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Effects of Pb Toxicity on the Membrane Stability and Fatty Acids Changes of *Zea mays* Seedlings Root

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Abstract: In this research we exposed four day old seedlings of *Zea mays* were exposed to different concentrations of 0.25, 0.5, 1, 2 mM $Pb(NO_3)_2$ with pH 6 in the controlled condition. After 72 h the root of seedlings were harvested and the changes of fatty acids and total lipids percent of the samples were dermined. The percent of total lipids has been increased specially in 2mM Pb treatment. It was suggested that this was not because of inducing the process of lipids synthesis, but it was due to inhibition of growth, loss of metabolism activities and metabolite synthesis. The changes of fatty acids indicated elevated amount of unsaturated linoleic and linolenic fatty acids; which are considered as a tolerance mechanism to protect membrane systems against oxidative and heavy metals stress. But increased level of K^+-leakage according to Pb concentration referred to significant decline of membrane stability. So it was concluded that Pb is a major chemical pollutant that causes different damages to root cells structure and components and *Zea mays* is a Pb-tolerant plant with a high potential of adaptation mechanisms like producing higher unsaturated fatty acids and the ability of forming some advantious roots from the first node of shoot that helps it to tolerate stress conditions.

Key words: Pb toxicity, membrane stability, unsaturated fatty acids, *Zea mays*

INTRODUCTION

Heavy metals pollution have increased in recent decades. Studying their harmful effects on plants and tolerance mechanisms has been a major part of late investigations, which suggest plants respond against heavy metals toxicity in different ways. Most of them are sensitive; some are accumulators (Allysum and Thalaspi). These are small plants with a low biomass; because they use a lot of energy to absorb heavy metals[1]. Another group of plants are tolerant with many different tolerance mechanisms such as increased level of soluble sugars[2] and unsaturated fatty acids[3-5] elevated antioxidant enzymes activities and organic acid exudates[6-8]. Because of the importance of determination of tolerant Plants for using them in contaminated areas and understanding tolerance mechanisms to produce transgenic plants with higher potential in heavy metals accumulation or extrusion heavy metal, the effect of Pb toxicity as a major chemical pollutant on *Zea mays* (as a Pb- tolerant plant) seedlings root cells membrane stability and fatty acids changes.

MATERIALS AND METHODS

Seeds of *Zea mays* Cv. single cross 704 were obtained from Agricultural Research Center of Urmia. They were cleaned, selected by the size, washed with water and detergent and finally with distilled water 3 times and incubated in 25°C to germinant. Four day-old seedlings with the same size were selected again and exposed to 50 mL of 0.25, 0.5, 1, 2 mM $Pb(NO_3)_2$ in growth solution adjusted to pH 6. at the aerated and controlled condition (day/night temperature 24/20°C, light density 16000 lux and humidity 60%). After 72 h root of seedlings were harvested, Oven dried at 70°C for three days, grounded to the powder and used to fatty acids analysis.

Effect of Pb on root membrane stability: To do this experiment, 3 day old seedlings of *Zea mays* were exposed to 10 mL KCl (20 mM). After 24h seedlings were treated with different concentrations of Pb $(NO_3)_2$ for 48 h.At the end of treatment the amount of K-leakage in growth solution of each replicate was determined by flame photometer (model 405 made by Fater Electronic Com.).

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Total lipids of *Zea mays* seedlings root: To extract total lipids of *Zea mays* seedlings root exposed to different concentrations of Pb, 0.1 g grounded dry material of the root was poured in a glass tube; 10 mL Diethyl Ether (DEE) (99.9%) was added; sealed tightly and was left in 30°C for 10 h, then centrifuged in 3500 rpm. Ether was removed in another glass tube that is weighed before. This process was repeated three times until all of lipids was extracted completely and was added to previous supernatant. Ether was evaporated at 40°C. Remained lipid extracts were weighed again with its glass. The amount of total lipids was calculated by subtracting the weight of glass+lipid-weight of glass.

Preparing fatty acids to inject into GC apparatus: Five hundred microliter methanolated-KOH (5.6 g KOH dissolved in 50 mL methanol) and 500 µL N-heptan (1N) were added on lipid extract of 0.1 g dry mater of root as described before; heated in 80°C for 20 min. It was allowed to separate in two different phases. N-heptan phase is filtered through dry Na_2SO_4 and is kept in sealed 2 mL vials in freezer until to be injected in GC apparatus.

Fatty acid analyzing by GC: One microliter of fatty acid prepared as mentioned before was injected in GC apparatus (model GC-1000, DANI, Italy), with the polar EC column (30 cm, I.D. 0.25 mm with flame ionized detector). Carrier gas (N_2) was generated by the generator model: NG 2081, Gliand, Italy. The detector fuels were hydrogen and air which hydrogen was generated by the generator model: HG 2200, Gliand, Italy and the air is maintained by an air capsule. To determine the fatty acids a special mixed standard solution from SUPELCO-(USA) was used.

RESULTS AND DISCUSSION

Treatment of *Zea mays* seedlings with different concentrations of Pb caused an apparent decline in root cells membrane stability (Fig. 1). It is because of Pb accumulation in root (as our previous studies indicated). Yoko Yamato[9] points to the effects of Al on lipid peroxidation, loss of membrane integrity and finally inhibition of root elongation in pea. These events may occur in Zea mays exposed to Pb stress too; because, a lot of Pb absorbed by roots precipitate on cell walls cause some cracks on it decrease root elongation[10], inactivates many membrane-bound enzymes, ion carriers, channels and finally loss of membrane integrity and stability. The percent of total lipids increased specially in 2 mM Pb treatment (Fig. 2).We suggest that it is not because of inducing lipid synthesis, but it is due to inhibition of

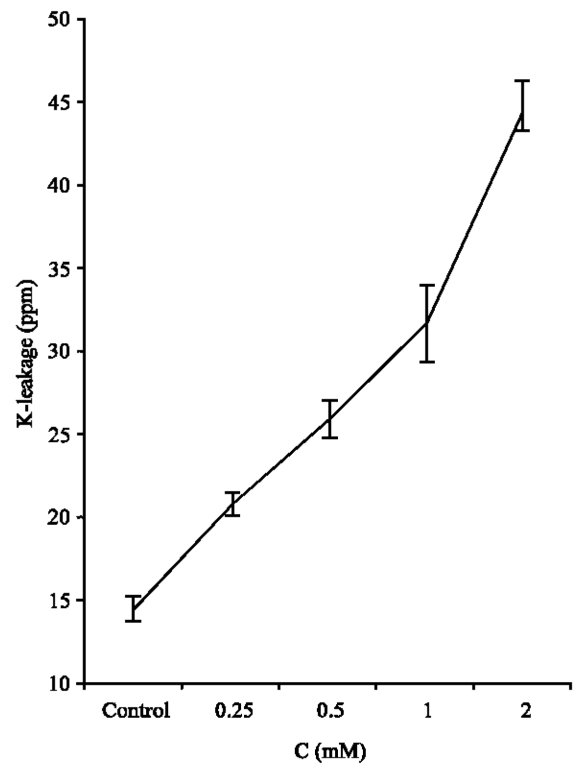

Fig. 1: Effects of different concentrations of $Pb(NO_3)_2$ with pH 6 on membrane stability on *Zea mays* seedlings during the period of 48 h. The values represent the mean of three replicates±SE

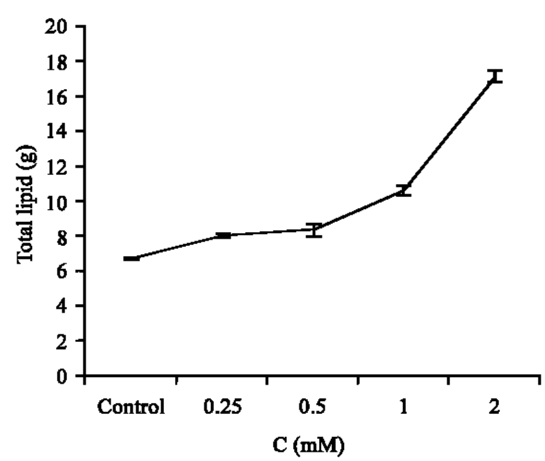

Fig. 2: The changes of total lipid percentage of *Zea mays* seedlings roots exposed to different concentrations of $Pb(NO_3)_2$ with pH 6 during the period of 72 h. The values represent the mean of three replicates±SE

Table 1: Changes in the percent of fatty acids in roots of *Zea mays* seedlings exposed to different concentrations of $Pb(NO_3)_2$ (% total fatty acids)

$Pb(NO_3)_2$ Fatty acids	Control	0.25 mM	0.5 mM	1 mM	2 mM
Palmitic acid	25.75±0.11c	24.66±0.17c	27.43± 0.38ab	26.04±0.53bc	28.19±0.80a
Stearic acid	1.96±0.09c	2.01b±0.1c	2.36±0.03a	2.25±0.08ab	2.03b±0.07c
Oleic acid	5.15±0.032d	7.06±0.28b	6.41b±0.08c	8.39±0.17a	6.22±0.22c
Linoleic acid	46.54±0.22d	50.06±0.862c	50.02±0.13b	54.21±0.27ab	55.41±0.14a
Linolenic acid	1.60±0.07d	2.54±0.25c	3.30±0.12b	3.88±0.05a	3.83±0.08a

Different letters (a,b,c,d)indicates that the difference between data are significant in 5%
The values represent the mean of three replicates±SE

Fig. 3: Shows at the left : the total root of *Zea mays* seedlings and at the right: only advantious roots of seedlings, grown from the bottom of the shoot

metabolic activities and metabolite synthesis. Increased levels of Linoleic and Linolenic fatty acids (Table 1) cause membrane fluidity[11] and protects membrane systems against stress damages[5]. Azaki[3] implies that salt induction of fatty acid elongase is a tolerance mechanism in hallotolerant alga Donalliela salina.

It was concluded that Pb is a major chemical pollutant that damages root cells structure and components and *Zea mays* is a Pb-tolerant plant with the potential of producing unsaturated fatty acid and the ability of forming some adventitious roots from the first node of shoot (Fig. 3) that help it to tolerate stress conditions.

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