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Photosynthesis and growth responses of pea *Pisum sativum* L. under heavy metals stress

Sabrine Hattab[1,*], Boutheina Dridi[1], Lassad Chouba[2], Mohamed Ben Kheder[3], Hamadi Bousetta[1,*]

1. Laboratory of Biochemistry and Environmental Toxicology, ISA Chott-Mariem, Sousse 4042. Tunisia. E-mail: sabrine_hattab1@yahoo.fr
2. Laboratory of Marine Biology, National Institute of Sea Sciences and Technologies Lagoulette, Tunis 2060, Tunisia
3. Technical Centre of Organic Agriculture, Chott-Mariem, Sousse 4042, Tunisia

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Abstract

The present work aimed to study the physiological effects of cadmium (Cd) and copper (Cu) in pea (*Pisum sativum*). Pea plants were exposed to increasing doses of cadmium chloride ($CdCl_2$) and copper chloride ($CuCl_2$) for 20 d. The examined parameters, namely root and shoot lengths, the concentration of photosynthetic pigments and the rate of photosynthesis were affected by the treatments especially with high metals concentrations. The analysis of heavy metals accumulation shows that leaves significantly accumulate cadmium for all the tested concentrations. However, copper was significantly accumulated only with the highest tested dose. This may explain the higher inhibitory effects of cadmium on photosynthesis and growth in pea plants. These results are valuable for understanding the biological consequences of heavy metals contamination particularly in soils devoted to organic agriculture.

Key words: *Pisum sativum*; heavy metals; photosynthesis; growth
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Introduction

Heavy metal contamination of soil and water resources is a growing problem in many areas around the world. Although, heavy metals are natural components of soils at trace levels, activities such as mining, industry, and localised agriculture have contributed to undesirable accumulations of these metals at toxic levels (Alloway, 1995; Kabada-Pendias and Pendias, 1992). Cadmium is of special concern due to its potential toxicity to biota at low concentrations (Das *et al*., 1997). Copper, although essential for plants at low concentrations, is toxic at high concentrations. Cadmium and copper are widespread heavy metals released into the environment by many sources such as power stations, heating systems, waste incinerators, and most phosphate fertilizers and pesticides (Alloway and Steinnes, 1999; Zhu *et al*., 1999; Mann *et al*., 2002).

The mechanisms of heavy metal toxicity on photosynthesis is still a matter of speculations, this may be partly due to the differences in experimental design, but some evidence points to the involvement of electron transport in light reactions (Giardi *et al*., 1997; Rashid, 1994) and enzyme activity in the dark reactions (Chugh and Sawhney, 1999; Van Assche and Clijsters, 1990). The efficiency of these seems to be highly affected by the presence of toxic metals such as cadmium and copper at high concentrations.

Deleterious effects of these metals on various photosynthetic processes, such as biosynthesis of chlorophyll (Padmaja *et al*., 1990; Stobart *et al*., 1985) functioning of photochemical reactions (Skorzynska *et al*., 1995) and the activities of enzymes of the Calvin cycle (Chugh and Sawhney, 1999), have been reported in many plants. Photosynthetic CO_2 fixation is significantly affected by heavy metals in a number of plant species (Romanowska, 2002).

Although the influence of excessive doses of heavy metals on photosynthetic activity of plants has been studied in many cultivated species (Prasad and Strzalka, 1999), *Pisum sativum* L. cv. Douce de Provence, a species potentially convenient to use in bioassays to monitor the manifestations of heavy metal toxicity in agroecosystems (Hattab *et al*., 2009) has been scarcely characterised. In the present study, we investigated the effects of increasing Cd and Cu concentrations on photosynthetic and growth parameters of *Pisum sativum*, a pea genotype widely used for food and for physiological and molecular studies (Repetto *et al*., 2003; Atta *et al*., 2004).

1 Materials and methods

1.1 Plant material and growing conditions

Pisum sativum L. seeds were obtained from the Tunisian Seed Control Agency. Plants were grown in the greenhouse

* Corresponding authors. E-mail: sabrine_hattab1@yahoo.fr (Sabrine Hattab); hamadi.bousetta@laposte.net (Hamadi Boussetta)

in aerated full-nutrient media in plastic pots under optimum conditions for 20 d (Sandalio *et al*., 2001). The treatments were either remained unsupplemented (control plants) or were supplemented with 7, 1.4, 0.7, and 0.35 mg/kg dry soil $CdCl_2$ (Cd-exposed plants) and with 700, 140, 70, and 35 mg/kg dry soil $CuCl_2$ (Cu-treated plants). Heavy metals were diluted in distilled water and added to the pots. Fifteen plants per treatment were used. The roots and shoots of control, Cd-exposed and Cu-exposed plants were collected after 20 d of exposure. The primary roots lengths as well as shoots lengths were determined. Leaves were collected and measured for fresh weight and then dried at 70°C during 3 d to measure dry weight (dw). The procedure was repeated in 15 independent plants.

1.2 Cadmium and copper analysis

The concentrations of Cd and Cu in plant samples were determined in the Marine Biology Laboratory of the National Institute of Sea Sciences and Technologies, Tunisia. The pea leave samples were carefully washed with deionized water and oven-dried at 105°C for 60 min followed by 60°C for 24 h, then grounded into fine powder, and sieved through a 1-mm nylon sieve. One gram per sample was digested by HNO_3:$HClO_4$ (3:1, *V/V*) in the microwave system. The concentrations of Cd and Cu were determined by a graphite furnace atomic absorption spectrophotometer (3300, Perkin-Elmer, USA). Standard materials, $CdCl_2$ and $CuCl_2$, were included for assurance control. Average concentration of Cd and Cu were calculated from triplicates analysis. The limit of detection (LOD) for Cd and Cu was 0.05 μg/g dw.

1.3 Chlorophyll content and rate of photosynthesis

Chlorophyll a (Chl-*a*) and b (Chl-*b*) and β-carotenes content of leaves was determined according to the method of Arnon (1956). Photosynthetic activity based on the rate of light-dependent oxygen evolution of excised leaves was determined at 30°C following the procedure of Greef *et al*. (1971) using a Biological Oxygen Monitor (YSI 5300A, Yellow Springs Instruments, USA).

1.4 Statistics

All investigated parameters are expressed as mean ± standard deviation. Data were analyzed by the Holm-Sidak ANOVA Multiple Comparison statistics using the Sigma-Stat 3.0 software (SYSTAT Software Inc, USA). Statistical significance was accepted at $p < 0.05$.

2 Results

2.1 Accumulation of heavy metals in leaves and its effect on root and shoot lengths

The accumulation of Cd in pea leaves increased significantly with increasing applied doses (Table 1). In control plant, no accumulation of Cd was detected in leaves, while in treated plants Cd tissue accumulation over 20 d exposure linearly increased reaching values ranging between (0.075 ± 0.017) and (0.243 ± 0.027) μg/g dw, respectively for plants exposed to 0.35 and 7 mg/kg medium. However, in plant exposed to increasing doses of Cu, we only found significant accumulation of Cu in leaves of plant subjected to the highest dose reaching the accumulation of (10.635 ±1.126) μg/g dw compared to the (7.319 ± 1.055) μg/g dw registered in control plants.

The increase in metal concentrations induced significant growth inhibition both in roots and shoots lengths of exposed plants (Table 2). The inhibitory effects increased with increasing doses. The most pronounced effect was observed in plants exposed to the highest cadmium concentration with lengths falling to (10.36 ± 1.87) cm and (14.92 ± 1.10) cm for root and shoot lengths, respectively. However, untreated plants grew (19.95 ± 2.46) cm in root lengths and (26.81 ± 2.87) cm in shoot lengths.

2.2 Effects of heavy metals on chlorophyll content and photosynthetic rate

The exposure of pea seedlings to Cd resulted in a reduction of chlorophyll and carotene content in leaves (Fig. 1a). The deleterious effect of Cd became more pronounced with increasing concentrations. In plants exposed to 7 mg Cd/kg growth media, Chl-*a*, Chl-*b* and carotene decreased by 50.63%, 51.89% and 45.33%, respectively compared to control plants. However, in the case of Cu, the content of the photosynthetic pigments was only altered in plants grown in presence of the highest concentration (Fig. 1b). Copper damaging effects resulted in a decline of up to 21.57%, 22.82% and 35.74%, respectively for Chl-*a*, Chl-*b*

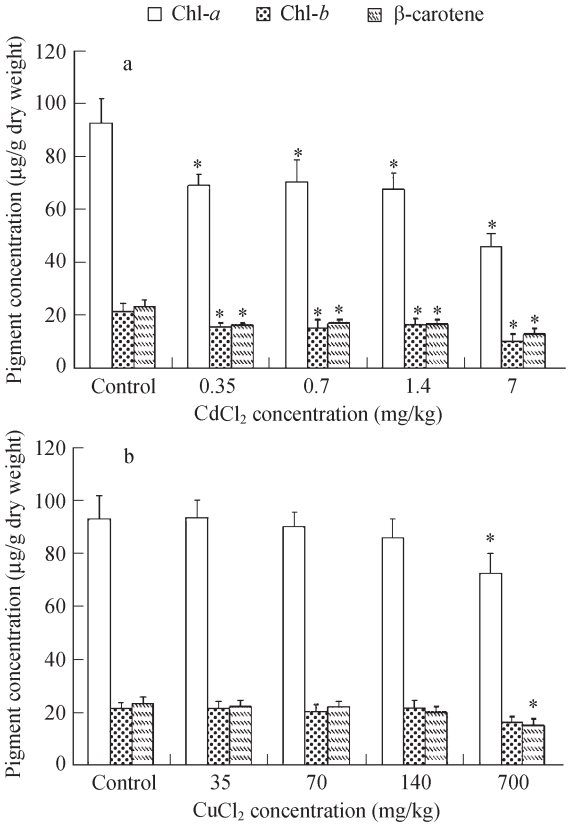

Fig. 1 Effect of Cd and Cu treatment on Chl-*a* and Chl-*b* and β-carotene content of pea leaves after 20 d exposure to different concentrations of $CdCl_2$ (a) and $CuCl_2$ (b). * Significance at $p < 0.05$ ($n = 15$).

Table 1 Cd and Cu uptake by pea leaves after exposure to increasing concentrations of $CdCl_2$ and $CuCl_2$

Treatment	Cd dosage (mg/kg dry soil)	Cd uptake (μg/g dw)	Treatment	Cu dosage (mg/kg dry soil)	Cu uptake (μg/g dw)
Control	0	ND	Control	0	7.319 ± 1.055
Cd1	0.35	0.075 ± 0.017*	Cu1	35	8.110 ± 0.843
Cd2	0.7	0.092± 0.011*	Cu2	70	8.023 ± 1.073
Cd3	1.4	0.168± 0.018*	Cu3	140	8.197 ± 0.923
Cd4	7	0.243± 0.027*	Cu4	700	10.635 ± 1.126*

ND: not detected. * Significance at $p < 0.05$ ($n = 15$).

Table 2 Influence of Cd and Cu on root and shoot lengths of pea plants after 20 d exposure to increasing concentrations of $CdCl_2$ and $CuCl_2$

Treatment	Root length (cm)	Shoot length (cm)	Treatment	Root length (cm)	Shoot length (cm)
Control	19.95 ± 2.46	26.81 ± 2.87	Control	19.95 ± 2.46	26.81 ± 2.87
Cd1	14.7 ± 2.22*	21.61 ± 1.22*	Cu1	16.67 ± 2.21	25.62 ± 2.27
Cd2	11.78 ± 1.21*	18.99 ± 1.43*	Cu2	16.45 ± 2.07	19.97 ± 1.36*
Cd3	10.97 ± 1.09*	16.12 ± 1.08*	Cu3	13.09 ± 1.22*	18.82 ± 2.12*
Cd4	10.36 ± 1.87*	14.92± 1.10*	Cu4	10.76 ± 1.67*	18.42 ± 1.32*

Data are the average values of at least 15 replicate experiments. * Values significantly different from control ($p < 0.05$) ($n = 15$).

and β-carotene pigments when compared to control plants.

The rate of photosynthesis as measured from the rate of light dependent CO_2 consumption per m^2 leaf area (Fig. 2a) in Cd treated pea seedlings, diminished progressively with increasing concentration of applied Cu. The decrease in photosynthetic rate was significant even with the lowest $CdCl_2$ concentration. The deleterious effect of Cd on photosynthetic rate became more pronounced with increasing concentrations falling to 35.32% of the control rate for 7 mg $CdCl_2$/kg growth media. Similar decreasing trend was observed when plants were exposed to increasing $CuCl_2$ concentrations (Fig. 2b). However, no significant reduction was observed for the two lowest applied concentrations (35 and 70 mg/kg dry soil). Paralleling the effect of heavy metals on the content of the photosynthetic pigments, the photosynthetic rate was minimal with the highest $CuCl_2$ concentrations reaching only 37.93% of the control photosynthetic rate.

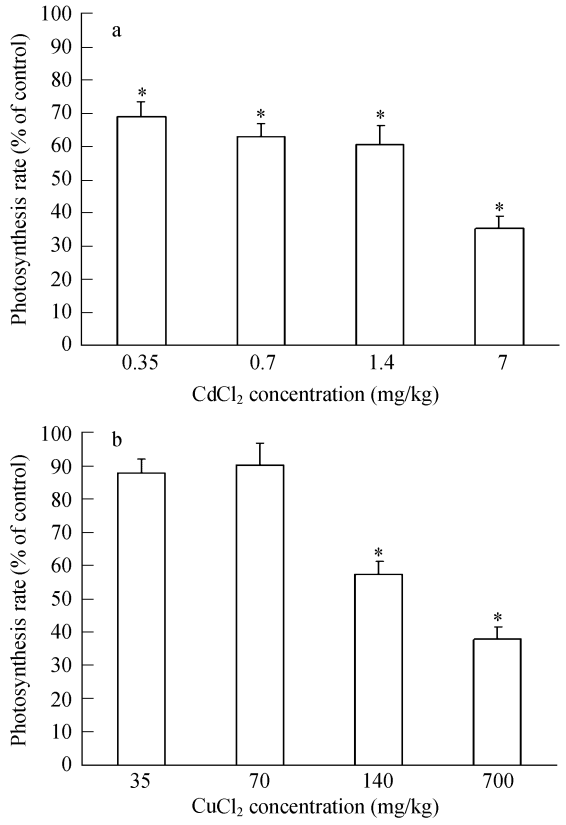

Fig. 2 Effect of Cd (a) and Cu (b) application on the rate of photosynthesis of treated pea seedlings expressed as the percentage of the control rate taken as 100%. Rate of photosynthesis of control 20 d old seedlings was (3. 19 ± 0.52) μmol CO_2 consumed/(cm^2·s). * Significance at $p < 0.05$ ($n = 15$).

3 Discussion

Our data demonstrated that Cd exposures yielded a linear increase in leaves tissue accumulation ($y = 0.029x + 0.047$; $R^2 = 0.927$), hence a higher degree of toxicity. Conversely, Cu was only significantly accumulated in leaves tissues when plants are exposed to the highest concentration. Several researchers have already described that increasing Cd concentrations induce significant growth inhibition in pea plants when exposed to heavy metals (Lozano-Rodriguez *et al*., 1997; Sandalio *et al*., 2001). However, our results evidenced the importance of the concentrations used in this work, since plants were exposed to environmentally relevant concentrations. Indeed, unpolluted soils are accepted to contain up to 7 mg/kg dry soil of Cd (Allaway, 1968), but heavy contaminated soils, such as those near smelters, may contain as high as 1700 mg/kg dry soil of this metal (Buchauer, 1973).

In the present investigation, the photosynthetic pigments content in pea declined in leaves of all plants grown in the presence of Cd and only with plants exposed to the highest Cu concentration. This fact has been previously reported (Padmaja, 1990; Stobart *et al*., 1985; Skorzynska *et al*., 1995) in some other plant species. Copper is reported to modify a number of physiological processes and particularly chlorophyll degradation. This is also evident from our present study as well as earlier studies (Devi and Prasad, 1998, 1999). In the case of Cu exposed plants, the loss in chlorophyll content could be due to the peroxidation of chloroplast membranes mediated by Cu (Baszynski *et al*.,

1988; Sandmann and Boger, 1980).

Leaf partial chlorosis was one of the most commonly observed effects of Cd and Cu toxicity. In agreement with earlier reports (Sawhney, 1990; Chugh and Sawhney, 1999), the photosynthetic activity of pea seedlings was markedly impaired by Cd. The deleterious effect on the rate of photosynthesis per cm^2 and per second could be a consequence of an overall reduction in growth with concomitant decrease in total leaf area (data not shown) or could be due to a more direct interference of the metal on photosynthetic reactions. The reduction in the rate of photosynthesis indicates that in addition to depressed growth, the heavy metal impedes the photosynthetic activity by directly interfering in the process of photosynthesis. Heavy metal toxicity is also related to the oxidative damage induced in living systems, which can be promoted both by directly increasing the cellular concentration of reactive oxygen species (ROS) and by reducing the cellular antioxidant capacity (Livingstone, 2001). ROS can be extremely harmful to organisms at high concentrations. They can oxidize proteins, lipids, and nucleic acids, often leading to alterations in cell structure and mutagenesis. Chloroplasts have a complex system of membranes rich in polyunsaturated fatty acids, which are potential targets for peroxidation. This could partially explain the deleterious effect of Cd and a higher Cu concentration on photosynthetic parameters and thus on the pea growth.

From an ecotoxicological point of view, the use of plants as bio-indicators may constitute an irreplaceable tool for investigation applied for detection and conservation of soil quality. These organisms are sedentary, sensitive to environmental variations and react, as first stages of the food chain, more rapidly to the presence of pollutants, especially heavy metals than organisms living at higher stages (Lovett Doust *et al*., 1994). In this study we are proposing a biological model; pea, a wildly used species for food, physiological and molecular studies as an alternative to evaluate the soil heavy metal contamination using a set of simple biological parameters related to growth and photosynthesis.

4 Conclusions

In summary, the long time exposure of pea plants to heavy metal seriously affects growth and photosynthesis as evidenced by the investigated parameters. Interestingly, the environmentally relevant concentrations of heavy metals used in the present work, considered as acceptable values in agriculture, affected the biological processes linked to photosynthesis. These results, together to the fact that it is a species with a short vegetative cycle, make pea plant as a good candidate plant species for biomonitoring soils contaminated with heavy metals.

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