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BRIEF COMMUNICATION

Impact of lead and cadmium on enzyme of citric acid cycle in germinating pea seeds

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Abstract

The present investigations were conducted to ascertain the influence of Pb^{2+} and Cd^{2+} both individually and in combination on selected enzymes of tricarboxylic acid (TCA) cycle. All the enzymes of TCA cycle examined (α-ketoglutarate dehydrogenase, isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase) were affected deleteriously by Pb^{2+} as well as Cd^{2+} and these metals in combination gave more or less an additive effect.

Additional key words: heavy metals, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase, succinate dehydrogenase.

It is well known that growth and economic yield of plants is significantly depressed when these are raised on soils contaminated with heavy metals (Foy *et al.* 1978, Lepp 1981, Woolhouse 1983). These heavy metals interfere with the activities of enzymes of respiration (Bansal *et al.* 2000), electron transport chain (Bansal and Sharma 2000), hydrolytic enzymes (Bansal *et al.* 2001) and overall seed germination and seedling growth (Bansal *et al.* 2000). Interference of Pb^{2+} with key plant processes like respiration (Bittel *et al.* 1974, Porter and Sheridan 1981) and nitrogen fixation (Brackup and Campe 1985) have been reported. Cd^{2+} has also been shown to exert deleterious effect on photosynthesis (Rani *et al.* 1990, Keshan and Mukherji 1992, Ali *et al.* 2000) and respiration (Reese and Roberts 1985, Sawhney *et al.* 1990). Under aerobic conditions bulk of the energy in a cell is derived from Krebs cycle. Therefore the present investigations were undertaken to observe the influence of these heavy metals on selected enzymes of TCA cycle in germinating seeds.

The present investigations were carried out on pea (*Pisum sativum* L. cv. Bonneville). Dry seeds of uniform size and colour were soaked in tap water for 30 min and then surface sterilized with 0.2 % (m/v) mercuric chloride solution for 5 min. After thoroughly washing them under running tap water, these were finally rinsed twice with distilled water. The seeds were germinated in Petri plates lined with two layers of *Whatman No. 1* paper at 25 °C in dark in *BOD* incubator. The seeds were germinated either in distilled water (control) or in aqueous solutions of nitrate salts of specified concentration of 0.5 mM $Pb^{2+}$and 0.25 mM Cd^{2+}. Streptomycin sulphate (25 μg cm^{-3}) was added to all these solutions to suppress microbial growth.

Effect of Pb^{2+} and Cd^{2+} on functioning of respiratory chain was determined in mitochondrial preparations. Mitochondria, from germinating pea seeds were isolated at an interval of 2, 4, 6 and 8 d after imbibition essentially by the procedure of Nawa and Asahi (1971). Mitochondria from supernatant were sedimented by centrifuging it at 25 000 *g* for 20 min. The supernatant obtained was carefully decanted and designated as "post mitochondrial fraction". The pellet was re-suspended in the homogenising media and re-centrifuged at 25 000 *g* for 200 min. This washed pellet was finally suspended in the extraction media and referred to as mitochondrial preparation. Further the activity of enzyme NADP-isocitrate dehydrogenase and succinate dehydrogenase was measured as per method of Kolloffel (1967), α-ketoglutarate dehydrogenase by method of Sanadi (1969) and malate dehydrogenase as per method of Nawa and Asahi (1971).

NADP-isocitrate dehydrogenase occurred in

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mitochondrial and extramitochondrial cellular compartments. The maximum activity of mitochondrial enzyme (Table 1) developed after 4 d of imbibition followed by a gradual decline at the concentrations used. Pb^{2+} and Cd^{2+} alone diminished the activity by 10 - 20 % whereas around 30 % decrease occurred in presence of both the metals. In contrast to mitochondrial NADP-isocitrate dehydrogenase activity in extra mitochondrial fraction increased progressively during 8 d of germination. The obtained results also suggest that this activity appeared to be slightly more susceptible to metals particularly during the later stages. For instance after 6 d Pb^{2+}, Cd^{2+}, and Pb^{2+} + Cd^{2+} depressed activity of mitochondrial enzyme by 10, 14 and 19 %, respectively, and the corresponding values for extra mitochondrial NADP-isocitrate dehydrogenase were 21, 27 and 38 %, respectively. Similar type of differential sensitivity of these two forms of enzymes to the metals is also apparent after 8 d. The development of mitochondrial and extramitochondrial NADP-isocitrate dehydrogenase activities in germinating pea seeds in the present investigations concurs with those reported previously by Chugh (1991) and Dua (1992).

Table 1. Effect of lead and cadmium on activities of different enzymes (d.m. - dry mass).

Enzymes	Metal	2 d	4 d	6 d	8 d
Mitochondrial isocitrate dehydrogenase [× 10^{-4} μmol(NADP reduced) g^{-1}(d.m.) s^{-1}]	0	8.87±0.82	17.83±1.20	14.06±1.20	13.63±0.91
	Pb^{2+}	7.39±0.51	14.21±1.10	12.61±0.81	12.03±1.00
	Cd^{2+}	6.81±0.40	14.79±1.30	12.03±0.72	12.61±1.10
	Pb^{2+} + Cd^{2+}	6.23±0.21	13.05±0.91	11.45±0.71	9.28±0.80
Extra mitochondrial isocitrate dehydrogenase [× 10^{-3} μmol(NADP reduced) g^{-1}(d.m.) s^{-1}]	0	26.90±2.10	30.01±2.20	37.41±2.10	44.34±2.40
	Pb^{2+}	20.01±1.20	26.39±1.70	29.58±1.20	34.22±2.20
	Cd^{2+}	22.18±1.40	25.23±1.60	27.40±1.30	32.19±1.70
	Pb^{2+} + Cd^{2+}	16.38±1.20	19.43±1.70	23.20±1.70	27.98±1.10
Mitochondrial malate dehydrogenase [× 10^{-3} μmol(NADH oxidised) g^{-1}(d.m.) s^{-1}]	0	89.75±7.00	184.90±11.0	205.90±20.5	211.40±11.2
	Pb^{2+}	68.58±2.30	126.70±10.2	169.07±10.1	179.65±10.2
	Cd^{2+}	73.95±4.30	110.92±9.20	142.68±8.10	174.29±8.90
	Pb^{2+} + Cd^{2+}	58.00±2.70	95.12±6.20	126.70±6.30	153.12±10.1
Extra mitochondrial malate dehydrogenase [× 10^{-3} μmol(NADH oxidised) g^{-1}(d.m.) s^{-1}]	0	5.17±0.41	6.91±0.42	7.81±0.37	5.85±0.32
	Pb^{2+}	4.06±0.22	6.03±0.47	7.11±0.73	3.05±0.20
	Cd^{2+}	3.95±0.31	5.80±3.22	6.59±0.25	4.21±0.26
	Pb^{2+} + Cd^{2+}	3.16±0.11	5.16±0.20	5.06±0.06	3.16±0.09
Mitochondrial α-ketoglutarate dehydrogenase [× 10^{-3} μmol(ferricyanide reduced) g^{-1}(d.m.) s^{-1}]	0	5.43±0.12	7.00±0.24	7.69±0.72	7.83±0.23
	Pb^{2+}	4.26±0.21	6.26±0.50	6.65±0.23	6.52±0.21
	Cd^{2+}	4.35±0.32	5.61±0.27	6.09±0.17	5.97±0.11
	Pb^{2+} + Cd^{2+}	3.36±0.11	4.39±0.18	5.22±0.16	4.87±0.13
Mitochondrial succinate dehydrogenase [× 10^{-4} μmol(DCPIP reduced) g^{-1}(d.m.) s^{-1}]	0	7.81±0.91	11.32±0.17	13.99±1.20	13.96±1.30
	Pb^{2+}	6.58±0.32	10.28±0.82	11.31±1.00	9.87±0.82
	Cd^{2+}	6.78±0.41	9.87±0.55	11.93±0.79	10.28±0.29
	Pb^{2+} + Cd^{2+}	5.75±0.16	8.01±0.43	10.07±0.73	7.81±0.60

Almost entire activity of α-ketoglutarate dehydrogenase in the germinating pea seeds was localized in mitochondria. It rose gradually up to 6 d and then remained more or less constant. Similar developmental profile of the enzyme was discerned in seeds germinated in Pb^{2+} and Cd^{2+}solutions except that they contained perceptibly lower activity than the control (Table 1). The activity of succinate dehydrogenase, like α-ketoglutarate dehydrogenase, was observed almost exclusively in mitochondrial fraction. In control seeds the activity rose progressively throughout the duration of experiment. In contrast, after increase upto 6 d, the activity declined by about 15 % in presence of either Pb^{2+} or Cd^{2+} in media and by around 25 % with Pb^{2+} + Cd^{2+}. The diminished activity of succinate dehydrogenase in response to Cd^{2+} treatment is in conformity with the results of Chugh (1991) in germinating pea seeds.

Activity of malate dehydrogenase was present in both mitochondrial as well as extra mitochondrial fractions. Activity of mitochondrial malate dehydrogenase in the control seeds rose rapidly up to 4 d of germination and then gradually up to 8 d. Presence of either Pb^{2+} or Cd^{2+} in the media diminished the activity of the enzyme. The decline in activity due to presence of Pb^{2+} and Cd^{2+} together was almost equal to their individual effects except on the 4th day. Individually Pb^{2+} and Cd^{2+} caused

31 and 40 % decrease but in combination they depressed activity by 49 %. Extramitochondrial activity increased up to 6th day and declined about 25 % on 8th day. Inclusion of either Pb^{2+} or Cd^{2+} in the germinating media exerted relatively a small effect of 15 - 20 % on the enzyme upto 6 d of germination but these caused a marked decrease of 48 and 46 % respectively, in the activity on the 8th day. Similar response was discernible when both metals were used together. Deleterious effect of Cd^{2+} on malate dehydrogenase in germinating pea seeds has also been reported earlier by Chugh (1991) who found that activity of this enzyme was depressed by about 30 % due to presence of 1 mM Cd^{2+} in the germination media.

Due to heterotrophic nature of germinating seed, its energy requirements have almost entirely to be fulfilled via respiration. The inhibition of citric acid cycle, in addition to curtailing supply of intermediates some of which serve as precursors for production of other biomolecules, would limit generation and supply of reducing equivalents for operation of the respiratory chain. This in turn is likely to diminish the capacity of these seeds to generate ATP through mitochondrial respiration, which is essential for rapid growth of embryonic axis and its development into a healthy seedling. These effects may partly contribute towards toxic effect of these metals on seed germination and early seedling growth.

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