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BRIEF COMMUNICATION

Soil pollution alters ATP and chlorophyll contents in *Pisum sativum* seedlings

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Abstract

Pisum sativum L. cv. Phenomen plants were grown in pots in greenhouse and their growth, and ATP and chlorophyll (Chl) *a* and *b* contents were assessed after 9-d exposure to sodium arsenate [0.04 and 0.07 mmol kg^{-1}(soil)], or to lead acetate [2.0 and 4.0 mmol kg^{-1}(soil)], or zinc acetate [5.3 and 9.3 mmol kg^{-1}(soil)]. The luciferin-luciferase method was used for ATP analyses. Soil pollution reduced significantly the growth, but the low toxicant concentrations elevated the cotyledon and shoot ATP concentrations per fresh matter content. The ATP/Chl ratio was increased in the zinc-treated seedlings as compared with the respective controls. The ATP concentration and a number of growth parameters were negatively correlated, and thus the high ATP content might contribute to the significantly reduced growth of seedlings.

Additional key words: arsenate, chlorophyll *a* and *b*, greenhouse, growth, lead acetate, pea, soil pollution, zinc acetate.

The total ATP content of a plant represents a balance between its utilisation and synthesis, and at any point in time, measurable ATP is only a minor portion of the total ATP produced (Mazor *et al.* 1984). The ATP/ADP ratio is an important regulating factor in the balance between the adenylate and phosphorylating pools. The reversible reaction between ATP and AMP in producing or utilising ADP is catalysed by adenylate kinase (Pradet and Raymond 1983). The adenylate energy charge, which expresses the phosphoanhydride bond status in the adenylate pool, influences the activities of many key enzymes of metabolism (Pradet and Raymond 1983, Lavergne and Champigny 1986).

Arsenate, lead, and zinc interfere in a number of ATP-producing or ATP-consuming reactions of plants. For example, arsenate uncouples phosphorylation in respiration and inhibits germination in *Lactuca sativa* (Speer 1973). Lead reduces photophosphorylation in *Spinacia oleracea*, and carbon dioxide fixation is inhibited *in vitro* in this species (Hampp *et al.* 1973), and *in vivo* in *Hordeum vulgare* and *Zea mays* (Stiborová *et al.* 1987). The activities of ATPases are intimately involved in the regulation of ATP concentration and also growth, through their function in cross-membrane transport (Kastrup *et al.* 1996). The ATP synthase, F_1F_0 ATPase, bound to the inner membranes of mitochondria and the thylakoid membranes of chloroplasts, synthesises and hydrolyses ATP from and to ADP and inorganic phosphate. During ATP synthesis, the rotation of one of its sub-units is coupled with translocation of four protons across the membrane per one ATP synthesized. The active domain of this ATPase contains magnesium and residues of a number of amino acids (Rastogi and Girvin 1999, Nath *et al.* 2000).

The hypothesis of this study was that the impacts of arsenate, lead, and zinc on the growth of *Pisum sativum* might be partially mediated by induced changes in ATP concentration.

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Abbreviation: Chl - chlorophyll

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Pisum sativum L. cv. Phenomen seedlings were grown in the greenhouse in plastic pots (three to four plants in each) with 755 ± 11 g dry mixture of peat, coarse granite sand, and quartz sand (4:3:3, m/m) as described by Päivöke and Simola (2001). The greenhouse conditions were not controlled; the recorded (minimum/maximum temperatures were 19/28 °C. Unsterilised seeds, pre-soaked in distilled water for 2 h, were sown directly into the pots (Päivöke and Simola 2001). Soil pollutant concentrations were: 0.04 and 0.067 (rounded to 0.07 in the text) mmol(disodium-hydrogen arsenate) kg^{-1}(dry soil), or 2.0 and 4.0 mmol(lead acetate) kg^{-1}(soil), or 5.3 and 9.3 mmol(zinc acetate) kg^{-1}(soil) (*Merck*, Darmstadt, Germany). Five parallel pots per toxicant and concentration were used, and two or three replicate cultures. At day 9, root and shoot length was measured, and the fresh and dry matter (103 ± 2 °C) contents of cotyledons, roots, and shoot determined. This time coincided with the maximum phytase activity of cotyledons (Päivöke and Simola 2001), and the axis was well developed. The ATP concentration was determined with the luciferin-luciferase (ATP monitoring reagent, *Wallac Oy*) according to (Myhrman *et al.* 1978, Thore and Rawlins 1980, Simpson and Hammond 1991) in the *LKB Wallac 1250* (Turku, Finland) luminometer with ATP disodium salt (*Boehringer*, Mannheim, Germany) as a standard. Triplicate or quadruplicate determinations were done on each sample, and three to four parallel plants were analysed from each toxicant concentration and replicate culture.

Chlorophyll (Chl) *a* and *b* concentrations were assessed according to Arnon (1949) of the three topmost internodes of the shoot, and the Chl *a/b* ratio was calculated. The data were subjected to Tukey's test to assess the significance of differences of means.

Germination was inhibited, up to 30 - 40 %, under the higher [0.07, 4.0, and 9.3 mmol, respectively, of arsenate, lead, or zinc kg^{-1}(dry soil)] toxicant concentrations. At day 9, the growth was more retarded at the lower [0.04, 2.0, and 5.3 mmol respectively, of arsenate, lead, or zinc kg^{-1}(dry soil)] concentrations than at higher concentrations (Table 1). However, the cotyledon dry matter was significantly higher in zinc-treated plants than in the controls.

At day 9, the low toxicant concentrations elevated the ATP content per fresh matter of the cotyledons and shoot, but the higher toxicant concentrations were without any significant effect (Table 2). The ATP content showed a number of correlations with growth parameters, yet these correlations were established nearly solely at the higher concentrations, with the exception of 0.04 mmol arsenate. In the controls, root ATP content was significantly related to shoot length (r = 0.88*; * - significant at 5 % level). At 0.07 mmol arsenate, root ATP content correlated negatively with root and shoot dry matter and shoot length in terms of mg dry matter per cm (r = -0.88*, r = -0.83*, and r = -0.81*, respectively), and at 0.04 mmol arsenate, the shoot ATP content was negatively related to shoot dry matter percentage (r = -0.81*). At 4.0 mmol lead, the ATP concentration of cotyledons and the shoot dry matter percentage were nagatively correlated (r = -0.91*). At 9.3 mmol zinc, the root ATP concentration showed significant positive correlation with root length (r = 0.87*), but negative with dry matter per cm (r = -0.90*).

Zinc lowered significantly also the Chl *a* and *b* contents as compared with the respective controls. The ATP/Chl ratio was increased at 5.3 mmol zinc (Table 2). The shoot ATP and Chl *a* or *b* contents were not related, except that at 4.0 mmol lead, the root ATP concentration was related to the Chl *a/b* (r = 0.82*).

The sensitivity of a plant's ATP content to a change in toxicant concentration of culture medium (Table 2) is known, for example, in solution cultures of *Phaseolus vulgaris* and *Fagopyrum esculentum*, in which the leaf ATP content is elevated at low, but reduced at higher lead concentrations (Sung and Yang 1979).

Activation of some catabolic reactions by the high ATP content (Table 2) may offer an explanation for poor dry matter yield (Table 1). Yet, the surplus of shoot ATP

Table 1. Length [cm] and fresh and dry matter yield [mg] of *P. sativum* seedlings, after 9-d exposure to soil with sodium arsenate, lead acetate, or zinc acetate in different concentrations [mmol kg^{-1}(soil)]. Mean ± SE, n = 6. Means marked with the same superscript letter do not differ at 5 % probability by Tukey's test.

Treatment	Length		Fresh mass			Dry mass		
	shoot	root	shoot	root	cotyledons	shoot	root	cotyledons
Control	6.2±0.3^{a}	23.6±2.0^{a}	550.3±48.9^{a}	757.8± 62.4^{ab}	411.0±18.0^{a}	54.0±5.3^{a}	38.8±2.7^{a}	50.6± 6.2^{a}
As 0.04	5.5±0.3^{ab}	13.0±1.1^{b}	370.6±37.1^{bc}	451.5± 59.5^{a}	442.5±34.9^{a}	36.5±3.8^{ab}	26.5±3.3^{a}	87.8±16.5^{ab}
As 0.07	5.0±0.2^{ab}	14.0±2.7^{b}	445.2±29.7^{ab}	461.0± 79.4^{a}	419.6±10.6^{a}	44.0±3.9^{ab}	26.2±4.4^{a}	63.2± 7.0^{ab}
Pb 2.00	5.0±0.3^{ab}	16.1±0.7^{ab}	452.7±44.8^{ab}	659.9± 51.0^{ab}	452.1±17.9^{a}	46.5±3.4^{a}	33.6±8.3^{a}	70.8± 8.2^{ab}
Pb 4.00	6.3±0.4^{a}	22.9±1.0^{a}	503.5±45.4^{ab}	830.0±108.2^{b}	487.0±19.3^{a}	52.3±7.1^{a}	44.9±4.4^{a}	56.5± 6.2^{ab}
Zn 5.30	4.2±0.5^{b}	13.7±1.9^{b}	259.3±28.7^{c}	448.8± 53.5^{a}	444.0±36.6^{a}	25.5±3.1^{b}	24.0±2.5^{b}	96.6±15.9^{b}
Zn 9.30	5.9±0.8^{ab}	18.4±2.2^{ab}	409.9±52.2^{b}	661.7± 24.5^{ab}	493.8±29.7^{a}	39.8±5.1^{ab}	34.6±2.4^{ab}	100.8±12.5^{b}

Table 2. Contents of ATP [mg kg^{-1}(f.m.)] of cotyledons, root, and shoot, ATP/Chl ratio [g(ATP) kg^{-1}(Chl)], fresh mass [g] of the three topmost internodes of the shoot, Chl *a* and Chl *b* contents [g kg^{-1}(f.m.)], and Chl *a*/*b* ratio of *P. sativum*, after 9-d exposure to soil with sodium arsenate, lead acetate, or zinc acetate in different concentrations [mmol kg^{-1}(soil)]. Mean ± SE, *n* = 6. Figures marked with the same superscript letter do not differ at 5 % probability by Tukey's test.

Treatment	ATP content			ATP/Chl	Fresh mass	Chl *a*	Chl *b*	Chl *a*/*b*
	cotyledons	root	shoot					
Control	103.2 ± 4.8^{a}	38.1 ± 5.9^{a}	75.6 ± 2.3^{a}	79.5 ± 2.4^{a}	0.43 ± 0.05^{a}	0.70 ± 0.05^{a}	0.25 ± 0.03^{a}	2.8 ± 0.1^{a}
As 0.04	234.1 ± 15.4^{b}	68.6 ± 5.4^{a}	124.3 ± 7.6^{b}	148.0 ± 9.1^{a}	0.28 ± 0.02^{abc}	0.62 ± 0.08^{a}	0.22 ± 0.02^{a}	2.8 ± 0.1^{a}
As 0.07	117.2 ± 17.8^{ac}	53.9 ± 7.9^{a}	69.5 ± 7.3^{a}	102.2 ± 10.8^{a}	0.22 ± 0.05^{bc}	0.50 ± 0.08^{ab}	0.18 ± 0.03^{ab}	2.7 ± 0.1^{a}
Pb 2.00	189.5 ± 20.3^{bc}	74.0 ± 7.5^{a}	127.0 ± 11.9^{b}	136.6 ± 12.8^{a}	0.38 ± 0.03^{ab}	0.66 ± 0.01^{a}	0.27 ± 0.02^{a}	2.4 ± 0.2^{a}
Pb 4.00	107.4 ± 13.1^{a}	56.2 ± 16.4^{a}	84.9 ± 7.9^{a}	89.4 ± 8.4^{a}	0.38 ± 0.06^{ab}	0.70 ± 0.08^{a}	0.25 ± 0.03^{a}	2.8 ± 0.2^{a}
Zn 5.30	198.2 ± 17.5^{b}	68.9 ± 9.9^{a}	133.0 ± 15.0^{b}	350.0 ± 39.5^{b}	0.16 ± 0.04^{c}	0.29 ± 0.04^{b}	0.09 ± 0.01^{b}	3.0 ± 0.1^{a}
Zn 9.30	111.9 ± 24.9^{a}	41.2 ± 4.1^{a}	64.0 ± 7.1^{a}	152.4 ± 17.0^{a}	0.18 ± 0.02^{c}	0.31 ± 0.05^{b}	0.11 ± 0.02^{b}	2.9 ± 0.1^{a}

per total Chl content (Table 2) may reflect also the greater sensitivity of the carboxylating enzymes (Stiborová *et al.* 1987) than photophosphorylation to heavy metals, which phenomenon results in accumulation of ATP and NADPH (Krupa and Baszyński 1995 and references therein). In *P. sativum,* within 5 d from germination, photophosphorylation becomes predominant in the shoot (Kollöffel 1967), although increased mitochondrial activity may compensate for reduced photophosphorylation in *P. vulgaris* grown with zinc. In this species, significant negative correlation occurs between some mitochondrial enzymes and growth (Van Assche 1984). This observation supports the current results on arsenate, in terms of the negative relation between the ATP concentration and growth of seedlings. With zinc, the length and dry matter reacted differently towards ATP content, and the lack of correlation at the low zinc concentration suggests that these relations are valid only within a limited tissue ATP concentration. Arsenate as a chemical analogue to phosphate, at low concentration, may enhance ATP synthesis by making phosphorus more available, yet high tissue ATP content intensifies the toxicity of lead by making phosphate less and lead more available (Brierley 1977 with references therein).

Magnesium is involved in a number of reactions of ATP, some kinase (Pacold and Anderson 1975) and ATPase enzymes (Kastrup *et al.* 1996), including the ATP synthase (Rastogi and Girvin 1999, Nath *et al.* 2000). Zinc may replace magnesium, for example, in the substrate of the plasma membrane H$^+$-ATPase, but more importantly, even 0.02 mM free zinc tissue concentration inhibits the hydrolysis of ATP and the coupled proton transport (Kastrup *et al.* 1996), with a subsequent build-up of ATP and reduced nutrient availability.

In *Acer platanoides*, the breaking of dormancy is related to the ATPase activity of seeds (Krawiarz and Szczotka 2000); an initial small amount of ATP or ADP is necessary for the initiation of breaking the dormancy (Perl 1986). In *P. sativum,* the maturation of mitochondria, and the start of cytochrome-mediated respiration take place only in the presence of the embryonic axis (Solomos *et al.* 1972, Morohashi and Bewley 1980). Initially about 43 % of the total ATP is produced by glycolysis (Raymond *et al.* 1985), in which pyruvate kinase is a regulatory enzyme (Pradet and Raymond 1983 and references therein). In this work, however, the inhibition of germination is unlikely to be related to inhibition of the mitochondrial respiration, because it takes 2 to 3 d for it to take over glycolysis; during this period the axis has already emerged (Kollöffel 1967).

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