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Effect of Cadmium on Germination, Seedling Growth and Metal Contents of Sunflower (*Helianthus annus* L.)

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In this study, sunflower plants were grown by using the cadmium-added irrigation water at different concentrations. Increasing cadmium applications in irrigation water caused a decrease at dry weight in third week of the study. Cadmium concentration in shoot samples increase depending on cadmium in irrigation water while a stationary change was found in cadmium level of root samples after irrigation with 0.05 mg L^{-1} cadmium. As similar cadmium in shoot, iron in root samples increase by all cadmium dozes. Furthermore, a stationary change in iron of shoot and zinc of both root and shoot were found for all doses and weeks.

Key Words: Cadmium, Sunflower, Metal, Irrigation water, Plant seedling.

INTRODUCTION

Heavy metal toxicity is one of the major currrent environmental health problems and potentially dangerous due to bioaccumulation through the food chain and in plant products for human consumption. Therefore, heavy metal contamination of soils and plants has become an increasing problem. Particularly, amongst the heavy metals, cadmium is caused increasing international concern because its toxicity is generally considered to be much higher than those of other heavy metals and it is readily taken up by plants[1,2]. As a result, European community[3] has limited the maximum cadmium concentration in irrigation water as 5.0 ng mL^{-1}.

The numbers of factors including climate, atmospheric deposition, the nature of soil on which the plant is grown and the maturity degree of plant at time of harvesting influence the concentration of heavy metals on and within plants[4,5]. Heavy metal contents of food plants can also be affected by the antropogenic factors such as the application of fertilizers, sewage sludge or irrigation with wastewaters[6,7]. Heavy metal contamination of agricultural soils can pose long-term environmental problems and are not without health implications[8].

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Thus, increasing industrialization and urbanization have not only deprived production of crops through land degradation but also caused the contamination of our precious food resources. During recent years, studies on toxic effects of heavy metals, especially cadmium, on crop plants are being received considerable attention[9]. Translocation of cadmium from root to shoot has been studied in several plant species, showing that it is likely to occur *via* the xylem and to be driven by leaf transpiration[10]. Souza *et al.*[11] examined the effect of excess cadmium and zinc ions on roots and shoots of maize seedlings. They observed that cadmium inhibited root growth more strongly than the shoots and more effectively than zinc ions. On the other hand, Klejdus *et al.*[12] reported that maize kernels exposed to the highest cadmium concentration (100 μmol L^{-1} = 11.2 mg L^{-1}) germinated formerly and much better in comparison with the 10 μmol L^{-1} (1.12 mg L^{-1}) and the control groups. Furthermore, linearly increased glutathione content was observed with the exposure time at all the studied concentrations by Klejdus *et al.*[12]. Yildiz[13] investigated the effect of lead levels on growth and mineral contents of different corn cultivars grown in nutrient culture. Nan *et al.*[14] studied the cadmium and zinc interactions and their transfer in the soil-crop system under field conditions. They found that increasing cadmium and zinc contents in soil could increase their accumulations in the crops.

The studies on the determination of metal concentrations in plant species are not important only for their translocation to food chain, but also to examination the soil remediation by phytoextraction of toxic metals. Keller and coworkers[15] studied phytoextraction efficiency of different plant species under the field conditions to remove toxic metals including cadmium, copper and zinc from the contaminated soils. Nehnevajova *et al.*[16] examined the probability of sunflower usage for metal phytoextraction in a contaminated field. The extent of environmental cadmium contamination and the risk from this metal posses to human health have stimulated interest in the development of sensitive and reliable analytical techniques for its determining in foods, biological and environmental samples such as vegetables, fruits, urine, tissues, surface waters and soils[17].

The aim of this study is to determine the impact of different cadmium levels in the irrigation water on germination and early seedling growth in sunflower. Furthermore, the concentrations of trace metals including cadmium, iron, zinc and copper in roots and shoots of sunflower were determined and examined to obtain their relations with the cadmium dozes in irrigation waters. For metal determinations, flame atomic absorption spectrometry (FAAS) was used. To improve the sensitivities of cadmium and copper in FAAS determinations, slotted tube atom trap (STAT) was used as accessory.

EXPERIMENTAL

An ATI UNICAM 929 Model flame atomic absorption spectrophotometer (FAAS) equipped with ATI UNICAM and KOTTO hollow cathode lamps was used for metal determinations. The optimum conditions for FAAS were applied as mentioned in manual book. A slotted tube atom trap (STAT) was used to improve the cadmium and copper sensitivity by FAAS.

Unless stated otherwise, all used chemicals were of analytical-reagent grade. Throughout all analytical work, doubly distilled water was used. All glass apparatus (Pyrex) were kept permanently full of 1 mol L^{-1} nitric acid when not in use. In the digestion procedures, concentrated nitric acid (65%, Merck) and hydrogen peroxide (35 %, Merck) were used. Stock solutions of metals (1000 mg L^{-1}) were prepared by dissolving their salts (Merck) in 1.0 mol L^{-1} nitric acid.

Germination and emergence

The experiment, seeds of cv VANKO (hybrid) were used, was conducted in the laboratory of Programme of Field Crops, Bingöl Trade College, University of Firat. Daily mean temperature was 29.8 ºC (27.2-32.9) during the study.

Four replicates of 25 seeds treated with fungicide were germinated in 2 rolled Whatman filter papers. A seed was considered to have germinated when the emerging radicle elongated to 1 mm. Germination percentages were recorded every 24 h for 3 d to determine germination speed and 7 d for germination rate[18]. Three replicates of 15 seeds were sown in seedling viols containing sterile turf for the emergence test. Emergence percentages were recorded for 7 d. The seedlings were selected for uniformity and transplanted onto pots (20 cm diameter) containing sterile turf. The seedlings were thinned to 5 plantlets per pot after emerging for every treatment whole 3 weeks. At the end of every week, fresh weights of shoot and root, dry weights of shoot and root, total weight and root/total weight were measured. Dry matters were measured after the samples were dried at 70 ºC for 48 h in an oven.

Cadmium was added to irrigation water to obtain four different concentrations: 10, 20, 50 and 100 ng mL^{-1}. These concentrations were chosen as regarding to the maximum allowable cadmium levels (5.0-10.0 ng mL^{-1}) in irrigation water by international regulators[3,19]. In emergence, every plant was irrigated with 80 mL water. In pots, every plant was irrigated with 400, 600 and 840 mL water in the first, second and third weeks, respectively.

Preparation of samples: The plant samples were carefully washed with deionized water and oven-dried at 75 ºC. Then, the dried samples (in range of 0.1-0.4 g) were ashed at 480 ºC for 4 h. The ashed samples were digested by using the mixture of HNO_3/H_2O_2 as described elsewhere[17]. After adding 2.0 mL of HNO_3 solution (1 mol L^{-1}), the mixture was centrifuged. The clear solution was measured by using FAAS for metal determinations.

Calibration graphs: Calibration curves were obtained by using the solutions at different concentrations of the studied elements. The obtained graphs were linear in the concentration ranges described below and the equations of the curves were as follows:

$Y = 2.4972\ X + 1.12$ $R^2 = 0.9999$ for Cd (4-100 ng/mL by STAT-AAS)

$Y = 0.3278\ X - 0.21$ $R^2 = 1$ for Cu (25-400 ng/mL by STAT-AAS)

$Y = 302\ X + 0.75$ $R^2 = 0.99$ for Zn (0.1-1.0 mg/L)

$Y = 64\ X + 0.43$ $R^2 = 1$ for Fe (0.20-3.0 mg/L)

RESULTS AND DISCUSSION

The accuracy of the method was studied by examining the recovery of the studied metals from plant samples fortified with various amounts. The following metal amounts were added: 50 ng/g of cadmium, 0.4 mg/kg of copper, 5 mg/kg of zinc and 10 mg/kg of iron, on dry weight basis. After digestion by dry ashing method, the recoveries were found to be at least 95 % for all studied metals. Levels of the metals including cadmium, copper, zinc and iron in the reagent blanks in total analytical steps were found to be 0.5; 10; 40 and 60 ng mL^{-1} with standard deviations of 0.1; 1.5; 9.0 and 8.0, respectively. Therefore, the detection limits for these elements, defined as three times the s values of blanks were calculated as 0.3; 4.5; 27 and 24 ng mL^{-1}. Related with precision, the standard deviations for 10 portions of the same sample were found to be less than 10 % for all studied elements.

Germination and emergence: The effects of cadmium contamination on growth parameters were assessed as germination speed, emergence rate and root length in emergence. The obtained results were given in Table-1. Variable effects were observed in the germination and emergence depending on cadmium doses. But these effects were not found important when compared to the control. An increase in root length in emergence was found for all cadmium doses except 0.02 ppm (Cd-2) compared to the control group.

TABLE-1
EFFECT OF DIFFERENT CADMIUM LEVELS ON THE GERMINATION, EMERGENCE AND ROOT LENGTH OF SEEDLINGS IN EMERGENCE

Dose (ppm)		Observations			
		Germination speed (%)	Germination rate (%)	Emergence rate (%)	Root length in emergence (cm)
Control		85	86	87	5.6
0.01	Cd-1	89	90	89	7.8
0.02	Cd-2	77	88	82	4.8
0.05	Cd-3	83	85	85	7.3
0.10	Cd-4	90	90	91	6.9

Early seedling growth: The effect of cadmium contamination on growth parameters were assessed as fresh and dry weight measurements. The obtained results were shown in Table-2.

TABLE-2
EFFECTS OF DIFFERENT CADMIUM LEVELS ON THE EARLY SEEDLING GROWTH IN SUNFLOWER. STANDARD DEVIATIONS WERE FOUND IN RANGE OF 10-15 %

Dose (ppm)	Fresh weight (g)			Root/ total weight (%)	Dry weight (g)			Root/ total weight (%)
	Shoot	Root	Total		Shoot	Root	Total	
I Week								
Cont.	1.4791	0.1064	1.5855	6.71	0.0839	0.0077	0.0916	8.41
0.01	1.0520	0.1997	1.9049	10.49	0.0770	0.0094	0.0864	10.88
0.02	1.8922	0.1982	2.0904	9.50	0.0986	0.0097	0.1083	8.96
0.05	1.7826	0.2253	2.0079	11.22	0.0929	0.0087	0.1016	8.56
0.10	1.6917	0.1055	1.7972	5.85	0.0954	0.0095	0.1049	9.06
II Week								
Cont.	2.7582	0.2491	3.0073	9.04	0.1390	0.0124	0.1514	8.92
0.01	2.3550	0.2726	2.6276	11.58	0.1254	0.0125	0.1379	9.97
0.02	2.0400	0.2124	2.2524	10.42	0.1066	0.0110	0.1176	10.32
0.05	2.2014	0.2423	2.4437	11.01	0.1294	0.0142	0.1436	10.98
0.10	2.5229	0.5189	3.0418	20.52	0.1925	0.0338	0.2263	17.56
III Week								
Cont.	3.5683	0.4977	4.0660	13.95	0.3349	0.0423	0.3772	12.63
0.01	3.5710	0.5259	4.0969	14.73	0.2623	0.0331	0.2994	12.43
0.02	3.5266	0.5073	4.0339	14.39	0.2514	0.0296	0.2810	11.78
0.05	3.7494	0.3527	4.1021	9.41	0.2209	0.0195	0.2404	8.83
0.10	3.4305	0.3736	3.8041	10.89	0.1966	0.0179	0.2145	9.11

In the first week of the growth, a slightly increase tendency in the fresh weight of shoots was observed, in compared with the control. The similar increase were found for dry weight of both shoots and roots in the first week. Although, the seedlings exposed to 0.01 mg L^{-1} cadmium had the total lowest dry weight, it was observed that, the highest ratio of root to total dry weight in comparison with the other doses was found in this group. An increase in the ratio of root to total fresh weight of seedlings were observed depend on increasing cadmium, but, the same trend wasn't found in Cd-4 treatment.

In the second week of the growth, a slightly decrease tendency in dry weights of shoots were observed for the first three doses of cadmium additions, when compared to the control. But these decreases were found as disproportional with cadmium doses. On the other hand, an increase was found in treatment of these samples with cadmium-4 dose. In addition, it was found that the ratio of root to total dry weight of seedling irrigated with water fortified 100 ng L^{-1} cadmium increased as high as 2-fold, in comparison with the control. A decrease was observed in fresh weight of shoots in the second week.

In the third week of the growth, a significantly decrease in dry weights of shoots and roots were found while a significant change was not observed in fresh weights of these samples. The ratio of root to total dry weight of seedling irrigated with water fortified 100 ng mL^{-1} cadmium decreased as high as 1.4-fold, in comparison with the control. In a study conducted with sunflower by Gallego *et al.*[20], it was found that cadmium ions can inhibit (and sometimes stimulate) the activity of several antioxidaditive enzymes. They have reported cadmium enchaded lipid peroxidation, increased lipoxygenase activity and decreased the activity of some antioxidative enzymes. With the same trend, decreasing ratio of root to total dry weights was observed with the increasing cadmium levels. Similar results have been reported in the previous studies. Lin *et al.*[21] suggest that cadmium enters first the roots and consequently they are likely to experience cadmium damage first. Normally cadmium ions are mainly retained in the roots and only small amounts are transported to the shoots[22] and in a general way of cadmium in plants causes leaf roll and chlorosis and reduces growth, both in roots and in stems[23].

Chemical analysis: The results obtained related to cadmium, iron, zinc and copper concentrations of the seedlings roots and shoots were given in Table-3.

It is known that the quantity of cadmium accumulation in plants have changed depending on the plant species. In emergence, it was observed that cadmium accumulation in the seedlings have increased linearly with increasing cadmium doses for shoots while a constant value observed after Cd-3 doses (0.05 ppm). This increase in cadmium accumulation are as high as 1.5, 5.0 and 9.0 -fold for irrigation water with 0.01, 0.02, 0.05 mg L^{-1} cadmium, respectively, in compared with control. Similar results were observed in 3 weeks of the studies. Azevedo *et al.*[24] found that, most of the cadmium taken up by sunflower plant was retained in the root, but the portion of cadmium that was translocated to the shoot increased with the increase in cadmium concentration in the medium. So, the obtained data in this study are harmonious with their results.

It was seen that the change in cadmium concentrations of root samples were similar to total samples. However, the changes in cadmium concentrations of shoot samples were found different from total and root samples. Briefly, cadmium concentrations of root and total samples were increased up to first two cadmium-dozes (a decrease was seen for Cd-3 and Cd-4 doses).

TABLE-3
DIFFERENT METALS CONCENTRATION OF SEEDLINGS (mg kg^{-1})

Growth Period	Control			Cd-1			Cd-2			Cd-3			Cd-4		
	Roots	Shoots	Total	Roots	Shoots	Total	Roots	Shoots	Total	Roots	Shoots	Total	Roots	Shoots	Total
Cadmium															
Emergence		75			97			102			135			280	
7 day	205	103	308	329	221	550	997	345	1342	1843	562	2405	1762	699	2461
14 day	394	167	561	1097	383	1480	1896	435	2331	1655	616	2271	1357	692	2049
21 day	492	192	684	654	220	874	841	293	1134	2145	492	2637	2056	617	2673
Iron															
Emergence		163			181			174			208			246	
7 day	280	77	357	460	107	567	944	101	1045	976	92	1068	1094	129	1223
14 day	525	98	623	627	97	724	833	105	938	931	97	1028	1028	66	1094
21 day	596	103	699	502	95	597	710	98	808	800	94	894	959	89	1048
Zinc															
Emergence		83			70			73			62			80	
7 day	123	57	180	239	84	323	237	60	297	163	80	243	170	69	239
14 day	254	53	327	305	59	364	167	47	214	242	64	306	218	61	279
21 day	168	63	231	183	36	219	244	54	298	93	54	147	124	62	186
Copper															
Emergence		27			23			24			23			23	
7 day	5	14	19	5	14	19	5	14	19	4	15	19	5	13	18
14 day	7	9	16	9	8	17	8	9	17	8	8	16	6	7	13
21 day	4	4	8	4	4	8	6	5	11	4	5	9	5	6	11

In the third week, cadmium concentrations of root and total samples were found approximately stationary after Cd-2 dose, while the increase continue up to Cd-4 dose for shoot samples. Cadmium concentrations in shoot samples were increased by increasing cadmium in water.

An increase in iron concentrations of roots and total samples were observed by increasing cadmium concentration in irrigation water while invariable (generally stationary) results were found for corresponding shoots samples. In all weeks, it was observed that the change in iron concentrations of root and total samples were the same as similar to cadmium concentrations. In the second and third weeks, iron concentrations in shoot samples were not changed appreciably compared to the root and total samples. In respect of zinc accumulation, it was found that zinc concentration of seedlings in emergence decreased with all cadmium treatments, in compared to the control. Although an increase in zinc concentration in the seedlings for Cd-1 treatment was found for roots, a decrease was observed for other cadmium doses, in the first 2 weeks. On the other hand, a stationary/decreasing change was observed in zinc concentrations of seedlings for shoots in the first 2 weeks. In all weeks, it was seen that the change in zinc concentrations of root samples were similar to total samples. In a previous study conducted with 15 sunflower cultivars by Nahnevajove *et al.*[16]. The metal concentration (mg kg^{-1} DW) in sunflower cultivars varied from 0.5-1.7 for cadmium, 2.8-26.5 for lead and 128-408 for zinc, depending on individual cultivar and fertilization (ammonium sulphate and ammonium nitrate) treatment. The observed results in this study are in these ranges, particularly for zinc. The researchers obtained that, metal accumulation in sunflower cultivars was found to be much lower than in hyperaccumulator plants such as *Thlapsi caerulescens*. But, a significantly higher biomass makes this high-yielding oil crop promising for the phytoextraction of toxic metals.

Copper accumulation of seedlings was not noticeably changed by cadmium treatments. But, a decreasing tendency in copper accumulation of seedlings was observed throughout the growth period from emergence to the third week particularly for shoots.

Conclusion

Cadmium, iron and zinc concentrations in the roots were found significantly higher than the shoots throughout the growth period. Some authors reported that, the highest metal concentration was found in leaves, whereas flowers accumulated relatively low amounts of metals, which even decreased during the ripening process[16]. Singh *et al.*[25] observed that, accumulation of chromium, iron, zinc and manganese was found to be maximum in the roots followed by the shoots and leaves and at initial exposure period (30 d), the metal accumulation followed the order roots > shoots > leaves. It has also been reported that, iron accumulated more effectively in the plants of

H. annuus in comparison to chromium, zinc and manganese. In a study conducted with *Helianthus annus* and *Brassica napus* by Solhi *et al.*[26], significant differences were found in shoot and root lead and zinc concentrations of sunflower. Root lead concentrations of sunflower were higher than the shoot lead concentrations, but root zinc concentrations were lower than the shoot zinc concentration, in their study, on the contrary of present results. This can be attributed to the antagonistic effect between lead and zinc. In respect to copper distribution in different seedlings tissues throughout the growth period, roots had lower copper than the shoots in the first two weeks. It was reported that shoot metal accumulation depends on three key factor: metal solubility, metal absorption by roots and metal translocation from roots to shoots[27]. Furthermore, soil factor, such as pH, organic matter content strongly affect the plant available metal fractions[28,29].

Consequently, the results obtained from the present study showed that, final germination and emergence percentages were not noticeably changed by cadmium treatments, but root length was slightly increased with increasing cadmium doses in irrigation water. In the first two weeks of the growth, cadmium treatments increased dry weight of seedlings but decreased dry weight of seedlings for both root and shoot, in the last week of the study. Generally two groups of plant species are considered for metal phytoextraction: (i) hyperaccumulating species able to accumulate and tolerate extraordinary metal levels and (ii) high biomass producing species, such as, maize, tobacco and sunflower, compensating moderate metal accumulation by high biomass yield[30-33]. Sunflower is a fast-growing oil crop with a high biomass production that makes it interesting especially for metal phytoextraction[16] (metal extraction = accumulation × yield).

Increasing cadmium levels in the irrigation water caused an increase in cadmium, iron and zinc accumulation of seedlings, but did not affect on the copper concentration. Azevedo *et al.*[24] reported that, in cadmium-treated plants, cadmium significantly caused decreases in the contents of iron in roots and copper in leaves. Pinto *et al.*[34] found that organic matter promoted the translocation of cadmium to plant shoots and decreased the uptake of copper, zinc and iron. Consequently, a great part of the accumulated metals have been storaged in the root parts of the seedlings.

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