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Sorption of Cadmium and Their Effects on Growth, Protein Contents, and Photosynthetic Pigment Composition of *Veronica anagallis-aquatica* L. and *Ranunculus aquatilis* L.

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Toxic metal pollution of waters is a major environmental problem. Pollution et al. 1987), *Azolla pinnata* (Jackson et al. 1990), *Lemna minor* (Mo et al. 1989), of the biosphere with toxic metals, has accelerated dramatically since the beginning of the industrial revolution (Niriago 1979; Settle and Patterson 1980).The primary sources of this pollution are the mining and smelting of metalliferous ores, burning of the fossil fuels, municipal wastes, fertilizers, pesticides and sewage (Kanbata and Pendias 1989). Cadmium enters the aquatic environment through anthropogenic sources such as industry and agriculture (Kay et al.1986). Cadmium is not known to be an essential element to plants. Although a limited transport of Cadmium to shoots and binding to cell walls occur in the roots (Balsberg 1989). In many ways living plants can be compared to solar driven pumps which can extract and concentrate certain elements from their environment (Raskin et al.1994). Aquatic plants and algae are known to accumulate metals and other toxic elements from contaminated water (Wang et al. 1995). All plants have the ability to accumulate from soil and water, those heavy metals (Fe, Mn, Zn, Cu, Mg, Mo and Ni) which are essential for their growth and development (Raskin et al. 1994). Certain plants also have the ability to accumulate heavy metals (Cd, Cr, Pb, Co, Ag, Se and Hg) which have no known biological function (Raskin et al.1994; Baker and Brooks 1989). However excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate them to unusually high concentrations has evolved both independently and together in a number of different plant species (Ernst et al. 1992; Banuelas et al. 1990). The aquatic plants are often the first link in aquatic food chains, the metal concentrations of a few plant species have been analysed in relation to metal contents of aquatic environments (Baker et al. 1989).

Some aquatic or semiaquatic macrophytes such as *Eichornia crassipes* (Dierberg at al.1987) ,*Azolla pinnata* (Jackson at al.1990), *Cladophora glomerata* (Mc Hardy 1990), *Spirogyra fluviatilis* (Saygıdeğer,1998) can take up Zn, Pb, Cu, Cd, Fe and Hg from contaminated solutions. They are also known to be tolerant to these metals. This study was carried out to investigate, the short-term uptake of Cd by *Veronica anagallis-aquatica* L. and *Ranunculus aquatilis* L. Effects of Cadmium on growth, protein contents and photosynthetic pigment composition, as well as to determinate tolerance to Cadmium were investigated.

MATERIALS AND METHODS

Two aquatic plants were used as test species. The wide spread and often prolific occurence of *Veronica anagallis-aquatica* L. and *Ranunculus aquatilis* L. İn Çukurova region freshwater has made it an ideal choice as test organism for pollution studies. Test species were collected from the River Seyhan (Adana,Turkey) and kept under laboratory conditions at a temperature of 22±2°C (day) and 18±2°C (night)), (pH 7,5) and with a daily photoperiod of 16h of light (6000 ±200 lux) and 8h darkness.

V.anagallis and *R.aquatilis* plants were cultured aseptically for 7, 14 and 28 days in eight beherglasses containing 500 ml solution in the absence (distilled water) or in the presence treated of 0.5, 1.0, 5.0 ppm Cd for each plant species. Four plants were than added into each beherglasses. After exposure to either 0.0, 0.5, 1.0, 5.0 ppm Cd for *V.anagallis* and 0.0, 0.5, 1.0, 5.0 ppm Cd for *R.aquatilis*. This procedure was repeated twice.

After 14 days two plants were removed from the each beherglasses to measure to levels of protein and chlorophyll and sorption of Cd. The remaining Cd contaminated (14 days) plants were than kept in clean water (two plants for each concentrations) and modified Hoagland solution for (by diluting it tenfold with distilled water) 7 and 14 days. For each exposure period and concentrations, two plants were analyzed to determine Cadmium, protein and chlorophyll concentrations and morphological observations for each plant species.The test media were changed every third day to replenish either the Cadmium or Hoagland medium (Hasman 1972).At the end of the experiments plants were washed thoroughly in distilled water, and each sample was dried at 80°C for 24 h abaut 1g each samples were separately digested in 14 M HNO_3, the acid evaporated and residues redissolved in 1 M HCl Cadmium concentrations of the organs were measured using an Atomic Absorption spectrophotometer (AAS) Perkin Elmer Model 3100. Percent total Nitrogen analyzed by an ammonia distillation process using a micro Kjeldahl methods (Kacar 1972) and protein account was multiplyed with 6,25 factor. Total chlorophyll was measured according to the method of Kirk,(1968).

Student Newman Keul's Test (SNK) was used to determine the effects of clean water and Hoagland on the elimination Cadmium from plants and changes biomass, protein and chlorophyll level (P=0.01).

RESULTS AND DISCUSSIONS

Mean Cadmium concentrations in the organs and their associated standard deviations in *V.anagallis* and *R.aquatilis* after treatment with Hoagland medium and clean water are given in Table 1 and 2 respectively for each exposure concentration and period.

Table 1. Cadmium concentration(µg/g dry wt) in the organs of Cd *V.anagallis* after treatment with Hoagland solution and clean water

Treatment	N	Exposure Period(days)	Root x±sx	Leaf x±sx	Stem x±sx
Control	8	14	ND	ND	ND
0.5 ppm Cd	8	14	1.20±0.82a	1.10±1.07a	0.81±0.19a
Hoagland	2	7	0.98±0.15a	0.86±0.56a	0.64±0.26a
	2	14	0.76±0.37a	0.61±0.75a	0.42±0.32a
Clean Water	2	7	1.06±0.68a	0.93±0.69a	0.68±0.41a
	2	14	0.91±0.35a	0.84±0.74a	0.57±0.48a
0.1 ppm Cd	8	14	6.07±0.90c	4.16±0.83c	2.21±0.73c
Hoagland	2	7	3.83±1.02b	2.02±0.86b	1.84±0.78b
	2	14	3.34±0.86b	1.86±0.91b	1.56±0.51b
Clean Water	2	7	4.19±0.75b	2.54±0.94b	1.93±0.66b
	2	14	3.93±0.87b	2.15±0.90b	1.84±0.42b
5.0 ppm Cd	8	14	11.54±0.63d	6.17±0.62d	3.56±0.19d
Hoagland	2	7	6.74±0.79c	3.48±0.46c	2.38±0.22c
	2	14	6.18±0.10c	3.05±0.59c	1.92±0.30b
Clean Water	2	7	9.22±0.28d	4.06±0.50c	2.64±0.35c
	2	14	7.56±0.33c	3.78±0.92c	2.51±0.40c

X±Sx:Mean±Standard Error ND: Not Detectable N: Number of plant in each group
SNK:a,b,c,d show the differences among control, Cadmium and Hoagland data shown with different letters are significantly different at the P<0.01 level.

Table 2-Cadmium concentration(μg/g dry wt) in the organs of Cd *R.aquatilis* after treatment with Hoagland solution and clean water

	N	Exposure Period(Days)	Root X±Sx	Leaf X±Sx	Stem X±Sx
Control	8	14	ND	ND	ND
0.5 ppm Cd	8	14	1.75±0.66 a	1.28±1.32 a	1.05±0.05 a
Hoagland	2	7	1.46±0.90 a	1.17±0.46 a	0.85±0.76 a
	2	14	1.19±0.75 a	1.10±0.31 a	0.68±0.82 a
Clean Water	2	7	1.54±0.42 a	0.94±0.69 a	0.78±0.44 a
	2	14	1.39±1.05 a	0.84±0.48 a	0.72±0.58 a
1.0 ppm Cd	8	14	7.10± 0.55 c	5.36±0.86 d	2.87±0.50 d
Hoagland	2	7	4.54±0.69 b	1.93±0.29 b	2.16±0.47 c
	2	14	4.28±0.94 b	1.79±0.38 b	1.75±0.55 b
Clean Water	2	7	4.60±1.03 b	1.88±0.30 b	1.90±0.69 b
	2	14	4.47±1.00 b	1.84±0.42 b	1.26±0.48 b
5.0 ppm Cd.	8	14	12.60±0.38 d	6.90±0.45 d	3.65±0.52 d
Hoagland	2	7	7.43±0.71 c	4.18±0.61 c	2.50±0.77 c
	2	14	7.10±0.80 c	3.91±1.08 c	2.47±0.15 c
Clean Water	2	7	8.38±0.74 c	3.99±1.01 c	2.40±0.24 c
	2	14	7.80±0.59 c	3.84±0.79c	1.98±0.33 b

X±Sx;Mean±Standard Error ND: Not detectable N:Number of plant in each group
SNK: a,b,c,d show the differences among control, Cadmium and Hoagland data shown with different letters are significantly different at the $P<0.01$ level.

Cadmium accumulation in tissues of plants icreases with increasing exposure periods and concentrations (Balsberg 1989). Cadmium accumulation in the *V.anagallis* and *R.aquatilis* plants increased significantly after exposure to 1.0 and 5.0 ppm for 14 days ($P<0.01$). Cadmium generally accumulates to high levels in organs which have high metabolic activities such as root and leaf (Balsberg 1989). In this study the highest Cadmium accumulation also occured in the root fallowed by the leaf and stem.

In a study carried out with *Raphanus sativa*, results showed that Cadmium concentrations were much higher in the shoots than the roots (Han et al. and Lee JH 1996).

Cadmium uptake by the *V.anagallis* and *R.aquatilis* increased with increasing Cd concentration of test solution. Cadmium uptake by the two species were similar but Cadmium uptake was higher in the *R.aquatilis* than the *V.anagallis*.

Other investigators have noted Cadmium was rapidly adsorbed by *Asterionella* and *Fragilaria* in the first 5-10 min. (Conway and Williams1979;Conway 1978). Uptake of Cadmium in *Spirogyra* was completely dependent upon physico-chemical adsorption on the cell surface, uptake being very rapid in the first 30 min.(Sakaguchi et al.1979).

The strong affinity of Cadmium ions for sulphydryl groups of several compounds and phosphate groups involved in plant metabolism might explain the great toxicity.

A number of growth and biomass studies on exposure of plants to elevated Cadmium levels show similar results.

When growing *Alnus rubra* seedlings for 11 weeks in a nutrient solution, containing 3 to 122 μg Cd L^{-1} the growth of both leaves, stem and roots was stimulated slightly at the lower Cadmium

concentrations. A certain biomass decrease was noted at 0,60 μg Cd L^{-1} but leaf biomass was not significantly reduced below 122 μg Cd L^{-1} (Pahlsson 1989).

Photosynthesis and transpiration are inhibited by Cadmium (Pahlsson 1989). Measured as CO_2 uptake the photosynthetic rate in spruce (*Picea abies*) was significantly decreased by 112 μg Cd L^{-1} in the growth medium.

Cadmium is one of the most active metals on chromosome substances and in poisoning of the cell cytoplasma. Thus, cytological abnormalities have been observed in Cd treated plants was supposed to be due to a lowered cell division and cellular growth (Pahlsson 1989).

In *Acer sacharium* seedling grown in nutrient solution with 5 ppm Cadmium leaf, stem and root dry weight decrease, height decrease and chlorophyll content changes (Pahlsson 1989).

Elimination of Cadmium in Hoagland medium and clean water increased with increasing exposure period ($P<0.01$). The greatest cadmium elimination was in the *R.aquatilis* in Hoagland medium. In all experimental conditions, the elimination of Cadmium from *V.anagallis* and *R.aquatilis* was greater in Hoagland solution than clean water. Hoagland solution significantly inhibited the effects of Cadmium in test species. Cd concentrations in the organs significantly decreased after 14 days of exposure to Hoagland solution.

In this study, Cd exposure of *V.anagallis* and *R.aquatilis* caused decrease in total protein and chlorophyll concentration of the leaf (Table 3 and 4).

Table 3- Protein(mg/g wet wt) and Chlorophyll (μg/L) content and biomass(mg dry wt) in the leaf of Cd-accumulated *V.anagallis* after treatment with Hoagland solution and clean water.

Treatment	N	Exposure Period(days)	Protein X±Sx	Chlorophyll X±Sx	Biomass X±Sx
Control	8	14	18.75±0.28 a	3.01±1.10 a	32.80±0.67 a
0.5 ppm Cd	8	14	15.56±0.60 b	1.96±0.38 b	27.21±0.33 b
Hoagland	2	7	17.75±0.71 a	1.46±0.47 a	30.70±0.25 a
	2	14	18.50±0.44 a	2.85±0.11 a	32.61±0.41 a
Clean water	2	7	17.18±0.42 a	2.22±0.14 a	29.66±0.70 a
	2	14	17.50±0.73 a	2.54±0.82 a	30.40±0.62 a
1.0 ppm Cd	8	14	12.50±0.86 c	1.72±0.45 c	26.78±0.95 b
Hoagland	2	7	15.62±0.90 b	1.89±0.68 b	27.90±1.09 b
	2	14	17.00±0.81 a	1.93±1.05 b	28.32±0.19 b
Clean water	2	7	15.31±0.43 b	1.84±1.17 b	27.62±0.27 b
	2	14	15.56±0.22 b	1.80±0.85 b	28.04±0.35 b
5.0 ppm Cd	8	14	9.68±0.31 d	1.60±0.95 c	25.08±0.42 c
Hoagland	2	7	10.75±0.68 c	1.68±0.83 c	25.96±0.49 c
	2	14	11.43±0.43 c	1.71±0.96 c	25.73±0.55 c
Clean water	2	7	10.62±0.75 c	1.64±0.34 c	25.44±0.61 c
	2	14	10.85±0.72 c	1.66±0.70 c	25.79±0.74 c

X±Sx:Mean ±Standard Error N:Number of plant each group
SNK: a,b,c,d show the differences among control, Cadmium and Hoagland data shown with different Letters are significantly different at the $P<0.01$ level.

Table 4- Protein(mg/g wet wt) and Chlorophyll (µg/L dry wt) content and biomass (mg dry wt) in the leaf of Cd accumulated *R.aquatilis* after treatment with Hoagland solution and clean water

Treatment	N	Exposure Period(days)	Protein X±Sx	Chlorophyll X±Sx	Biomass X±Sx
Control	8	14	10.40±1.10 a	3.40±1.04 a	18.90±1.02 a
0.5 ppm Cd	8	14	9.82±0.75 a	3.06±0.11 a	17.05±0.99 b
Hoagland	2	7	9.90±0.81 a	3.28±0.47 a	17.85±0.48 a
	2	14	10.22±0.92 a	3.41±0.84 a	18.20±0.79 a
Clean water	2	7	9.37±0.79 a	3.20±0.61 a	17.85±0.44 a
	2	14	9.69±0.65 a	3.35±0.46 a	18.01±0.12 a
1.0 ppm Cd	8	14	8.64±0.57 b	2.78±0.75 c	16.40±0.66 b
Hoagland	2	7	8.90±0.48 b	2.90±0.73 b	16.80±0.65 b
	2	14	10.0±0.55 a	2.82±0.91 b	16.95±0.79 b
Clean water	2	7	8.30±0.61 b	2.97±0.92 b	16.69±0.86 b
	2	14	8.85±0.78 b	2.91±0.79 b	16.63±0.90 b
5.0 ppm Cd	8	14	7.05±0.70 c	2.56±0.19 d	14.80±0.69 d
Hoagland	2	7	7.63±0.67 c	2.84±0.27 b	15.10±0.47 c
	2	14	7.88±0.76 c	2.93±0.31 b	15.70±0.43 c
Clean water	2	7	7.56±0.82 c	2.67±0.29 c	14.95±0.54 d
	2	14	7.60±0.91 c	2.72±0.35 c	15.08±0.59 c

X±Sx: Mean±Standard Error N.Number of plant in each group
SNK:a,b,c,d show the differences among control, Cadmium and Hoagland data shown with different Letters are significantly different at the P<0.01 level.

Protein and chlorophyll concentration in the *V.anagallis* and *R.aquatilis* leaves decreased significantly after exposure to 1.0 and 5.0 ppm Cd (P<0.01). The highest protein decrease occured in the *R.aquatilis*. Also the highest chlorophyll and biomass decreases occured in the *R. aquatilis* after exposure to 1.0 and 5.0 ppm Cd (P<0.01).

Protein and chlorophyll synthesis in the leaf due to 14 days of Cd exposure was normalized after Hoagland treatment in the *V.anagallis* and *R.aquatilis* . In this study, plants treated with higher concentrations of Cd usually become stunded in growth. The leaves are smaller, curled and chlorotic and leaf margins and veins snow a red-brown coloration.

The first signs of toxic effects were noted in both V.anagallis and R. a*quatilis* exposed to 1.0 ppm Cd. The most conclusive evidence of damage was found for 5.0 ppm Cd solutions were *V.anagallis* and *R.aquatilis* samples had predominantly damaged at 14 days.

Results of this study showed that Hoagland solution increased elimination of Cadmium from the organs of Cd-contaminated plant and also cadmium exposure of *V.anagallis* and *R.aquatilis* caused decreased protein and chlorophyll concentrations of the leaves.

Uptake of cadmium was completely dependent upon physico-chemical adsorption on the cell surface (Sakaguchi et al. 1979)

The strong affinity of Cadmium ions for sulphydryl groups of several compounds and phosphate groups involved in plant metabolism might explain the great toxicity (Balsberg 1989).

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