibility of tissues observed in our experiments in the presence of lead confirms the possibility of Pb^{++} acting by way of increasing the rigidity of the cell wall, thus depressing its extensibility. This might result in the reducing influence of lead on water uptake, observed in the present experiments.

The present investigations show a distinct difference between green tissues and etiolated ones in their reaction to the toxic action of lead. The green tissues, namely, tested by us were more susceptible to Pb^{++} than etiolated ones. Our experiments, however, have not definitively elucidated this problem.

Acknowledgement

This work was supported by a grant from the Institute of Soil Cultivation and Fertilisation in Puławy, problem No. 09.4.06.

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Wpływ ołowiu na indukowane auksyną wydłużanie komórek

Streszczenie

Praca przedstawia wpływ chlorku ołowiawego na wzrost tkanek roślinnych. Ołów obniżał wydłużanie etiolowanych wycinków koleoptyli pszenicy, fragmentów zielonych epikotyli grochu oraz etiolowanych i zielonych hypokotyli słonecznika. Zielone tkanki były bardziej wrażliwe na działanie ołowiu niż tkanki etiolowane. $PbCl_2$ w stężeniu 10^{-4} M istotnie zmniejszał rozciągliwość plastyczną i elastyczną ścian koleoptyli pszenicy jak również zmniejszał uwodnienie wycinków hopokotyli słonecznika. Auksyna (kwas indolilo-3-octowy — IAA) podana w optymalnych stężeniach dla wzrostu poszczególnych tkanek osłabiała częściowo hamujące działanie ołowiu na wydłużanie, plastyczną i elastyczną rozciągliwość oraz absorpcję wody. Auksyna stosowana w stężeniach ponadoptymalnych nie znosiła hamującego działania ołowiu na wzrost tkanek.