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Studies on the effect of heavy metal (Cd and Ni) stress on the growth and physiology of *Allium cepa*

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ABSTRACT

The toxicological and physiological effects of various concentrations of Cadmium (Cd) and Nickel (Ni) on the different growth and physiological parameters viz. dry weight, biomass accumulation, weight of onion bulb, diameter of onion bulb and chlorophyll content of Allium cepa at different stages of growth was studied. Three different concentrations viz. T_1 *(25 ppm),* T_2 *(50 ppm) and* T_3 *(100 ppm) of Cd and Ni were used along with* T_0 *control plant. Negative correlation was observed between different growth parameters and increasing concentration of Cd and Ni at all stages of growth. Toxic effects of heavy metal uptake in plants was clearly visible with wilting of leaves, decrease in weight and size of the onion bulb, decrease in biomass accumulation, decrease in the overall chlorophyll content. The intensity of such decrease increased with passage of time and increase in heavy metal concentration. Maximum toxicological effects were seen on 70 Days after sowing (DAS). Ni was identified as a potential inhibitor of photosynthesis. It was also seen that heavy metals compete with micronutrients and inhibit their uptake thus disturbing the growth and physiology of the plants.*

Keywords: Heavy metals, toxicity, Cadmium, Nickel, *Allium cepa*

INTRODUCTION

Protection of the environment is the most vital issue today. Explosive population growth, rapid progress in science and technology, massive industrialization, use of various chemicals in agriculture and most importantly anthropogenic sources are some of the factors threatening the very quality of life [16]. The tremendous progress in every sphere of science and technology has led ultimately to detrimental environmental impact resulting in extreme unhygienic conditions modifying our living environment. Today the world is facing both an environmental and developmental crisis and both these crisis seems to be intensifying and interacting to reinforce each other. Mans increased effectiveness in industrialization has brought him in contact with rare minerals of the earth for which evolution provided no effective homeostatic mechanism. At the same time man's megalopolophilla accentuates his environmental pollution from industrial and agribusiness wastes. Industrialization has led to increased introduction of several heavy metals into the soil and water systems. The major environmental problem crops out from waste disposal, either in form of air pollutants or water pollutants [12] of which heavy metals are a major component. The pollutants are usually coming out from industrial, mining and milling operations and nuclear wastes. Heavy metals as pollutants have received escalating attention due to their possible injurious effects to man, animals and plants. Heavy metals are conventionally defined as elements with metallic properties viz. conditivity, ductility, stability as cations, ligand specificity, etc. and atomic number greater than 20. Heavy metals form a major group of toxic pollutants as they have the potential to tamper the harmony of the ecosystem [14].

Cd and Ni are heavy metals of great importance. Cd is an important toxic metal pollutant. It is a non-essential element with reported toxic effects on plants especially at ionic balance [15], enzymatic activity [19] and pollen germination and germ tube elongation [5]. Ni does not occur in nature in pure form. It is a ubiquitous trace metal and has been detected in all parts of the biosphere. The human dietary uptake of Ni is 50-80 mg/gm of diet. Higher quantities of Ni are known to be injurious to human health [13].

In this paper, studies have been made on different growth parameters of *Allium cepa* and their response to heavy metal (Cd and Ni) stress has been reported.

MATERIALS AND METHODS

The seeds of *Allium cepa* were obtained from the Rajasthan State Seed Corporation, Kota. Garden soil of average fertility was used for the study. Four kilograms of soil was filled in each pot of 30 cm height and 25 cm diameter. Soil was dried in the oven at 105^0 C, ground properly in mortar and pestle and mixed thoroughly. Three different concentrations of Cd and Ni were prepared by taking corresponding amounts (calculated on the basis of their atomic weights) of $CdCl_2$ and $NiCl_2$ (E. Merck G. R.) and mixed thoroughly in soil. The Cd and Ni concentrations applied were 25ppm, 50ppm and 100ppm represented as T_1, T_2 and T_3 respectively. Pots without any added metal constituted the control plant represented as T_0. Four inoculated seeds were sown per pot and watering was done every day. Two sets of triplicates of each concentration of both metals were used and triplicate of control plants were also set up. Sampling was done at different stages of growth viz. 28, 42, 56 and 70 Days after sowing (DAS) and observations were noted for dry weight, biomass accumulation, weight and diameter of bulb and chlorophyll content. Dry weight was obtained by washing the plant sample with distilled water and drying in a hot air oven at 90^0C for 24 hrs. The plant samples were then cooled in desiccators for about 15 minutes and then weighed. Chlorophyll content was estimated using the Hiscox and Israelstam (1978) method [8] using Di Methyl Sulphoxide (DMSO).

RESULTS AND DISCUSSION

Table No. I: Dry weight (gm) of *Allium cepa* under Cd and Ni stress

Conc. (ppm)	Symbol	Cd DAS				Ni DAS			
		28	42	56	70	28	42	56	70
Control	T_0	1.6	2.0	6.3	7.6	2.6	4.0	6.3	7.6
25	T_1	1.5	1.7	2.5	6.6	2.2	3.8	3.2	6.2
50	T_2	1.2	1.5	2.0	4.3	1.8	3.4	2.1	4.4
100	T_3	0.4	0.9	1.2	2.7	1.6	2.9	1.9	1.0

Table No. II: Biomass Accumulation (gm) of *Allium cepa* under Cd and Ni stress

Conc. (ppm)	Symbol	Cd DAS				Ni DAS			
		28	42	56	70	28	42	56	70
Control	T_0	6.9	8.7	15.3	18.6	6.7	8.7	15.3	18.6
25	T_1	5.5	6.0	9.7	12.9	4.6	5.2	14.3	16.5
50	T_2	5.1	4.2	9.1	11.7	2.4	4.0	9.2	13.6
100	T_3	3.7	3.6	7.5	9.3	2.1	3.4	8.1	9.8

Table No.III: Weight of Onion Bulb (gm) of *Allium cepa* under Cd and Ni stress

Conc. (ppm)	Symbol	Cd DAS				Ni DAS			
		28	42	56	70	28	42	56	70
Control	T_0	6.2	10.2	15.2	18.2	7.9	12.6	15.2	18.2
25	T_1	6.1	9.8	12.6	14.5	7.1	10.1	14.6	17.1
50	T_2	5.5	7.7	7.6	6.2	6.2	8.7	8.4	11.3
100	T_3	5.4	5.3	4.0	5.1	4.9	5.0	7.8	11.0

Table No. IV: Diameter of Onion Bulb (cm) of *Allium cepa* under Cd and Ni stress

Conc. (ppm)	Symbol	Cd DAS				Ni DAS			
		28	42	56	70	28	42	56	70
Control	T_0	4.3	5.6	9.0	11.1	4.3	4.9	9.0	11.1
25	T_1	4.1	5.3	7.2	8.7	2.1	4.8	8.8	10.3
50	T_2	3.8	4.2	4.7	6.3	2.0	4.3	6.9	8.4
100	T_3	3.1	3.9	4.1	3.9	1.8	3.9	5.6	7.8

Table No. V: Chlorophyll Content (mg/gm Fresh Weight) in leaves of *Allium cepa* under Cd and Ni stress

Conc. (ppm)	Symbol	Cd DAS				Ni DAS			
		28	42	56	70	28	42	56	70
Control	T_0	1.3	1.6	2.9	3.4	1.4	1.6	2.9	3.4
25	T_1	1.0	1.1	1.6	2.3	0.9	0.9	2.7	3.1
50	T_2	1.9	1.1	1.4	2.1	0.8	0.8	1.7	2.5
100	T_3	0.5	0.6	1.3	2.0	0.4	0.7	1.5	2.2

Results are presented in tables I-V. On the basis of the results obtained, it is clearly evident that Ni uptake by *Allium cepa* plants is relatively lower as compared to Cd uptake: thus plants under Ni stress show better yield and growth as compared to plant under Cd stress. Lower uptake of Ni is because the organic and phosphate matter in soil reduce the availability of Ni to plants. Similar result was given by Halstead et al. [7].

***Allium cepa* as affected by Cd stress**

Dry weight of T_0 plant on 28 DAS was 1.6 gm and that of T_1, T_2 and T_3 was 1.5, 1.2 and 0.4 gm showing a reduction of 6%, 25% and 75% respectively. Decrease in dry weight further increased on 70 DAS where T_0 plant was 7.6gm and T_1, T_2 and T_3 plants were 6.6, 4.3 and 2.7gm respectively showing a reduction of 13%, 43% and 64% respectively (Table No. I). Dry weight reduction is often used as a measure of heavy metal toxicity [10] [18]. Reduction in dry weight could be due to the lesser translocation of food materials from the seeds to the plants under subjection of metal stress. Cd has also been reported to inhibit the uptake of micronutrients causing harmful after-effects to the plants. Similar reports have also been given by [17].

Biomass of *Allium cepa* also showed a decrease up to 50% on 70 DAS (Table No. II). Reduction in biomass of a plant is a clear indication of it being contaminated and under heavy metal stress. Reduction in biomass accumulation could be due to a possible retention of Cd in plant system, resulting in physiological damage to the plant. Similar results were given by [26]. Hidden injuries and divergence in the metabolic pathway due to Cd affected plants, causing decrease in biomass accumulation have also been reported by [11]. Concentration dependent decrease in weight and diameter of bulb was observed (Table No. III & IV). Weight of onions bulbs in T_0 plants on 28 DAS was 6.2gm while in T_1, T_2 and T_3 plants was 6.1, 5.5 and 5.4 gm respectively Maximum decrease was noticed in T_3 plants with a reduction of 13%. This reduction increased to 83% in T_3 plants as compared to T_0 on 70 DAS. Similar concentration dependent results were observed in case of diameter of onion bulbs. Onion bulbs in T_3 plants showed a major decrease of 65% with respect to T_0 plants on 70 DAS. Decrease in weight and diameter of onion bulbs is a direct consequence of heavy metal stress. As concentration steadily increased, weight and diameter of bulbs decreased. Cd, even at low levels has been found to be hazardous [26]. Cd toxicity also affects the plants to the extent that the plant is forced to change its metabolic pathway [11]. Micronutrients are the pre-requisite for healthy development of the bulbs, heavy metals compete with micronutrients and inhibit their uptake thus disturbing the growth and physiology of the plants. Similar results have been reported [2], [1].

Chlorophyll content of T_0 plants on 28 DAS was 1.34mg / gm Fresh Weight (F.W.) and T_1, T_2, and T_3 was 0.1, 0.9 and 0.5 mg /gm F.W. respectively showing a maximum reduction of 60% in T_3 with respect to T_0 plants. This reduction was further seen on 70 DAS, chlorophyll content in T_0 was 3.4 mg/gm F.W. and in T_3 plant was 2.0mg / gm F.W. showing a reduction of 41% (Table No. V). Reduction in the Chlorophyll content brings about multidimensional impact on the plant. Such reduction in Chlorophyll content under heavy metal stress has been earlier studied by [6], [4], [9]. Decrease in Chlorophyll content is also concentration dependent and is inversely proportional to increasing heavy metal concentration. Metal toxicity reduces the rate of photosynthesis [24] and Chlorophyll content in plants [22]. The reduction in the Chlorophyll content was directly related to the accumulation of the metal ions in leaf tissues. [23] also reported the reduction in Chlorophyll content in vascular plants with Cd treatments. Decrease in Chlorophyll content may be due to inhibition of cytochrome oxidase which regulates Chlorophyll synthesis and production [28], [25].

***Allium cepa* as affected by Ni stress**

Similar effects of toxicity were seen in Ni contaminated plants. Distinct morphological and physiological alterations under metal stress were noticed. Dry weight of T_0 plants on 28 DAS was 2.6 gm whereas that of $T_{1,}$ T_2 and T_3 plants was 2.2, 1.8 and 1.6 gm, respectively showing a reduction of 15%, 31% and 38% respectively (Table No. I). Reduction further increased on 70 DAS up to a maximum of 86% in T_3 with respect to T_0 plant. The constant reduction in dry weight could be due to lesser translocation of reserve food materials from the seeds to the plants due to Ni stress. [17] also reported similar results. Decreasing biomass in Ni subjected plants is a clear indication of Ni toxicity (Table No. II). Ni at lower concentrations has been found to enhance plant growth and act as a micronutrient but Ni at elevated levels has been found to be phyto-toxic [21]. This may be due to the retention of Ni in the plant system. Enzyme activity which is directly responsible for plant growth may be directly inhibited due to Ni accumulation. Severe cases of acute Ni toxicity have been found to divert the metabolic pathway and may even cause death of the plant, [11]. The decrease in weight and diameter of onion bulb (Table No. III & IV) is also attributed to the possible retention of Ni in the plants thus showing corresponding toxicity. Micronutrients essential for normal plant growth are inhibited due to Ni uptake thereby affecting the overall growth and physiology of the plants [27]. Reduction in chlorophyll content under Ni stress was evident. Ni as an inhibitor of photosynthesis has also been reported by [20], [13]. On 28 DAS the chlorophyll content showed a maximum decrease of 64% in T_3 plants with respect to T_0 plants. Similar results were seen on 70 DAS. Ni induced reduction in chlorophyll content is basically through an indirect method in which Ni reduces photosynthesis by affecting the electron transport mechanism [20]. Due to reduction in the rate of photosynthesis, the production of chlorophyll content was hampered. Chlorophyll content was also reduced by Ni subjected decrease in stomatal conduction [13].

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