Effect of indole-3-acetic acid on lead accumulation in maize (*Zea mays* L.) seedlings and the relevant antioxidant response

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Abstract

The effect of indole-3-acetic acid (IAA) on lead (Pb) accumulation in maize (*Zea mays* L. cv. TY2) seedlings, and the responses of maize to Pb, IAA or Pb + IAA stress were investigated under hydroponic conditions. Exogenous IAA significantly increased Pb accumulation in roots, but significantly decreased Pb accumulation in shoots over the culture period of 5 days. Increased Pb uptake in maize roots partly took place through calcium and potassium channels, and also depended upon membrane potential. Lipid peroxidation in terms of malondialdehyde (MDA) content was enhanced, indicating that Pb and/or IAA caused oxidative stress in maize seedlings. Under stress conditions the antioxidant defense mechanism was activated. Some antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were up-regulated compared to the control. Pb, IAA or Pb + IAA treatments resulted in a general reduction of macro- and micronutrient contents, especially of K, Ca and Mn. Compared to a single Pb or IAA treatment, treatment with a combination of Pb + IAA induced more severe oxidative stress on maize roots and shoots as inferred from the higher observed MDA and lower nutrient contents. A Fourier transform infrared (FTIR) spectroscopic study indicated that Pb reacted with the carboxylic acid group of the IAA pyrrole cycle.

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1. Introduction

Lead (Pb) is a highly toxic metal which can be potentially harmful to microbes, plants, animals and humans at elevated levels. Therefore, uptake and accumulation of Pb in plants poses a potentially serious health risk to humans through contamination of the food chain. Pb causes multiple direct and indirect effects on plant growth and metabolism. Auxins are organic substances that regulate intracellular processes and modify physiological processes in plants. Auxinic herbicides such as 2,4-dichlorophenoxy acetic acid (2,4-D) and auxin phytohormones have been widely used in agriculture. Indole-3-acetic acid (IAA) is the most abundant naturally occurring auxin, well known for its regulatory function in plant growth. López et al. (2004) reported that 0.2 mM Pb + 10 mM gibberellic acid

increased Pb concentration in alfalfa (*Medicago sativa* L.) roots by about 40% compared to treatment with Pb only. Treatment with 0.2 mM EDTA + 100 μM IAA increased Pb accumulation in leaves of alfalfa by factors of 28 and 6, respectively, when compared to the Pb content in leaves when exposed to Pb only or to Pb + EDTA in combination (López et al., 2005).

Reactive oxygen species (ROS) such as the superoxide anion ($\text{O}_2^{\bullet-}$), singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\bullet\text{OH}$) all induce oxidative damage because they are highly reactive molecules that can oxidize organic compounds and cause lipid peroxidation in a cellular environment. Malondialdehyde (MDA) is one of the major decomposition products of the polyunsaturated fatty acids in biomembranes and has been widely used as an indicator of lipid peroxidation. Many heavy metals, including Pb, are known to induce over production of ROS and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membranes of several plant species (Halliwell and Gutteridge, 1999; Malecka et al., 2001). IAA also generates

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ROS, excites oxygen intermediates (De Melo et al., 1992; Joo et al., 2005) and increases MDA content in various rat tissues (Celik and Tuluce, 2006).

Plants have evolved a variety of mechanisms to deal with the effects of ROS in cellular compartments. This is generally handled through the production of various antioxidative enzymes, such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). For example, SOD, a metallo-enzyme, can catalyse the dismutation of $O_2^{\bullet-}$ into H_2O_2 and O_2 , and subsequently H_2O_2 can be effectively scavenged by CAT and POD. Heavy metal ions can increase the activity of SOD in oat (*Avena sativa* L.) (Luna et al., 1994) and in rice (*Oryza sativa* L.) (Verma and Dubey, 2003). Heavy metals may also decrease (Mishra and Choudhuri, 1996) or not affect at all the SOD activity (Cardinales et al., 1984). Peroxidases utilize H_2O_2 in the oxidation of various organic and inorganic substrates. Peroxidases utilizing guaiacol as an electron donor *in vitro* are called guaiacol peroxidases. A strong induction of POD activity in response to Pb was reported in thale cress (*Arabidopsis thaliana* (L.) Heynh) (Lummerzhim et al., 1995), rice (*Oryza sativa* L.) (Verma and Dubey, 2003) and maize (*Zea mays* L.) calli (Zacchini et al., 2003). However, the inhibition of POD activity due to heavy metals has also been observed in oat leaves (Luna et al., 1994). CAT is a heme-containing enzyme that converts H_2O_2 into H_2O and O_2 . Generally, CAT activity is increased in plants due to heavy metal stress (Mishra and Choudhuri, 1996). The effect of IAA on antioxidant enzymes is mostly concerned with animals. De Melo et al. (2004) reported that rat neutrophils and lymphocytes in the presence of IAA (1 mM) increased the activities of SOD, CAT and glutathione peroxidase, which could be an attempt at self-protection.

Pb is one of the most abundant heavy metal pollutants in both aquatic and terrestrial environments while IAA exists in various biologically active compounds, such as herbicides, but there is little information on its negative effect on higher plants. Therefore, an understanding of Pb and IAA interaction in plants is essential. López et al. (2004, 2005) studied the effect of IAA on Pb accumulation in alfalfa, but the relevant mechanisms were not discussed. To date there is very little information on the specific responses of maize seedlings to either IAA only or the combined effect of Pb + IAA in combination. We hypothesized that exogenous IAA affects Pb accumulation, causes oxidative stress and activates antioxidant defense mechanisms. The aim of the present research was to study the effect of IAA on Pb accumulation in maize, and to gather more information on the relevant mechanisms involved. The question of how maize responds to Pb, IAA or Pb + IAA stress was also addressed by examining some biochemical changes in the plants including lipid peroxidation, antioxidant enzyme concentration and nutrition level.

2. Materials and methods

2.1. Plant cultivation and treatment

Maize (*Zea mays* L. cv. TY2) seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China). Seeds were first surface-sterilized in 3% (v/v) H_2O_2 for 30 min,

rinsed with deionized water thoroughly and soaked in 2.8 mM $Ca(NO_3)_2$ for 4 h. Seeds were then germinated on moist filter papers for 4 days in dark and subsequently transferred to a hydroponic solution containing the following nutrients: 0.33 mM $Ca(NO_3)_2$, 0.17 mM $Ca(H_2PO_4)_2$, 0.17 mM K_2SO_4 , 0.33 mM $MgSO_4$, 0.5 mM NH_4NO_3 , 75 μ M EDTA-Fe, 46 μ M H_3BO_3 , 9 μ M $MnSO_4$, 0.8 μ M $ZnSO_4$, 0.3 μ M $CuSO_4$ and 0.8 μ M Na_2MoO_4 . Selected maize seedlings were floated in culture vessels (three seedlings per vessel). The vessels were covered with black polyethylene film to minimize illumination of the growth solution and fitted with aeration tubes. The solution was buffered to pH 5.8 with 2 mM Mes-Tris. Maize seedlings were grown in a plant growth chamber with day and night temperature of 22 °C (16 h) and 15 °C (8 h), respectively. A relative humidity between 50 and 60% and a photon flux intensity of 300 μ mol $m^{-2} s^{-1}$ were maintained throughout the entire experiment. Nutrient solutions were replaced with fresh nutrient solutions every other day. In all treatment studies, 10-day-old uniform maize seedlings were used.

Six treatments were established: control (neither addition of Pb or IAA), 250 μ M IAA, 500 μ M IAA, 20 μ M Pb as $Pb(NO_3)_2$, 20 μ M Pb + 250 μ M IAA and 20 μ M Pb + 500 μ M IAA. For the treatment solutions $Ca(H_2PO_4)_2$ was omitted to avoid the possibility of lead phosphate precipitation. Treatment solutions were changed daily. Seedlings were harvested after treatment for 1, 3 and 5 days, rinsed with deionized water, and separated into roots and shoots. The root and shoot samples were dried at 70 °C for 2 days and the content of metals including Pb was determined.

2.2. Effect of cation channel blockers on Pb influx

To better understand the transport pathway of the increased Pb influx, we examined the physiological functions of Ca^{2+} channel inhibitor of La^{3+} and K^+ channel inhibitor of Cs^+ . The original experiments were repeated with 10-day-old uniform maize seedlings, which were now incubated in 20 μ M Pb treatment solution amended with either 0.2 mM La^{3+} as $La(NO_3)_3$ or Cs^+ as $CsCl$ for 4 h. $La(NO_3)_3$ and $CsCl$ have previously been identified as cation channel blockers by White (1997), who also reported appropriate application concentrations. The treatment with 20 μ M Pb or 20 μ M Pb + 250 μ M IAA was used as a control.

2.3. Influence of vanadate on Pb uptake

The effect of vanadate on Pb uptake was examined by treating 10-day-old uniform maize seedlings with 0.1 mM vanadate for 15 min and then exposing the seedlings to a 20 μ M Pb treatment solution, as defined in Section 2.1, for an additional treatment period of 4 h.

2.4. Extraction of enzymes and activity assays

2.4.1. Extraction of enzymes

Fresh root and shoot samples (0.5 g) from control and treated maize seedlings were ground in liquid nitrogen using a mor-

tar and pestle. The ground samples were homogenized on an ice-bath in 10 mL of solution containing 50 mM potassium phosphate buffer and 1% (w/v) polyvinylpyrrolidone (pH 7.8) and were extracted at 4 °C. The homogenate was centrifuged at $8000 \times g$ for 15 min at 4 °C. The supernatant was stored at 4 °C prior to analysis of the SOD, CAT and POD activities.

2.4.2. SOD activity

SOD activity was measured by the spectrophotometric method of Giannopolitis and Ries (1977). The assay was performed in terms of SOD's ability to inhibit reduction of ρ -nitroblue tetrazolium chloride (NBT) to formazan by the superoxide radical. One unit of SOD activity is defined as the amount of enzyme required to inhibit NBT reduction by 50% when measured at 560 nm. All spectrophotometric analyses were conducted on an ultraviolet-visible spectrophotometer (U-3010, Hitachi Ltd., Tokyo, Japan).

2.4.3. POD activity

POD activity was assayed following the method of Polle et al. (1990). Changes in the absorbance of brown guaiacol at 460 nm in the presence of H_2O_2 were recorded for calculating POD activity. The enzyme activity was defined as the nmol of H_2O_2 oxidized $\text{min}^{-1} \text{mg}^{-1}$ protein.

2.4.4. CAT activity

CAT activity was assayed after Aebi (1983). A reaction mixture consisting of supernatant (666 μL) and 73 mM H_2O_2 (334 μL) was assayed for 3 min at 240 nm. The activity was assayed by monitoring the decrease of absorbance as a consequence of H_2O_2 consumption. The enzyme activity was expressed as decomposed nmol of H_2O_2 $\text{min}^{-1} \text{mg}^{-1}$ protein.

2.4.5. Soluble protein

Total soluble protein content of the enzyme extracts were determined by reaction with Coomassie Brilliant Blue G-250 according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

2.4.6. Analysis of MDA in maize roots and shoots

Lipid peroxidation in roots and shoots was measured in terms of MDA content by the thiobarbituric acid (TBA) reaction. The maize roots and shoots were homogenized in 5% trichloroacetic acid (TCA) solution (3 mL). The homogenate was centrifuged at $2500 \times g$ for 10 min and the supernatant was assayed for MDA concentration according to the method of Heath and Packer (1968). MDA content was expressed as nmol g^{-1} fresh weight (FW).

2.5. Determination of metals

Root and shoot samples were digested with 3 mL of concentrated HNO_3 – HClO_4 (2:1, v:v) under high pressure conditions (Zhang and Shan, 1997). Concentrations of Pb, K, Ca, Mg, Fe, Mn, Cu and Zn in digested samples were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Optima 2000 DV, Perkin-Elmer Co., Wellesley,

MA, USA). A certified reference material: peach leaves (GBW 08501, China) was used to ensure the quality of analyses. Good agreement was obtained between the determined and the certified values (data not shown).

2.6. FTIR spectroscopic study

A bundle of 10-day-old uniform maize seedlings were transferred to nutrient treatment solution with or without 20 μM Pb, 250 μM IAA or 20 μM Pb + 250 μM IAA. The seedlings were harvested 1 day after treatment. The fresh maize roots were freeze-dried and ground in an agate mortar and pestle. A portion of the ground roots (1 mg) and 100 mg of KBr (FTIR Grade, Alfa Aesar) were mixed and pressed into pellets. Infrared spectra were obtained on a Perkin-Elmer GX/2000 Fourier transform infrared (FTIR) spectrometer equipped with deuterated triglycine (DTGS) and mercury-cadmium-telluride (MCT) detectors, a KBr beam splitter and a sample bench purged with dry air. The resolution of FTIR spectra was 4.0 cm^{-1} , and a total of 32 scans were collected for each spectrum. Spectral analysis was performed using OMNIC (Thermo Nicolet) software.

2.7. Statistical analysis

Data analysis was performed with Microcal origin 7.0 (Microcal Software, Northampton, MA, USA) or SPSS 12.0 for windows (SPSS, Chicago, IL, USA). Data were statistically evaluated by ANOVA and significant differences were assessed by Tukey's test. Comparison of multiple means was performed using Duncan's multiple range test with $P < 0.05$. All values reported in this work were means of at least three independent experiments performed in triplicate.

3. Results

3.1. Pb accumulation in maize as affected by IAA

Table 1 shows Pb content as affected by IAA in both maize roots and shoots after 1, 3 and 5 days treatment. Compared to the Pb only treatment, exogenous IAA (250 and 500 μM) significantly increased Pb content in roots, but significantly decreased Pb content in shoots, especially for the treatment periods of 3 and 5 days. The 500 μM IAA treatment triggered higher Pb content in roots and lowered Pb content in shoots, compared to the treatment of 250 μM IAA. In maize roots application with 250 and 500 μM IAA increased Pb content by 26 and 41, 42 and 57, 78 and 144% after 1, 3 and 5 days treatment, respectively, compared to the Pb only treatment. In contrast, the corresponding Pb content in maize shoots decreased by 27 and 43, 41 and 60, 34 and 47%, after 1, 3 and 5 days treatment, respectively.

3.2. Effects of cation channel blockers and vanadate on Pb uptake

Fig. 1 shows the effects of La^{3+} (the Ca^{2+} channel inhibitor), Cs^+ (the K^+ channel inhibitor) and vanadate on Pb uptake and indicates that La^{3+} and Cs^+ significantly inhibited Pb influx

Table 1

Pb contents ($\mu\text{mol g}^{-1}$ DW) in the presence and absence of 250 μM or 500 μM IAA in maize roots and shoots after 1, 3 and 5 days (d) treatment

Treatment	Root			Shoot		
	1 d	3 d	5 d	1 d	3 d	5 d
Pb	5.03 $\pm$ 0.02	10.64 $\pm$ 0.04	14.88 $\pm$ 0.98	0.79 $\pm$ 0.02	1.99 $\pm$ 0.04	3.55 $\pm$ 0.07
Pb + 250 μM IAA	6.32 $\pm$ 0.07	15.02 $\pm$ 0.03**	26.42 $\pm$ 0.05**	0.58 $\pm$ 0.04	1.18 $\pm$ 0.13*	2.33 $\pm$ 0.16*
Pb + 500 μM IAA	7.06 $\pm$ 0.13*	16.65 $\pm$ 0.14**	36.33 $\pm$ 0.18**	0.45 $\pm$ 0.03*	0.79 $\pm$ 0.03**	1.88 $\pm$ 0.15*

Values are means $\pm$ S.D. ($n = 3$).* Significant difference between Pb + IAA and Pb treatments for 1, 3, 5 days at $P < 0.05$.** Significant difference between Pb + IAA and Pb treatments for 1, 3, 5 days at $P < 0.01$.

($P < 0.01$). Compared to the controls of 20 μM Pb and 20 μM Pb + 250 μM IAA, La^{3+} decreased the Pb content in maize roots by 61 and 59%, respectively. Analogously, Cs^+ decreased the Pb content by 33 and 39%, respectively. Pre-treatment of maize roots by vanadate, a H^+ -ATPase inhibitor, for 15 min, decreased Pb content in maize roots by 58 and 54%, respectively, relative to the 20 μM Pb, and 20 μM Pb + 250 μM IAA controls ($P < 0.01$).

3.3. Antioxidant enzyme activity

The activities of antioxidant enzymes SOD, POD and CAT in maize roots and shoots under Pb, IAA or Pb + IAA treatments are shown in Fig. 2. Although not statistically tested, the level of SOD activity in roots (Fig. 2A) was quite similar to that in shoots (Fig. 2B), while the level of POD activity in roots (Fig. 2C) was higher than in shoots. CAT activity (Fig. 2E) was lower in roots than in shoots (Fig. 2F). The SOD, POD and CAT activities in roots and shoots treated with 250 μM IAA, 500 μM IAA or 20 μM Pb steadily increased with increasing treatment time, and the highest activities were observed on day 5. On the 5th day, SOD and POD activities with Pb + IAA treatments in roots were lower than either Pb or IAA only treatments. Results of SOD and POD activities in shoots were less definitive. CAT activities in roots with Pb + IAA treatments were higher than CAT activities observed in either Pb or IAA only treatments, but conversely in shoots, CAT activities were lower in the Pb + IAA

treatment when compared to either the Pb or IAA only treatments. The highest CAT activities were observed in roots treated with Pb + 500 μM IAA and these were 2.1, 5.8 and 3.9 times that of the CAT activities in the controls following treatment for 1, 3 and 5 days, respectively. After the 3 and 5 days treatments, CAT activity in shoots with Pb or IAA treatments were significantly higher than CAT activity in the controls, while CAT activity in shoots with Pb + IAA was not significantly different from controls.

3.4. Effect on lipid peroxidation

Our results indicated a progressive increase in the MDA content of maize roots (Fig. 2G) for Pb and IAA treatments from day 1 to 5. The MDA content of roots was higher than that of shoots from the third day. The increase in MDA content ranged from 1.2 to 4.2 and 1.3 to 2.2 times the controls in roots and shoots, respectively. In maize roots on day 1 a significant increase in MDA content compared to the control was observed only with 20 μM Pb + 500 μM IAA treatment. From day 3 all treatments significantly promoted MDA content.

3.5. Effect on nutrition level

There was a general decline of all the macro- and micronutrient levels in maize roots and shoots after Pb, IAA or Pb + IAA treatments when compared to their respective controls (Tables 2 and 3). In roots, treatment with Pb only significantly decreased Mn content from day 3 to 5, but did not significantly decrease K content. Treatment with Pb only decreased Ca content in roots on day 5. On day 5, both the IAA and Pb + IAA treatments caused a significant decrease in K, Ca, Mn and Zn contents of roots. Significant decreases in Cu and Zn contents were observed in maize shoots and roots due to IAA and Pb + IAA treatments.

In shoots, treatment with Pb only resulted in a decrease in Mn content on day 5 and had no effect on the other nutrients during the whole treatment period. However, treatment with IAA only or Pb + IAA caused significant decreases in K, Ca, Mn and Cu contents of the shoots. Mg and Fe contents in roots and shoots were not affected by Pb, IAA or Pb + IAA treatments.

Moreover, as to the general distribution of nutrients in roots and shoots for the six treatments, Ca, Mg and Zn contents in roots were lower than those in shoots, while Fe and Cu contents in roots were higher than those in shoots.

Fig. 1. Effect of cation channel inhibitors La, Cs and the H^+ -ATP inhibitor vanadate on Pb uptake in maize roots with and without 250 μM IAA application. The uptake time is 4 h.

Fig. 2. SOD, POD, CAT activity and MDA content in maize roots (A, C, E, G) and shoots (B, D, F, H), respectively. The vertical bars represent means $\pm$ S.D. of three experimental replicates ($n=3$). Columns with different letters mean significant differences between the treatments on the same day according to Duncan's multiple range test ($P < 0.05$).

3.6. FTIR spectral analysis

The FTIR spectra of maize roots of control (a), treated with 250 μM IAA (b), 20 μM Pb (c) and 20 μM Pb + 250 μM IAA (d) are shown in Fig. 3. The broad bands at 3373, 3381, 3363 and 3377 cm^{-1} , respectively, are dominated by $-\text{OH}$ stretching vibrations. Absorbance changes were observed at 1653, 1638, 1653 and 1642 cm^{-1} , which were assigned to the antisymmet-

rical $-\text{COO}^-$ stretching. The IAA and Pb + IAA treatments resulted in a red shift of 15 and 11 cm^{-1} , respectively, compared to the control, probably due to the conjunction of $-\text{COOH}$ group and the formation of hydrogen bonds (H-bonds). The peaks at 1384, 1383, 1378 and 1376 cm^{-1} are the vibrations of the $-\text{NO}_2$ group. The Pb and Pb + IAA treatments caused red shifts of 6 and 8 cm^{-1} , respectively, when compared to the control.

Table 2

Nutrient content ($\mu\text{mol g}^{-1}$ DW) in maize roots with different treatment and exposure period, 1, 3 and 5 days (d)

Treatment	K	Ca	Mg	Fe	Mn	Cu	Zn
1 d							
Control	390.1 $\pm$ 55.1	50.5 $\pm$ 3.4	28.0 $\pm$ 4.2	7.04 $\pm$ 1.33	0.30 $\pm$ 0.04	0.59 $\pm$ 0.07	1.75 $\pm$ 0.15
250 μM IAA	271.0 $\pm$ 24.2	45.7 $\pm$ 1.3	25.6 $\pm$ 3.0	5.10 $\pm$ 1.48	0.24 $\pm$ 0.01	0.47 $\pm$ 0.14	1.69 $\pm$ 0.27
500 μM IAA	280.9 $\pm$ 6.6	56.5 $\pm$ 4.4	25.5 $\pm$ 1.5	6.15 $\pm$ 1.22	0.32 $\pm$ 0.09	0.58 $\pm$ 0.05	2.05 $\pm$ 0.21
Pb	281.2 $\pm$ 46.3	54.7 $\pm$ 6.5	30.1 $\pm$ 4.0	9.33 $\pm$ 2.36	0.27 $\pm$ 0.04	0.60 $\pm$ 0.02	1.82 $\pm$ 0.28
Pb + 250 μM IAA	272.5 $\pm$ 37.5	50.3 $\pm$ 6.1	23.3 $\pm$ 1.9	7.33 $\pm$ 1.10	0.24 $\pm$ 0.07	0.46 $\pm$ 0.12	1.70 $\pm$ 0.41
Pb + 500 μM IAA	212.4 $\pm$ 6.8*	44.2 $\pm$ 1.8	24.4 $\pm$ 1.3	5.40 $\pm$ 1.07	0.23 $\pm$ 0.02	0.42 $\pm$ 0.01	1.72 $\pm$ 0.21
3 d							
Control	537.6 $\pm$ 12.9	78.7 $\pm$ 7.5	32.1 $\pm$ 6.3	7.37 $\pm$ 1.84	0.75 $\pm$ 0.11	0.67 $\pm$ 0.17	1.76 $\pm$ 0.42
250 μM IAA	508.7 $\pm$ 6.9	74.4 $\pm$ 3.7	28.1 $\pm$ 2.7	6.31 $\pm$ 0.70	0.42 $\pm$ 0.09*	0.58 $\pm$ 0.11	1.45 $\pm$ 0.31
500 μM IAA	406.6 $\pm$ 75.1	68.7 $\pm$ 6.6	30.1 $\pm$ 2.3	6.09 $\pm$ 0.35	0.36 $\pm$ 0.01*	0.53 $\pm$ 0.04	1.28 $\pm$ 0.21
Pb	473.4 $\pm$ 11.1	73.5 $\pm$ 11.3	35.5 $\pm$ 4.6	6.91 $\pm$ 1.65	0.39 $\pm$ 0.10*	0.58 $\pm$ 0.17	1.29 $\pm$ 0.23
Pb + 250 μM IAA	334.8 $\pm$ 42.2	68.1 $\pm$ 5.9	29.2 $\pm$ 5.1	4.90 $\pm$ 1.48	0.36 $\pm$ 0.07*	0.61 $\pm$ 0.12	1.27 $\pm$ 0.53
Pb + 500 μM IAA	329.1 $\pm$ 12.5*	69.4 $\pm$ 8.7	30.7 $\pm$ 2.3	5.99 $\pm$ 1.51	0.35 $\pm$ 0.05*	0.56 $\pm$ 0.17	1.38 $\pm$ 0.14
5 d							
Control	705.8 $\pm$ 101.4	69.2 $\pm$ 7.5	36.5 $\pm$ 3.4	6.92 $\pm$ 0.71	0.94 $\pm$ 0.16	0.63 $\pm$ 0.14	1.73 $\pm$ 0.10
250 μM IAA	415.2 $\pm$ 18.7*	50.6 $\pm$ 9.1	31.7 $\pm$ 3.9	7.30 $\pm$ 0.68	0.35 $\pm$ 0.04*	0.50 $\pm$ 0.08	1.14 $\pm$ 0.15*
500 μM IAA	321.8 $\pm$ 10.0**	42.3 $\pm$ 8.8*	35.9 $\pm$ 3.1	5.36 $\pm$ 1.34	0.25 $\pm$ 0.03**	0.40 $\pm$ 0.07	1.21 $\pm$ 0.01
Pb	686.1 $\pm$ 75.5	47.3 $\pm$ 13.1*	33.8 $\pm$ 5.4	6.18 $\pm$ 1.73	0.31 $\pm$ 0.03**	0.58 $\pm$ 0.07	1.26 $\pm$ 0.13
Pb + 250 μM IAA	356.9 $\pm$ 21.5**	42.9 $\pm$ 7.3*	26.4 $\pm$ 1.0	6.88 $\pm$ 1.36	0.24 $\pm$ 0.02**	0.48 $\pm$ 0.03	1.06 $\pm$ 0.24*
Pb + 500 μM IAA	312.9 $\pm$ 12.7**	38.3 $\pm$ 0.9*	27.5 $\pm$ 3.8	6.40 $\pm$ 1.83	0.25 $\pm$ 0.03**	0.47 $\pm$ 0.02	1.16 $\pm$ 0.03

Data are means $\pm$ S.D. ($n = 3$).* Significant difference between IAA, Pb, Pb + IAA treatments and controls for given time and element at $P < 0.05$.** Significant difference between IAA, Pb, Pb + IAA treatments and controls for given time and element at $P < 0.01$.

4. Discussion

Our results indicated that an exogenous plant growth regulator, IAA, affected Pb accumulation in maize seedlings. With the addition of IAA, Pb content in maize roots was significantly

enhanced, but Pb content in maize shoots was significantly decreased (Table 1). It seems that the increased Pb accumulation, along with decreased Ca content in maize roots, may be a result of competition between Pb^{2+} and Ca^{2+} for binding sites on the transporting transmembrane proteins. Consequently,

Table 3

Nutrient content ($\mu\text{mol g}^{-1}$ DW) in maize shoots with different treatment and exposure period, 1, 3 and 5 days (d)

Treatment	K	Ca	Mg	Fe	Mn	Cu	Zn
1 d							
Control	643.7 $\pm$ 35.5	72.7 $\pm$ 4.4	129.4 $\pm$ 4.5	3.44 $\pm$ 0.14	0.27 $\pm$ 0.02	0.21 $\pm$ 0.02	1.88 $\pm$ 0.20
250 μM IAA	568.5 $\pm$ 93.4	63.8 $\pm$ 6.9	136.2 $\pm$ 25.7	2.93 $\pm$ 0.24	0.25 $\pm$ 0.08	0.18 $\pm$ 0.02	1.83 $\pm$ 0.29
500 μM IAA	517.6 $\pm$ 22.4	59.7 $\pm$ 11.0	123.1 $\pm$ 15.9	2.79 $\pm$ 0.38	0.23 $\pm$ 0.04	0.16 $\pm$ 0.04	1.45 $\pm$ 0.18
Pb	595.8 $\pm$ 82.3	65.5 $\pm$ 2.5	135.2 $\pm$ 13.2	3.34 $\pm$ 0.40	0.24 $\pm$ 0.08	0.20 $\pm$ 0.05	2.23 $\pm$ 0.55
Pb + 250 μM IAA	449.8 $\pm$ 56.4	59.0 $\pm$ 2.9	118.8 $\pm$ 10.6	2.36 $\pm$ 0.73	0.24 $\pm$ 0.06	0.16 $\pm$ 0.02	1.55 $\pm$ 0.44
Pb + 500 μM IAA	384.7 $\pm$ 35.5*	55.1 $\pm$ 4.4	114.7 $\pm$ 4.5	3.02 $\pm$ 0.23	0.22 $\pm$ 0.02	0.14 $\pm$ 0.02	1.45 $\pm$ 0.10
3 d							
Control	783.3 $\pm$ 86.6	110.9 $\pm$ 19.9	131.0 $\pm$ 7.8	3.78 $\pm$ 0.43	0.53 $\pm$ 0.22	0.23 $\pm$ 0.03	2.07 $\pm$ 0.41
250 μM IAA	613.4 $\pm$ 38.1	104.0 $\pm$ 3.4	115.1 $\pm$ 17.0	4.04 $\pm$ 0.94	0.45 $\pm$ 0.03	0.25 $\pm$ 0.05	1.86 $\pm$ 0.60
500 μM IAA	459.6 $\pm$ 27.9*	95.7 $\pm$ 14.0	98.4 $\pm$ 5.8	2.86 $\pm$ 0.58	0.32 $\pm$ 0.03*	0.21 $\pm$ 0.01	1.25 $\pm$ 0.29
Pb	719.2 $\pm$ 94.0	99.7 $\pm$ 13.1	142.0 $\pm$ 13.9	3.63 $\pm$ 1.48	0.42 $\pm$ 0.05	0.21 $\pm$ 0.03	1.95 $\pm$ 0.37
Pb + 250 μM IAA	498.0 $\pm$ 44.7*	99.0 $\pm$ 21.6	132.2 $\pm$ 6.8	2.85 $\pm$ 0.61	0.30 $\pm$ 0.10*	0.17 $\pm$ 0.04	1.85 $\pm$ 0.25
Pb + 500 μM IAA	425.2 $\pm$ 37.8*	91.1 $\pm$ 11.3	120.5 $\pm$ 0.1	3.52 $\pm$ 0.45	0.24 $\pm$ 0.07*	0.13 $\pm$ 0.02*	1.58 $\pm$ 0.20
5 d							
Control	1009.2 $\pm$ 162.1	138.4 $\pm$ 10.7	130.6 $\pm$ 20.0	4.18 $\pm$ 1.19	0.91 $\pm$ 0.09	0.22 $\pm$ 0.04	1.96 $\pm$ 0.50
250 μM IAA	363.9 $\pm$ 37.1**	62.5 $\pm$ 9.4*	116.1 $\pm$ 21.8	3.98 $\pm$ 0.54	0.26 $\pm$ 0.08**	0.21 $\pm$ 0.07	1.83 $\pm$ 0.45
500 μM IAA	337.0 $\pm$ 10.6**	50.1 $\pm$ 4.8*	107.0 $\pm$ 15.9	2.41 $\pm$ 0.69	0.24 $\pm$ 0.04**	0.14 $\pm$ 0.03*	1.42 $\pm$ 0.28
Pb	942.1 $\pm$ 38.9	82.4 $\pm$ 5.8	118.6 $\pm$ 9.9	2.40 $\pm$ 0.42	0.37 $\pm$ 0.12*	0.18 $\pm$ 0.02	1.73 $\pm$ 0.17
Pb + 250 μM IAA	355.7 $\pm$ 27.9**	51.3 $\pm$ 0.1*	133.2 $\pm$ 5.1	2.74 $\pm$ 0.88	0.26 $\pm$ 0.01**	0.16 $\pm$ 0.02	1.52 $\pm$ 0.20
Pb + 500 μM IAA	248.9 $\pm$ 18.0**	39.9 $\pm$ 0.1**	109.9 $\pm$ 11.7	2.30 $\pm$ 0.14	0.19 $\pm$ 0.09**	0.14 $\pm$ 0.04*	1.37 $\pm$ 0.15

Data are means $\pm$ S.D. ($n = 3$).* Significant difference between IAA, Pb, Pb + IAA treatments and controls for given time and element at $P < 0.05$.** Significant difference between IAA, Pb, Pb + IAA treatments and controls for given time and element at $P < 0.01$.

Fig. 3. FTIR spectra of maize roots after 1 day treatment: control (a), with 250 μM IAA (b), 20 μM Pb (c) and 20 μM Pb + 250 μM IAA (d).

stronger Pb^{2+} interaction with the transporting proteins may result directly in the observed higher accumulation of Pb.

It has been found that Pb significantly inhibited voltage-gated Ca^{2+} channel activity in the plasma membrane of wheat roots (Huang and Cunningham, 1996). The inhibition of the Ca-channel by Pb^{2+} could result either from the competitive transport of Pb^{2+} through the Ca-channel or from its blockage by Pb. In maize and wheat plasma membrane vesicles the Ca^{2+} channel is very sensitive to La^{3+} (Tyerman and Skerrett, 1999). Bregante et al. (1997) reported that 1 mM Cs^+ was able to block, in a very fast and voltage-dependent manner, up to 96% of the potassium current in protoplasts of maize roots. Our results suggest that La^{3+} and Cs^+ significantly inhibited the uptake of Pb by maize roots whether IAA was applied or not (Fig. 1). It is generally recognized that Cs^+ is a K^+ channel blocker, while not affecting any Ca^{2+} channels. La^{3+} is a broad range Ca^{2+} channel blocker, but also blocks non-selective cation channels, anion channels and metabolism. Interestingly, Pb^{2+} can bind to Ca^{2+} -binding sites in regulatory proteins such as calmodulin. Recently, the tobacco plasma membrane protein *NtCBP4* and the Arabidopsis gene *CNGC1* were reported to be responsible for Pb^{2+} entry into plant cells (Sunkar et al., 2000). The involvement of *CNGC1* and *CNGC2* in the transport of potassium has also been reported (Leng et al., 1999). These previous results support our current findings that the uptake of Pb^{2+} was mediated, at least in part, by the K^+ and Ca^{2+} channels.

Uptake of cationic solutes is likely to be driven largely also by the negative membrane potential across the plasma membrane, which is generated in part by metabolically dependent processes such as proton extrusion via the plasma membrane H^+ -ATPase (Kochian, 1991). The pmf generated by plasma membrane H^+ -pumping ATPase powers transport through a variety of carriers. The plasma membrane H^+ -ATPase is a P-type ATPase. All P-type ATPase membranes are inhibited by orthovanadate (H_2VO_4^-). In our study, pre-treatment of maize roots by vanadate significantly inhibited Pb uptake with or without IAA application (Fig. 1), suggesting that the enhanced Pb uptake

into the cytosol of maize protoplasts was dependent upon physiological functions of the plasma membrane P-type ATPase.

Some antioxidant enzymes have been stimulated by individual or joint stress of Pb and IAA (Fig. 2). The increase in SOD activity could possibly be a result of de-novo synthesis of enzyme protein (Shah et al., 2001). The activity of guaiacol peroxidase was also enhanced. This enzyme serves as an intrinsic defense tool to resist Pb and/or IAA induced oxidative stress. The role of peroxidases in responses to environmental stress in plants has been widely accepted (Castillo, 1986). In maize roots the SOD and POD activities with Pb + IAA treatments were the same as those of Pb or IAA only treatments up to day 3. After day 3, SOD and POD activities decreased and were lower than those of Pb or IAA only treatments on the day 5. The decrease in SOD and POD activities with Pb + IAA treatments was probably due to a decline in enzyme synthesis or a change in the assembly of enzyme subunits under Pb + IAA stress. CAT activities in maize roots with Pb + IAA treatments was consistently higher than either Pb or IAA only treatments, suggesting that CAT may be more tolerant to the joint stress of Pb and IAA than either SOD and POD, markedly elevating the capability of the plant to decompose H_2O_2 and protect the roots from H_2O_2 stress. In maize shoots, the SOD, POD and CAT activities with Pb + IAA treatments were generally lower than the Pb or IAA only treatments presumably because Pb + IAA caused heavier oxidative stress and inhibited the antioxidant defense mechanism. Even on day 5 the SOD, POD and CAT activities with most treatments (except CAT with 20 μM Pb + 500 μM IAA), whether in roots or shoots, were still higher than the controls, indicating that the antioxidant capacity was promoted. The response of maize to oxidative stress by Pb and/or IAA at the biochemical level was a process commencing as soon as treatment was initiated.

Lipid peroxidation is a biochemical marker for free radical mediated damage. The treatments with Pb and/or IAA induced oxidative stress in both maize roots and shoots as evidenced by increased MDA content compared to the controls. The oxidative stress was more severe in maize roots than in maize shoots. Moreover, MDA content with the 20 μM Pb + 500 μM IAA treatment was higher than that observed with the Pb only treatment (Fig. 2H), suggesting that exogenous IAA caused heavier oxidative stress to the Pb stressed maize seedlings.

The present results suggest a general decrease of nutrition content due to Pb and/or IAA treatments in maize seedlings (Tables 2 and 3). According to Huang and Cunningham (1996), treatment with 20 μM Pb significantly inhibited Ca, Mg, K, Fe, Zn and Mn accumulation in shoots of corn and ragweed (*Ambrosia artemisiifolia* L.). Pb causes the imbalance of minerals K, Ca, Mg, Mn, Zn, Cu and Fe within the tissues by physically blocking the access of these ions to the absorption sites of roots (Godbold and Kettner, 1991). On the other hand, a number of studies have shown that plant hormones in general, and auxins in particular, can significantly affect the uptake and further transport of nutrients within plants by regulating the sink action of developing tissues (Arteca, 1996; San-Francisco et al., 2005). Brummer and Parish (1983) hypothesized that an ionic gradient of the differential transmembrane, due to auxin-induced transport processes, resulted in changes in permeability

of various ions. Those previous studies supported our results (Tables 2 and 3). It can also be seen that in maize roots and shoots the levels of mineral nutrients with Pb + IAA treatments were generally lower than Pb or IAA only treatments.

FTIR spectroscopy was used to clarify the reaction of Pb in maize roots (Fig. 3). The characteristic changes in the FTIR spectra were detected as a response to reactions between Pb and IAA. According to Kamnev et al. (2001), it is not the benzene ring in IAA but the IAA pyrrole moiety that is involved in coordination with Fe^{III}. Coordination is likely involved of the π -electron of the pyrrole ring conjugated with the non-shared electron pair at the heteroatom. The observed shift from 1653 to 1642 cm⁻¹ was probably due to the coordination of Pb with the –COOH group of IAA.

We conclude that exogenous IAA significantly affected the patterns of Pb accumulation. Compared to Pb or IAA only treatments, IAA + Pb caused a synergistic effect on the oxidative stress on maize seedlings evidenced by higher MDA and lower nutrient contents. The increase in lipid peroxidation observed in this work could be considered as an indicator of oxidative stress caused by Pb and/or IAA. The antioxidant enzymes SOD, POD and CAT were stimulated to counteract the oxidative stress, which could serve as important indicator of an antioxidant defense mechanism.

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