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Effects of Pb-Surplus and P-Deficiency on ATP Content in Plant Leaves

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植物葉의 ATP含量에 미치는 Pb過剩 및 P缺乏의 影響

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ABSTRACT

Kidneybean (*Phaseolus vulgaris* L.) and buckwheat(*Fagopyrum esculentum* Mönch) seedlings grown with Hoagland solution to a height of 7 to 10 cm in the earthen pot containing sand were used for experimental plants. One group of the experimental plants was irrigated with Hoagland solution composed of various Pb-concentrations of 0 to 1000 ppm containing 30 ppm, and the other group was irrigated with Hoagland solution composed of various P-concentrations of 0 to 1,000 ppm containing 30 ppm Pb for a month during June and July in 1979. By Pb-surplus over 100 ppm, the margins of buckwheat were curled down and turned into yellow-brown chlorosis, but the leaves of kidneybean were become dark-green at an early stage, and then developed chlorosis, finally shedded earlier than the control. Pb-toxicity was similar to the symptoms of P-deficiency. The ratio, 3/1 of chlorophyll a/b at an early stage, was unaffected by Pb-surplus or P-deficiency, but at the later stage it was altered.

ATP content with Pb-surplus of 100 ppm was decreased by 69% in kidneybean leaves and by 38% in buckwheat leaves, and it with P-deficiency was decreased by 75% in kidneybean leaves and by 43% in buckwheat leaves. In the assay of ATP content of the leaves at intervals of three hours for one day of July in the year, the rhythms of ATP level were unaffected by light or temperature although the amplitude of the level was modified. The rhythms of Pb-surplus and P-deficiency plants were observed at the lower range of ATP content than that of control.

INTRODUCTION

It was reported that by the interaction between Pb^{2+} and phosphate groups microcrystalline precipitates of $Pb_3(PO_4)_2$ were formed in the internal or on the external surface of the cell (Malone *et al.*, 1974; Schulze and Brand, 1978; Sung, 1976; Sung and Jung, 1977). The toxic effects of Pb differed according to plant types (Sung, 1976; Sung and Jung, 1977; Takahashi *et al.*, 1976). Generally, some metals like Hg^{2+}, Ag^{+}, Pb^{2+}, and Zn^{2+} are poisonous because they combine with and inactivate ligands containing SH, PO_4 and COOH groups of biopolymers and membranes; imidazole and amino-groups have little importance for Pb^{2+} complex formation (Martin, 1968; Somers, 1960; Venugopal and Lucky, 1978). The inhibition of photosynthesis and respiration by Pb was recognized by several studies (Hung *et al.*, 1974; Koeppe and Miller, 1970, 1972; Milles *et al*., 1972). Phosphate plays a key role in ATP involved metabolism and is known as a component of sugar phosphate, nucleotides, coenzymes and phospholipids (Lin and Hanson, 1974; Noggle and Fritz, 1976). Because of P-deficiency induced by Pb the phosphorylation may be impared (Hung *et al*., 1974; Venugopal and Lucky, 1978).

Several studies on ATP have confirmed the unaffected ATP levels by light (Gower and Posner, 1979), the decrease of respiration and ATP level by cadmium (Keck, 1978), the increase of ATP content and higher energy charge in cold treated leaves (Sobczyk and Alina, 1978), the energy source of ATP for potassium phosphate absorption (Lin and Hanson, 1974), and the inhibition of cyclic photophosphorylation by diethylstilbestrol (Balke and Hodges, 1979), etc. The various studies were conducted on the inhibition of ATPase by heavy metal (Spalding, 1979; Tyler, 1974) and dichlorophenyl dimethylurea (Gower and Posner, 1979), as well as on the stimulation of ATPase activity by K^+ and Mg^{2+} in plasma membrane (Fisher *et al*., 1970; Leonard *et al*., 1973; Leonard and Hotchkiss, 1976). On the other hand the rhythms of bioluminescence in *Gonyaulax* was reported to cause a large delay phase swift by exposure to vanillic acid (Kiessig *et al*., 1979). Recently, a study was conducted on Pb-toxicity and P-deficiency in *Chlamidomonas* (Schulze and Brand, 1978).

Effects of Pb-toxicity and P-deficiency on ATP content and the rhythm of ATP level in plant leaves have not been clearly understood. The purpose of the present study was to determine these effects on ATP content and the rhythm of ATP level.

MATERIALS AND METHODS

Plant Growth Kidneybean (*Phaseolus vulgaris* L.) and buckwheat(*Fagopyrum esculentum* Mönch) seeds were germinated and grown with Hoagland solution to a height of 7 to 10 cm in a 2 liter earthen pot containing sand previously washed with distilled water, under the greenhouse condition at 20 to 30°C for two weeks during June and July, 1979. After the two weeks the seedlings were thinned out to 8 plants per pot. These plants were grown for one month with the following treatments; for one group, the concentrations of 0, 0.1, 1, 10, 100 and 1000 ppm Pb solutions were prepared from $PbCl_2$ with Hoagland solution containing 30 ppm P from KH_2PO_4, and then 300 *ml* of each of the final solutions were supplied to each of the Pb treatments on alternate day; for the other groups, the concentrations of 0, 0.1, 1, 10, 100 and 1000 ppm P solutions were prepared from KH_2PO_4 with Hoagland solution containing 30 ppm Pb, and then the final solutions were supplied to each of the P treatments in the same way. The pH of culture solutions were adjusted to 6.5. The leaves of the cultured plants were collected to determine dry weight, chlorophyll content, respiration, ATP content and the rhythym of ATP level.

Chlorophyll Content The content of chlorophyll a and b was estimated by a modifications of the method of Arnon (1949) with UV-190 Spectrophotometer.

Leaf Respiration The CO_2 evolved by leaf respiration was measured as previously reported (Sung, 1979).

ATP Content Each leaf sample of 3 g was boiled with 30 *ml* tris buffer of pH 7.4 for three minutes to kill the cells rapidly and inactivate the ATPase contained in the cells themselves. The boiled leaf solution was replenished for evaporated moiety to 30 *ml* by adding some precooled tris buffer solution and transferred to ice-jacketed homogenizer vessel. After homogenizing the leaf in the solution at 4500 rpm, 0°C, for 10 minutes, the homogenate was filtered through two layers of gauge. This filtrate was used to measure the ATP content of the leaves. The ATP content of filtrate was determined at 0°C by using a SAI Technology Co. 2000 ATP-Photometer which measures the fluorescence of 560-580nm wavelength emitted by the hydrolysis of ATP contained in 1 *ml* of the mixture composed of 0.5 *ml* of the filtrate and 0.5 *ml* luciferin-luciferase, and then by referring the peak reading of the photometer to the standard curve of ATP.

Rhythm of ATP Level The leaves of both plants treated with Pb and P concentrations were collected at intervals of three hours for one day on July. To determine the rhythm of ATP level, the ATP content of the leaves was measured as soon as leaf collections. The light intensity and temperature in the greenhouse during leaf collection were showed.

RESULTS AND DISCUSSION

Symptoms of Pb-Toxicity The symptoms of Pb-toxicity in kidneybean leaves was characterized by dark-green leaves at early stage, later converted into chlorosis and finally shedded 15 days earlier than the control leaves. Newly formed kidneybean leaves were simillar in size and dark-green colered. The buckwheat leaves did not show the symptoms of Pb-toxicity at 100 ppm Pb unlike kidneybean, but the margins of leaves curled downward and developed marginal chlorosis. The toxicity symptoms by Pb was similar to that of P-deficiency in both plants. The tolerance to Pb-toxicity was different with plant species.

Pb-toxicity sometimes induce P-deficiency in plants as in the present study, because the lead-phosphate complexes are precipitated in and also on organisms (Malone *et al.*, 1974; Schulze and Brand, 1978; Sung, 1976). These symptoms, similar to Pb-toxicity, were also found in the case of Al-toxicity (Edwards *et al.*, 1976; Edwards and Horton, 1977) and P-deficiency (Gauch, 1973). It was thought that the chlorophyll synthesis was impared by inactivation of enzyme related to chlorophyll precursors, because aminoevulinic acid dehydratase and porpyrinogen decarboxylase in animal cell were inactivated by Pb (Venugopal and Lucky, 1978).

Dry Weight The change in total dry weight of shoot and root in kidneybean and buckwheat treated with 100 ppm Pb containing 30 ppm Pwas not significant, but the dry weight of those treated with 1000 ppm Pb containing 30 ppm P was decreased by 60% in kidneybean and by 80% in buckwheat (Fig. 1). In the low concentration of 0.1 ppm Pb containing 30 ppm P, a little increase of dry weight was

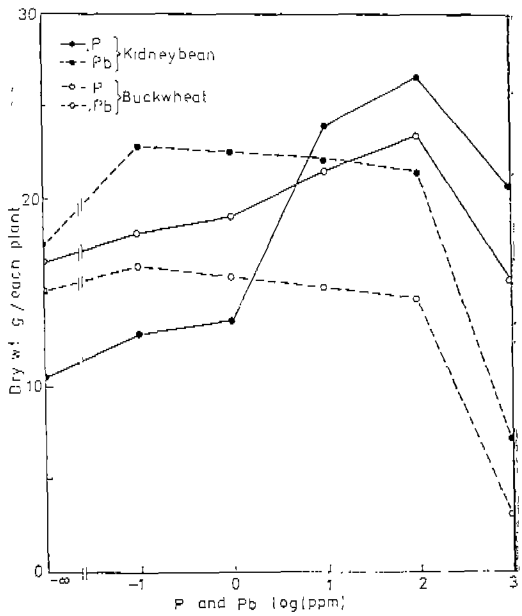

Fig. 1. The total dry weight of each plant in kidneybean and buckwheat plants grown by sand culture of various concentrations of P and Pb for a month. Significant at 1% level in 1,000 ppm Pb.

observed in both plants as previously reported (Takahashi *et al.*, 1976). It has been reported that the lead (30 mM) inhibited pod growth in fresh was thought soybean by 35% (Hung et al., 1974). It weight of that the decrease of dry weight by lead mightdepend on the equivalance between Pb and P concentration.

Chlorophyll a and b The application of low 30 ppm Pb with high 1,000 ppm P concentration increased chlorophyll a and b content in both kinds of plants slightly (Fig. 2). However the application of high 1,000 ppm Pb containing low 30 ppm P concentration did not affect significantly the content of chlorophyll a and b in both plants (Fig. 3). The ratio, 3/1 of chlorophyll a/b content, was not altered by Pb-surplus at early stage. However, at later stage, the chlorophyll a and b content was affected by Pb-surplus (Fig. 3).

It has been reported that lead inactivates aminoevulinic acid dehydratase involved in the conversion of δ-aminolevulinic acid to porphobilinogen and also inactivates porphyrinogen decarboxylase

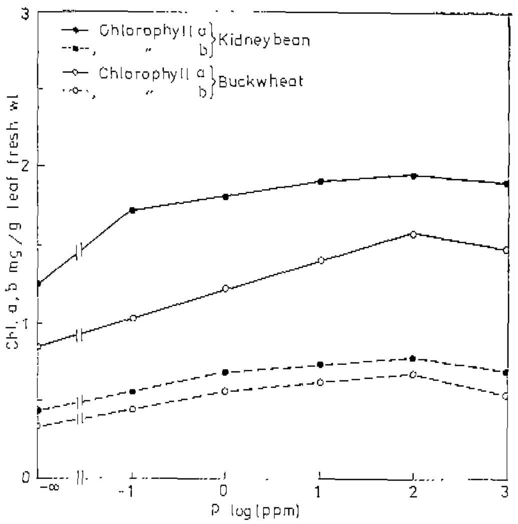

Fig. 2. Chlorophyll a and b content of the leaves in kidneybean and buckwheat plants grown by sand culture of various concentrations of P for a month. No significant among P concentrations.

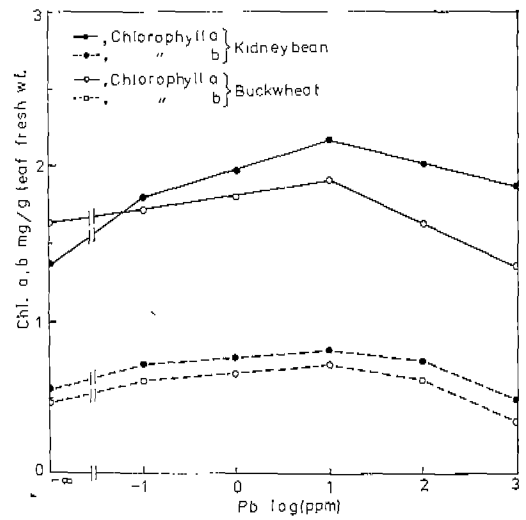

Fig. 3. Chlophyll a and b content of the leaves in kidneybean and buckwheat plants grown by sand culture of vaious concentrations of Pb for a month. No significant among Pb concentrations.

(Venugopal and Lucky, 1978). These results suggest that lead may affect the synthesis of chlorophyll.

Leaf Respiration Respiration of the leaves with P supply was increased, but decreased by 36% in kidneybean and by 10% in buckwheat with application of 10 ppm Pb containing 30 ppm P. By the application of 100 ppm Pb containing 30 ppm P, leaf respiration was decreased by 80% in kidneybean and by 30% in buckwheat (Fig. 4). By the application of 1000 ppm Pb contained 30 ppm P, the respiration was decreased significiantly, by 90% in kidneybean and by 50% in buckwheat (Fig. 4). The leaf respiration was inhibited by the application of low P and high Pb concentration, whereas by the application of high P and Pb concentration, the respiration was increased. The effect of Pb on leaf respiration in both plant was different from each other. The inhibition of respiration by Pb has been already reported (Koeppe and Miller, 1970, 1972) and the inhibition of respiration in plants by some heavy metals (Cd, Hg and Pb) has also been reported (Keck, 1978; Sung, 1979).

ATP Content The ATP content of leaves were increased in kidneybean treated with 0.1 ppm Pb containing 30 ppm P, and in buckwheat treated with 10 ppm Pb containing 30 ppm P. Although the ATP content with the application of 100 ppm

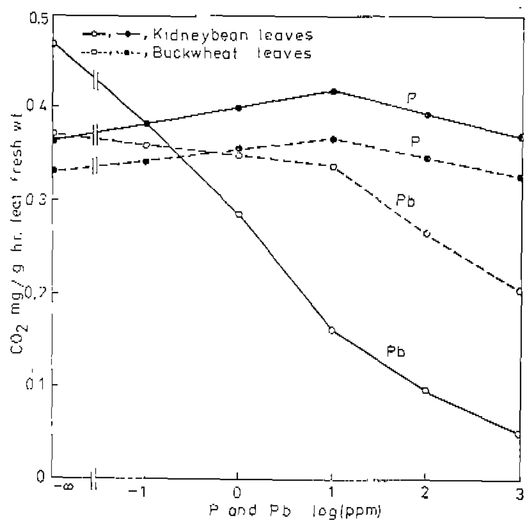

Fig. 4. The leaf respiration of kidneybean and buckwheat plants grown by sand culture of various concentrations of Pb for a month. Significant at 1% level in kidneybean of 100 ppm Pb.

Pb containing 30 ppm P was decreased by about 30% in kidneybean leaves, it was unaffected in buckwheat leaves. By the application of 1000 ppm Pb containing 30 ppm P, ATP content was decreased by 70% in kidneybean leaves and by 33 % in buckwheat leaves (Fig. 5). In the report similar to the present study, exogenously supplied adenosine and adenine were known to increase the adenine nucleotide pools (Anderson, 1977). Generally, phosphate plays a key role in ATP involved reaction in plants (Noggle and Fritz, 1976). In several studies, the decrease of ATP level similar to the present study was reported; the inhibition of phosphorylation by dichlorophenyl dimethylurea (Gower and Posner, 1979), the inhibition of ATP-ase by diethylstilbestrol (Balke and Hodges, 1979) and the decrease of ATP level by 1 mM $CdSO_4$ (Keck, 1978). It is likely that the decrease of ATP level by lead may be related to the inhibition of ATP metabolism by lead complex with SH-, PO_4- and COOH-carring ligands, and forming colloidal lead phosphate, diffusible lead peptides, or Pb-organic acid complexes (Venugopal and Lucky, 1978).

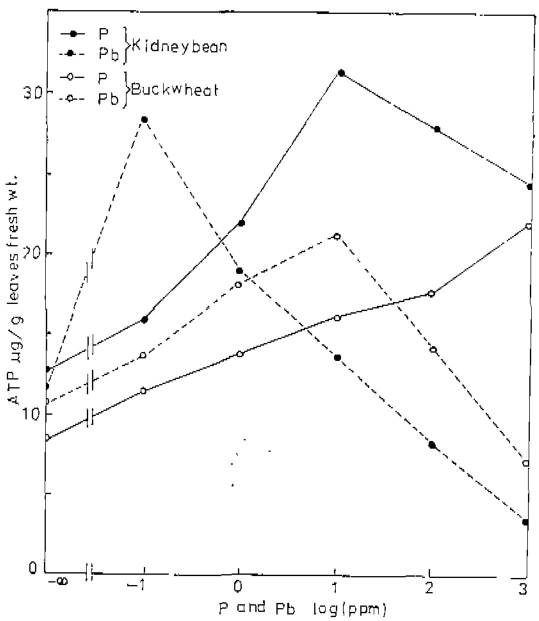

Fig. 5. ATP content in the leaves of kidneybean and buckwheat plants grown by sand culture of various concentrations of P and Pb for a month. Significant at 1% level in kidneybean of P and Pb.

Rhythm of ATP Level From the determination of ATP content in leaves at intervals of three

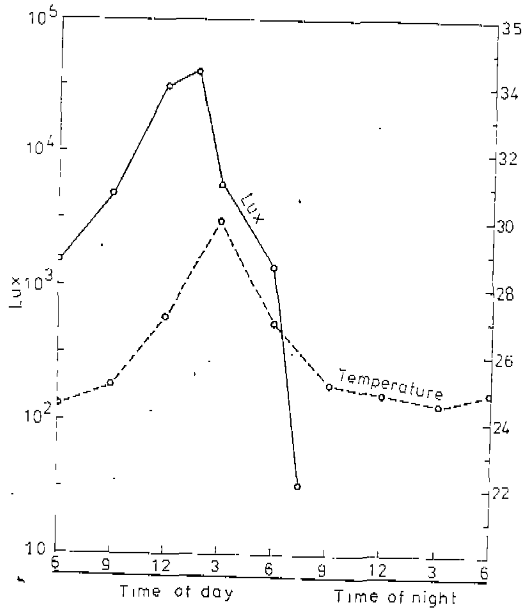

Fig. 6. The environmental conditions of sampling at intervals of three hours for measuring of ATP content in the leaves (July 25, 1979)

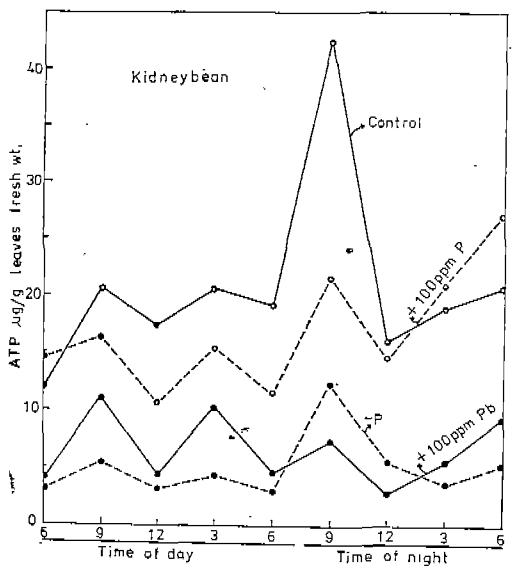

Fig. 7. The rhyhtm of ATP level in the leaves of kidneybean plant determined at intervals of three hours for a day. Significant at 1% level among the four groups.

hours for a day, it could be assumed that there was a certain rhythm of ATP level in both plants (Figs. 7 & 8). These rhythms showed self-reliance without being affected by light intensity or temperature under the greenhouse condition at 20 to 30°C (Fig. 6, Table 2) which resembles the previous study on ATP content being unaffected by low temperature (Sobczyk and Alina, 1978). The level of the ATP rhythm in the leaves of Pb-surplus(100 ppm) and P-deficiency was somewhat lower than that of control (Figs. 7 & 8). With Pb-surplus containing 30 ppm P, the average level of ATP was damped down by 69% in kidneybean and by 38% in buck wheat, and with P-deficiency it was damped down by 75% in kidneybean and by 43% in buckwheat (Table 1). The period of rhythm in ATP level was not altered by lead, although the average

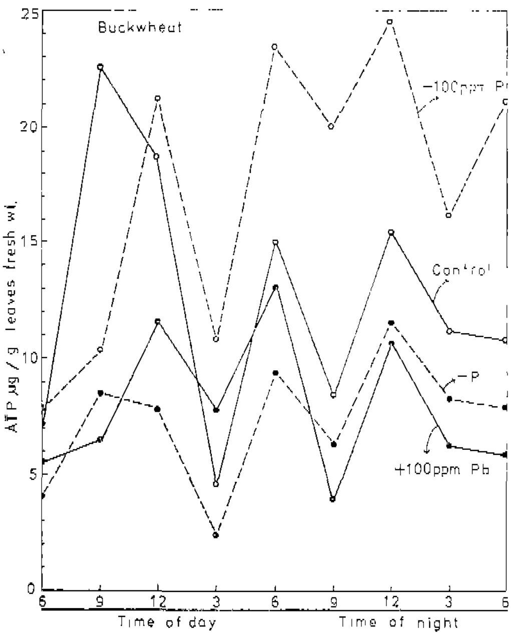

Fig. 8. The rhythm of ATP level in the leaves of buckwheat plant determined at intervals of three hours for a day.

Table 1. The average level of ATP content measured at intervals of three hours for a day in the leaves of plants grown with P and Pb treatments for a month

Supply \ Leaves	ATP(μg/g fresh wt.) Kidneybean	Buckwheat
Control	20.97	12.65
−P +30 ppm Pb	5.20	7.22
+P 100 ppm +30 ppm Pb	17.01	17.26
+Pb 100 ppm +30 ppm P	6.61	7.85
Mean	12.45	11.25
Significance	P<1%	P>5%

Table 2. Correlation(r) between the rhythms of ATP level and the variations of temperature(°C) or lightintensity (lux) under the greenhouse conditions during day and night

Plants	Temperature		Lux	
	r-Value	Significance	r-Value	Significance
Kidneybean Control	−0.008	none	+0.003	none
−P +30 ppm Pb	−0.213	none	+0.332	none
100 ppm P +30 ppm Pb	−0.272	none	−0.553	none
100 ppm Pb +30 ppm P	+0.258	none	+0.290	none
Buckwheat Control	+0.248	none	+0.004	none
−P +30 ppm Pb	+0.495	none	+0.210	none
100ppm P +30 ppm Pb	−0.102	none	+0.328	none
100ppm Pb +30 ppm P	+0.337	none	+0.448	none

level of the fluctuation of ATP level with lead treatment was lower than that of control (Fig. 7, 8). It was thought that the period of the rhythm may be controlled by the genes involved in ATP metabolism as already reported (Salisbury and Ross, 1979). The rhythm of the bioluminescence depending on heredity was also reported recently (Kiessig *et al.*, 1979).

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摘 要

강낭콩(*Phaseolus vulgaris* L.) 및 메밀(*Fagopyrum esculentum* Mönch)의 種子를 Hoagland solution으로 二週間 砂耕栽培한 후 Pb 및 P의 濃度別 培養液을 隔日로 灌水하여 1個月間 처리하였다. Pb 및 P의 濃度別 培養液은 Hoagland solution에 Pb 및 P를 각각 0~1,000 ppm에서 6個 濃度區로 만들고, Pb 濃度에는 P 30 ppm을, P 濃度區에는 Pb 30 ppm을 添加하였다.

100 ppm Pb 過剩處理에서 葉의 ATP 含量은 control에 比하여 강낭콩의 境遇 69%, 메밀의 境遇 38% 減少하였으며, P 缺乏에서는 강남콩의 경우 75%, 메밀의 境遇 43% 減少하였다. 葉中 ATP 含量의 變化를 하루동안 3시간 간격으로 測定한 結果, 溫室條件下의 照度와 溫度에 관계없이 ATP의 含量變化에 一定한 週期(rhythm)가 있음을 發見할 수 있었으며, Pb 過剩 P 및缺乏區는 control에 比하여 ATP rhythm의 水準이 낮게 나타났다.

植物葉의 Pb 過剩症狀과 P 缺乏症狀은 類似하게 나타났으며, 初期에는 黑綠色을 나타냈으나, 生長後期에는 chlorosis를 나타내면서 control 보다 15日 前에 落葉하였다. 呼吸에 미치는 Pb의 濃度別 影響은 100 ppm Pb 以上의 濃度에서 control 보다 呼吸量이 減少되었다.

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