Pak. J. Bot., 43(3): 1467-1470, 2011.

EFFECT OF CADMIUM ON GROWTH, PROTEIN CONTENT AND PEROXIDASE ACTIVITY IN PEA PLANTS

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Abstract

In this study the effects of different cadmium chloride concentrations (5, 10, 20, 50, and 100 μ*M*) on some physiological and biochemical processes including seed germination, root and shoot fresh and dry weight, protein content and peroxidase activity in peas (*Cicer arietinum* cv. pars) were investigated. Cadmium did not have any significant effect on the rate of pea seed germination. However, it affected the subsequent growth rate in these plants. Higher cadmium concentrations specially at 50 and 100 μ*M* reduced plant growth significantly. Leaf chlorosis, wilting and leaf abscission were observed in plants treated with cadmium. Protein content in pea roots reduced significantly in the presence of high cadmium concentrations. Low concentrations of $CdCl_2$ resulted in higher peroxidase activity both in roots and shoots of pea plants.

Introduction

Cadmium is a nonessential heavy metal that does not have any metabolic function in higher plants. Under natural conditions, it exists at low concentrations in most soils. It enters the soil with phosphorus fertilizers, sewage sludge and air pollutants. It has a great mobility in the soil as compared with other heavy metals, and is taken up in varying degrees by plants (Varo *et al*., 1980). The increasing amount of cadmium in the environment affects various physiological and biochemical processes in plants (Sanita di Toppi & Gabbrielli, 1999). Reductions in both biomass production and nutritional quality have been observed in crops grown on soils contaminated with moderate levels of heavy metal (Cottoine *et al*., 1976). Even at low concentration it inhibits plant growth and disturbs photosynthesis, sugar metabolism, sulphate assimilation and several enzyme activities (Van Assche & Clijsters, 1990; Sanita di Topi & Gabbrielli, 1999; Kevresan *et al*., 2003; Sottnikova *et al*., 2003). The objective of present study is to examine the effects of cadmium on germination, growth, protein content and peroxidase activity of pea plants.

Materials and Methods

Plant material and growth conditions

Seed germination: Pea seeds (*Cicer arietinum* L. cv. pars) were obtained from Agricultural Research Institute in Zarghan, Fars Province, Iran. The seeds were surface sterilized with 10% Sodium hypochlorite for 10 min., and then rinsed extensively with distilled water. Seeds were placed on filter papers moistened with different cadmium concentrations to germinate in germinator at 22 ± 1°C. After 7 days**,** the rates of seed germination were determined. For each cadmium chloride concentration, over 100 seeds were tested in three separate experiments.

Growth experiments: Pea seeds, after being surface–sterilized with Sodium hypochlorite, were germinated in vermiculite. After 10 days, seedlings were transferred to an aerated full strength Hoagland nutrient solution. The solutions pH, was adjusted to 5.0 by 0.1N HCl and 0.1N NaOH. After 3 days, Cd was added to nutrient solutions as $CdCl_2$ in a final concentrations of 5, 10, 20, 50 and 100 μM. Plants were kept in growth chamber set at 16/8 h light/dark cycles, and day/night temperature of 25/20^{0}C, under 9000 Lux fluorescent lights. The experiments were repeated three times and three replicates were used for each treatment. Twenty one-day-old pea plants were used to study the effects of Cd^{2+}. Plants were exposed to different concentrations of Cd^{2+} for 7 days. Shoots and roots were separated for their fresh and dry weight measurements. They were then dried at 75^{0}C for 48h in an oven for dry weight measurements.

The protein concentration was determined according to Lawry (1951) method using bovine serum albumin (BSA) as standards. Peroxidase activity of the 21-day-old pea plants grown in nutrient culture, plus different levels of Cd^{2+} for 7 days was bio-assayed according to MacAdam (1992) method.

Result and Discussion

The rate of seed germination was not affected by cadmium treatments significantly (Fig. 1). Cadmium inhibited growth rate. Roots and shoot fresh and dry weights decreased with the increase in cadmium concentrations. The rates of reduction were recorded for 50 and 100 μM Cadmium chloride concentrations (Figs. 2-5).

At higher cadmium concentrations, growth abnormalities such as root browning, leaf chlorosis, wilting and also leaf abscission occurred in pea plants. The rate of plant growth, decrease is a useful index of metal toxicity (Ernst *et al*., 1992). Cadmium is readily absorbed by plant roots within a few hours of its supply to roots media and from there is easily transported to other parts of the plants (Ghoshroy & Nadakavukaren, 1990; Rauser & Meuwly, 1995).

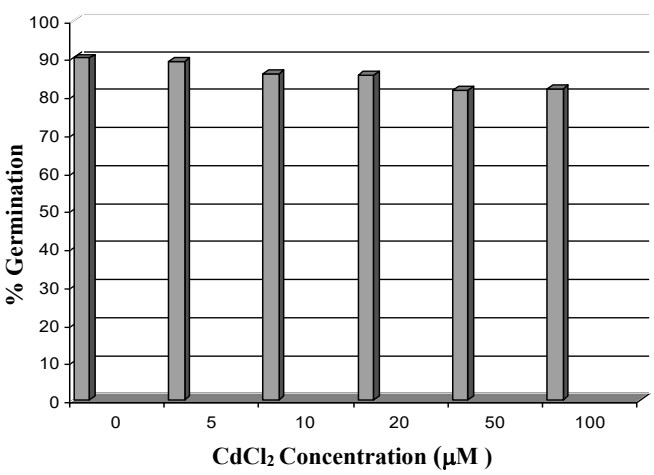

Fig. 1. Germination of pea under increasing cadmium concentrations. Germination was evaluated as radical emergence after 7 days.

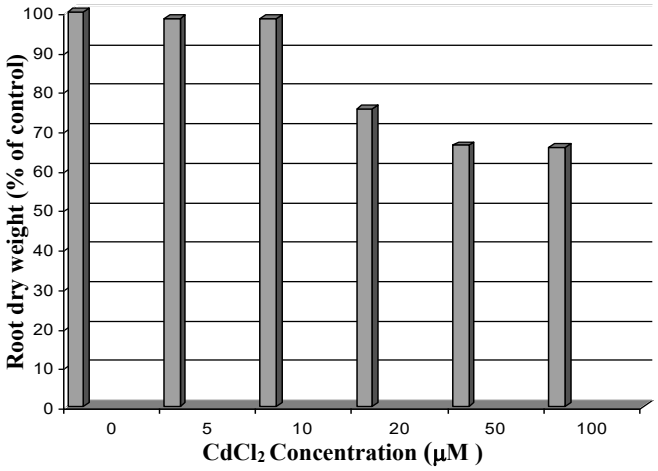

Fig. 2. Effect of increasing Cd supply on root dry weight of pea plants grown in nutrient solution for 7 days.

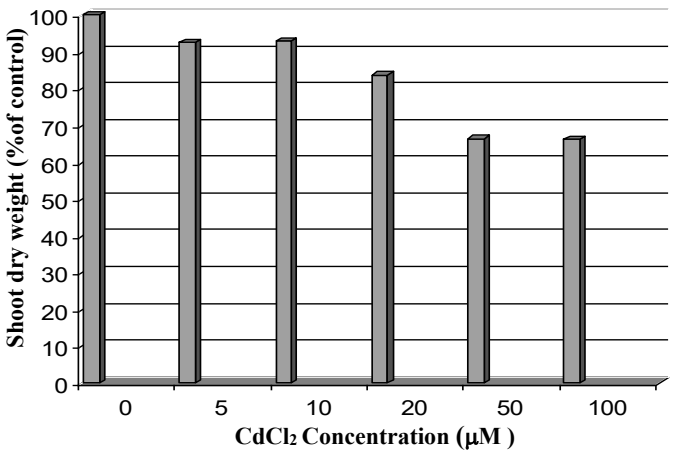

Fig 3. Effect of increasing Cd supply on shoot dry weight of pea plants grown in nutrient solution for 7 days.

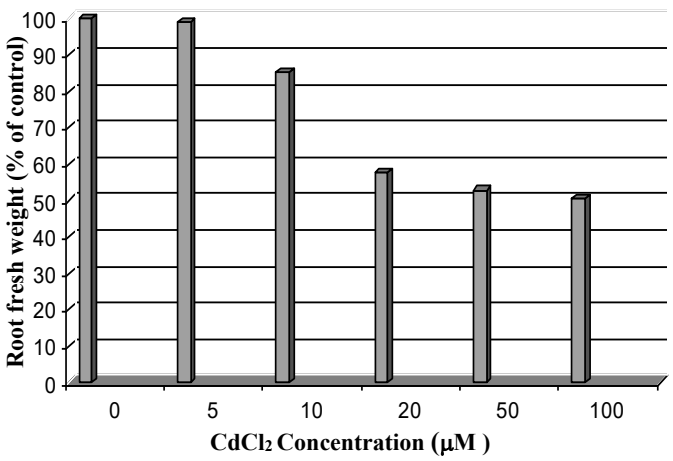

Fig. 4. Effect of increasing Cd supply on root fresh weight of pea plants grown in nutrient solution for 7 days.

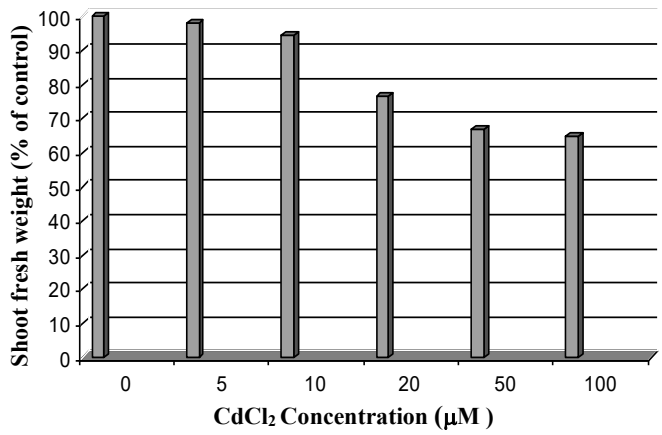

Fig. 5. Effect of increasing Cd supply on shoot fresh weight of pea plants grown in nutrient solution for 7 days.

High cadmium accumulation in plants may interfere with plant growth and development in several ways such as reduction of enzymatic activity (Ouarili *et al*., 1997; Van Assche & Clijsters, 1990), disorders both in dark respiration and photosynthesis (Vassilev & Yordanov, 1997), stomatal closure (Barcelo & Poschenrieder, 1990) and inhibition of nutrient uptake (Sanita di Toppi & Gabbrielli, 1999). The consequence of root growth inhibition by cadmium will be low nutrient and water uptake, low rate of transpiration and as a result low shoot growth rate (Chen *et al*., 2003). Protein synthesis was greatly affected by cadmium treatments. There was a significant difference in plants protein content between plants treated with cadmium and control. In the presence of 20, 50 and 100 µM $CdCl_2$, the reduction in root protein content was 31%, 30% and 38% respectively (Fig. 6). The reduction in the amount of protein could be due to decrease in protein synthesis or an increase in the rate of protein degradation (Blaestrasse *et al*., 2003). In roots of soybean plants cadmium treatments 50 and 200 µM have caused an increase in the rates of protease activity. However in nodule, higher concentrations of cadmium (200 µM) decreased both protease activity and total protein content (Balestrasse *et al*., 2003). This reflects the toxic effects of supra cadmium concentrations on protein synthesis machinery which results in both a decrease in protease activity and the content of other proteins. Possibly, by reacting with the SH-groups cadmium may result in protein denaturation (Fuhrer, 1982). Heavy metals are known to promote protein denaturation (Gadd & Griffith, 1978) and to increase the hydrolytic activities of proteases, RNAase and DNAase enzymes (Lee *et al*., 1976 a,b).

Peroxidases which constitute a wide variety of heme-containing enzyme act in a wide range of normal and stress related physiological processes in plants (Bruce *et al*., 1993). In *Phaseolus vulgaris* roots and leaves, 5µM Cd enhanced activities of guaiacol and ascorbate peroxidases and raised lipid peroxidation (Chaoui *et al*., 1997). Depending on concentration cadmium affected peroxidase activity differently. At low $CdCl_2$ concentrations (5, 10 and 20 µM), the rates of peroxidase activity in both roots and shoots were higher than at 50 and 100 µM $CdCl_2$ (Figs. 7-8). At such a high $CdCl_2$ concentrations the effects on plants defense mechanisms is so high that the plants protein synthesis machinery is reduced drastically and thus no peroxidase is synthesized. Cadmium cause production of reactive oxygene species (ROS) in plant and animal cells (Sobkowiak *et al*., 2004).

Cadmium, unlike other heavy metals, such as Cu, seems not to act directly on the production of ROS via Fenton and/or Haber–Weiss reactions (Sobkowiak *et al*., 2004). Peroxidase catalyzes the polymerization of phenolic compound into lignin and forms cross-links between lignin, extensin and feruloylated polysaccharides (Grisebach, 1981) and thus preventing plant growth and development. We consider the increase in peroxidase activity in Cd-treated pea plants as one of the important criteria in recognizing cadmium toxicity effects in plants leading to leaf senescence and plant death.

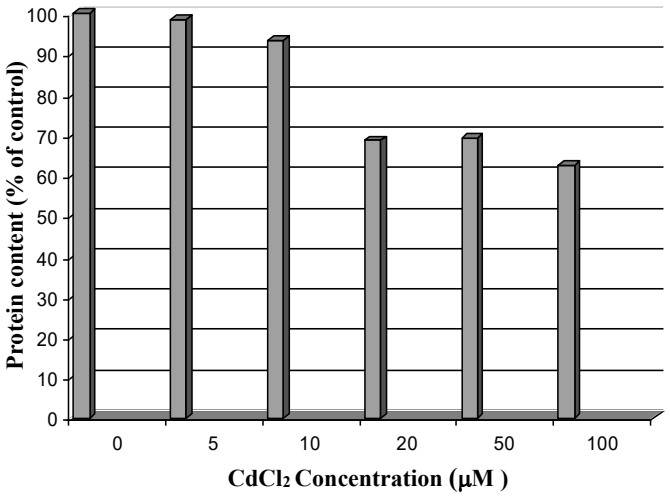

Fig. 6. Protein content in roots of pea plants grown in nutrient solution with different levels of Cd for 7 days.

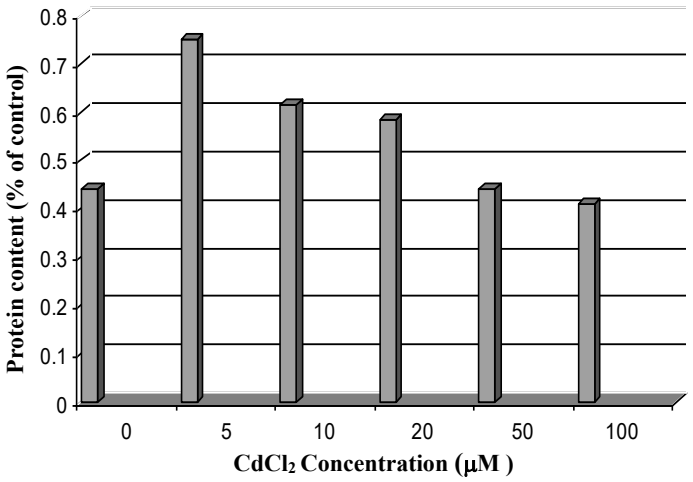

Fig 7. Effect of Cd treatments on the activity of peroxidase in the roots of pea plants.

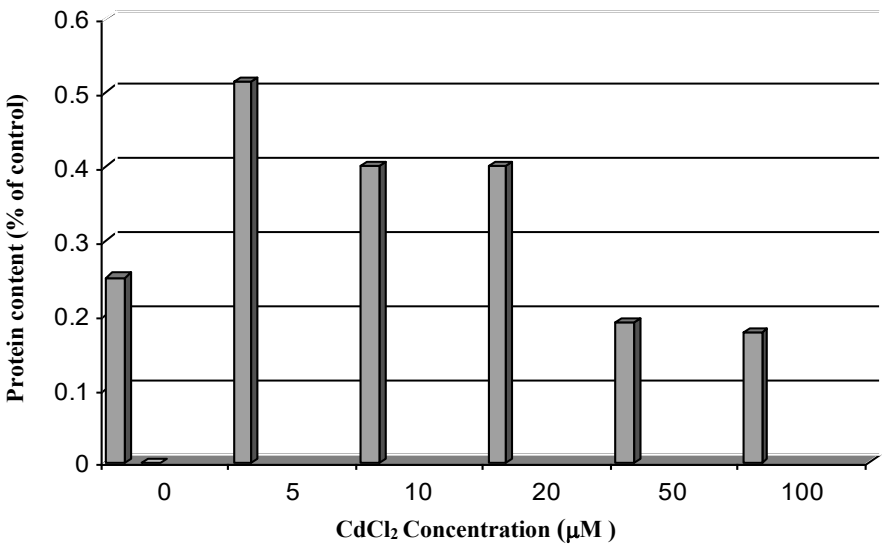

Fig. 8. Effect of Cd treatments on the activity of peroxidase in the leaves of pea plants.

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(Received for publication 9 October 2009)