quickly cooled in an ice bath for 15 min. After centrifugation at $10.000 \times g$ for 15 min, the absorbance of the supernatant was measured at 532 nm. A correction for non-specific turbidity was made by subtracting the absorbance value taken at 600 nm. The lipid peroxides were expressed as nmol MDA (mg protein)$^{-1}$, by using an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.7. Catalase assay

The activity of catalase was assayed according to the method of Aeby (1984) with some modifications. Fresh samples (1 g) were homogenized in 5 mL of 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10 g l^{-1} PVP, 0.2 mM EDTA and 10 mL L^{-1} Triton X-100. The homogenate was centrifuged at $12.000 \times g$ for 20 min at 4 °C and then, the supernatant was used for the enzyme assay. Activity of catalase was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm from a reaction mixture containing 2 mL 15 mM H_2O_2 in KPO_4 buffer (pH 7.0) and 30 µl extract. Activity was expressed as ΔE/min/mg protein.

2.8. Ascorbate peroxidase assay

Ascorbate peroxidase (APX) was measured according to Zhu et al. (2004). The reaction mixture, at a total volume of 2 mL, contained 25 mM (pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2 and 100 µl enzyme extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm ($E = 2.8$ mM^{-1} cm^{-1}) and activity was expressed as µM ascorbate oxidated min^{-1} mg^{-1} protein.

2.9. Protein extraction

In all the enzyme preparations, protein was determined by the method of Bradford (1976) using bovine serum albumin as standard and was expressed in mg.

2.10. Statistical analysis

The analyses of variance were computed on statistically significant differences determined based on the appropriate *F*-tests. The results are the means ± SD of at least three independent replicates. The mean differences were compared utilizing Duncan's range test. Three pools of five replicates each ($n = 3$) were taken for all analyses from each set of experiments.

3. Results

3.1. Hg content and seedling growth

The content of Hg in tissues of cucumber seedlings was exposure time- and concentration-Hg dependent (Table 1). Hg accumulated at a higher content in the roots than in the cotyledons. Hg content in the roots of 10 and 15-day-old seedlings was, respectively, about 7-fold and 5.6-fold higher than that in cotyledons. The maximum accumulation of Hg was 31 857 µg g^{-1} dry weight in roots treated with 500 µM $HgCl_2$ at 10 days.

Table 1
Mercury content of cucumber seedling growth under increasing concentrations of $HgCl_2$ for 10 or 15 days

Hg treatment (µM $HgCl_2$)	Hg content (µg g^{-1} dry wt.)	
	Cotyledons	Root
Day-10		
0	0.67 ± 0.17	0.60 ± 0.11
0.5	3.40 ± 1.47	6.13 ± 0.74
50	552.33 ± 43.5*	1284.33 ± 61.5*
250	1800.33 ± 50.5*	12498 ± 78*
500	4734.33 ± 63.5*	33377 ± 55*
Day-15		
0	1.4 ± 0.27	0.79 ± 0.05
0.5	3.38 ± 0.13	4.43 ± 0.1
50	759 ± 22*	1474.33 ± 21.5*
250	1816.33 ± 44.5*	12654 ± 45*
500	3698 ± 60*	20545 ± 42*

Data represent mean values ± SD based on independent determination.
* Different from control to $p < 0.05$.

The effect of Hg on the growth of cucumber seedlings, expressed as biomass and length of roots and shoot, are shown in Fig. 1. Hg-exposure induced a significant reduction of root (Fig. 1A) and shoot (Fig. 1B) length, and this effect varied with the time of exposure and the concentration of exogenous Hg. At the higher concentrations of Hg (250 and 500 µM $HgCl_2$), the root length of 10 and 15-day-old seedlings was, respectively, 96% and 98% less than that of the control. However, shoot length was completely impaired.

A low concentration of Hg conversely affected the production of fresh biomass, where, at about 50 µM $HgCl_2$, root fresh weight of 15-day-old seedlings increased (Fig. 1C). Moreover, only a concentration higher than 250 µM $HgCl_2$ reduced root fresh weight. For 10-day-old seedlings, the presence of Hg in substrate caused a continuous reduction in root fresh biomass (Fig. 1C), and shoot fresh biomass (Fig. 1D). At 15 days, only at 50 µM $HgCl_2$ was there no reduction observed in shoot fresh biomass (Fig. 1D). Contrary to the results observed for fresh biomass, the dry weight of roots (Fig. 1E) significantly increased as a function of Hg level in the substrate. In addition, 15-day-old seedlings showed greater dry weight than did 10-day-old seedlings. With relation to shoot dry weight, there was a significant effect at all concentrations of mercury tested (Fig. 1F).

3.2. Chlorophyll levels

The effects of Hg on chlorophyll levels are shown in Fig. 2A. The presence of Hg in the substrate caused a linear decrease of chlorophyll content in the cotyledons, but this

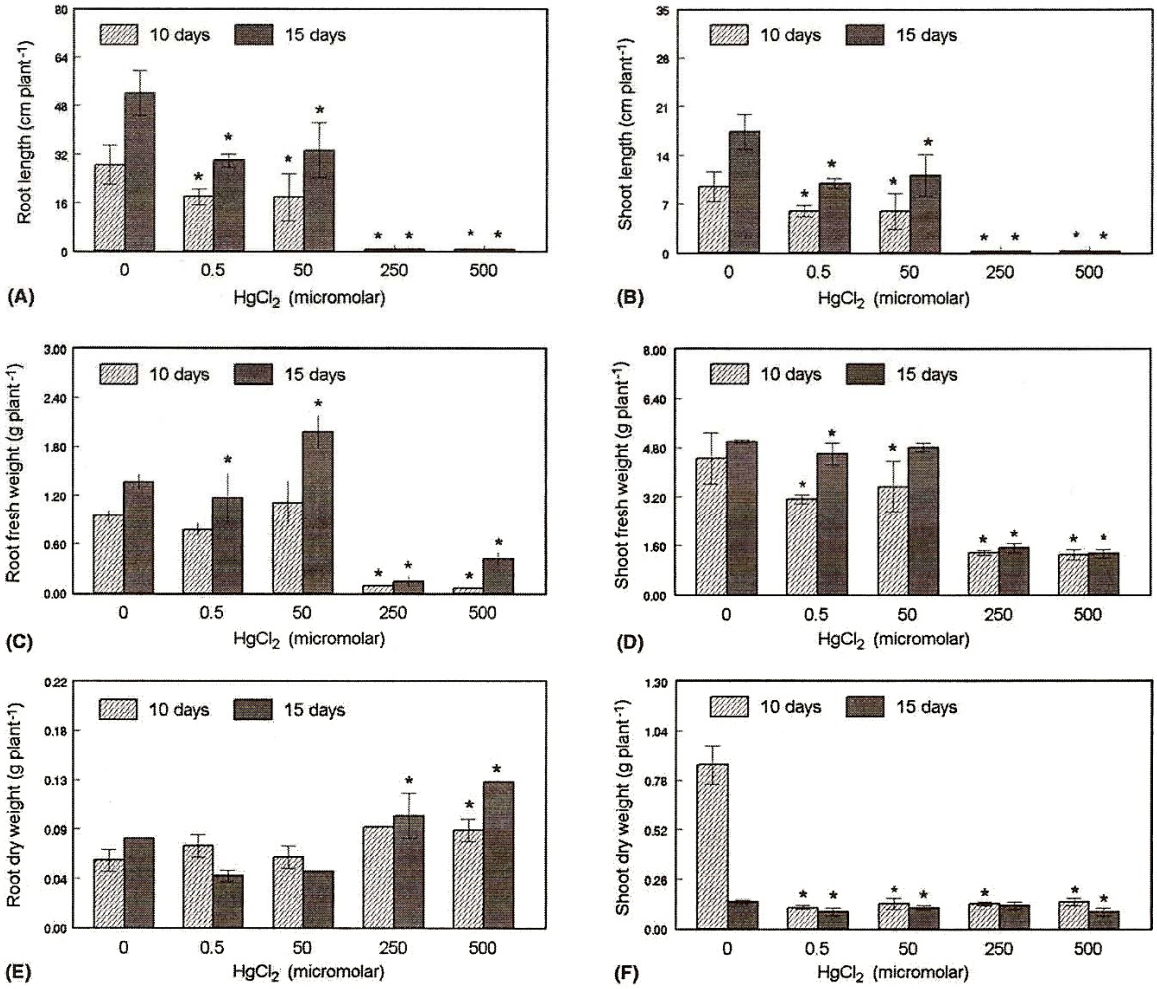

Fig. 1. Effect of increasing concentration of $HgCl_2$ in the growth medium on the length of roots (A), length of shoots (B), root fresh weight (C), shoot fresh weight (D), root dry weight (E) and shoot dry weight (F) of 10- and 15-day old cucumber seedlings. Data represent the mean ± SD of three different experiments. *Different from control to $p < 0.05$.

response varied with the time of exposure and the concentration of exogenous Hg. At the highest levels of Hg (500 μM $HgCl_2$), chlorophyll content was 59% and 94% lower, respectively, than that of the control in 10- and 15-day-old seedlings.

3.3. Lipid peroxidation and protein oxidation

The effects of Hg on lipid peroxidation and protein oxidation are shown in Fig. 2B and C. At the highest level of Hg (500 μM $HgCl_2$), the level of lipid peroxides, measured in terms of TBARS, increased 33% and 250%, respectively, in comparison with the control for both 10- and 15-day-old plants (Fig. 2B). At the concentrations lower than 250 μM $HgCl_2$, the lipid peroxide content was higher in 15-day-old seedlings than in 10-day-old seedlings.

Increasing Hg levels in the substrate caused an enhancement of protein oxidation at 250 and 500 μM $HgCl_2$ (Fig. 2C), where the highest carbonyl levels were found in the 15-day-old seedlings at the concentration of 250 μM $HgCl_2$.

3.4. Soluble protein content

The effects of $HgCl_2$ on soluble protein content are presented in Fig. 3A. The soluble protein content was exposure time- and concentration-Hg dependent. Plants treated with Hg for 10 days showed a higher soluble protein content than those treated for 15 days. In addition, regardless of Hg-exposure time, soluble protein content significantly increased as Hg increased.

3.5. Activities of some antioxidant enzymes

Catalase activity varied as a function of both exposure time and Hg concentration (Fig. 3B). For 10-day-old seed-