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Effects of Heavy Metals (Cu and Cd) on Growth of Leafy Vegetables- *Spinacia oleracea* and *Amaranthus caudatus*

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Abstract

The sewage water has been recommended for crop and in some areas this is also being used for irrigation of vegetables incase non-availability of water sources. The wastewater contains several hazardous chemicals including heavy metal which may cause serious issues of plant growth and human health. The aim of this study was to investigate the toxic impact of cadmium (Cd) and copper (Cu) on the growth parameters (shoot and root length, leaf number, shoot, root and leaf fresh and dry weight) of Spinacia oleracea and Amaranthus caudatus seedlings. The experiment was conducted using earthen pots and $CuCl_2$ and $CdCl_2$ salts were selected for experimental set-ups to test the toxicity of Cu and Cd, respectively in plants. The experimental set-ups were prepared as per standard protocol for plant toxicity testing. Two concentrations of heavy metals: low (50 ppm) and high (250 ppm) were tested for plant toxicity assessment. A set-up without metal was used as experimental control. As compare to control the set-up with Cu showed slight decrease in growth parameters but Cd causes high impact at same dose. While at higher doses both metals caused significant toxic impact and all studied parameters were low as compared to control set-up. Comparatively, Amaranthus caudatus was more sensitive towards metal toxicity as compared to Spinacia oleracea. Toxicity caused by selected heavy metals was in order Cd>Cu while heavy metal tolerance level was in the order Cu>Cd.

Keywords: Heavy metals, leafy vegetables, soil contamination, plant growth, ecotoxicity.

Introduction

Various industrial and commercial processes manipulated heavy metal compounds for numerous reasons. Heavy metals are toxic to environment as well as human health above certain concentration[1]. Heavy metals cause significant toxic impact on plants if available higher than the safe limits. A number of heavy metals at high concentrations have been reported to inhibit the vegetative growth and decrease in the productivity of crops[2]. The one of the big problem associated with the persistence of heavy metals is their potential for bioaccumulation and biomagnifications causing higher exposure in some plants and animals then they alone present in the environment. Exposures of heavy metals at higher concentration can result in damaged central and mental processes, reduction in body energy levels, and damage to blood composition, blood pressure, liver, kidney, lungs and various other vital organs. Long-term exposure may cause slow but highly damaging physical and neurological processes that mimic number of sclerosis.

Copper is an essential element for all living things, including humans. Various soils has been reported toxic level of Cu whereas other may contain high levels of Cu as a result of various anthropogenic activities. Copper is extensively used in agriculture in the form of fertilizers, growth promoters and bactericides[3]. This in turn creates copper toxicity in the soil. Normal growth of the plant requires 5-20ppm of Cu and less than 4ppm is deficient, more than 20ppm is considered to be toxic. Though Cu is an essential micronutrient required for plant growth, exposure to excess Cu has a damaging effect on vegetative plant growth. At concentrations beyond those required for optimal growth Cu was shown to inhibit growth and baffle the important physiological processes such as photosynthesis and respiration[4]. The toxic effects of excess copper in various plants also include reduction in growth, poorly developed and discolored root system, leaf chlorosis, stunted plants with reduced branches and reduction in economic yield due to male sterility. The major affect of Cu toxicity is on root growth and morphology. Cu tends to accumulate in the root tissue through soil with slow movement to the shoots[4]. Similarly, cadmium (Cd) is a nonessential element considered as an environmental contaminant because of its toxic effects on both plants and animals. It is a carcinogenic element and more available to the plants compared to other heavy metals and may thus very easily enter the human diet through food chain. Cd is well known among all other highly toxic environmental element because of its higher toxicological properties and high mobility from soil to root, root to higher plant parts and further down the food chain[5]. It can be easily accumulated in large amounts in the body of all organisms and alter physiological metabolism processes like photosynthesis, respiration, transpiration and nitrogen assimilation[6]. Processes like heating system, power station, metalworking industries or urban traffic tend to release Cd into the environment. The wide uses of Cd are electroplating, cadmium coatings, cadmium stabilisers, and nickel-cadmium batteries etc. It is also recognized as a significant pollutant due to its high toxicity and higher solubility in water[7]. Cadmium can alter the uptake of minerals from soil. The level of Cd in soil increases with increase in time. Plants can easily uptake cadmium and transfer it to other organs. In humans, Cd accumulates mainly in the kidney with a biological half-life about 20 years, and leads to pulmonary emphysema and renal tubular damage. Cd has been considered as an extremely significant toxic pollutant affecting all organisms because of its high great solubility in soil and water[7]. Developmental stages of plant such as seedlings and seed germination are more sensitive to various environmental factors like temperature, light intensity, heavy metals pollution[8].

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family of Amaranthaceae. It is an annual plant. Spinach may grow over winter in temperate regions. Though low in calories; it contains higher concentrations of minerals, vitamins and other. It has high contain of Niacin and Zinc, and also a very good source of dietary fiber, protein, and vitamins (A, C, K, thiamin, riboflavin, B6, and foliate) and essential micronutrients (Ca, Fe, Mg, P, K, Cu and Mn). Amaranthus (*Amaranthus caudatus*) is included in the family Amaranthaceae. Amaranthus has a higher content of the minerals i.e. Ca, Mg, Fe and of the amino acid Lysine. Plant species have a higher capacity of accumulating and removing heavy metals than any other life form. Some findings also suggested that plant species have variety of capacities to accumulate specific heavy metals. Roots and leaves of herbaceous plant retain higher concentration of heavy metal then stems and fruits[9].

The aim of the present study is to assess the interaction of different concentrations of Cadmium and Copper on germination of *Spinacia oleracea* and *Amaranthus caudatus*.

Material and Methods

Preparation, preservation and growth of vegetable samples: Leafy vegetables *Spinacia oleracea* and *Amaranthus caudatus* were used as experimental plant in this study. $CuCl_2$ and $CdCl_2$ salts were used a source of metals for this experimentation. All set-ups were prepared in earthen pots of 2 kg capacity. All pots, used for experimental set-ups, were filled with pre-sieved and dried garden soil (1 kg). Two concentrations: low (50 ppm) and high (250 ppm) were prepared for both metal salts. The metal salt was thoroughly mixed in soil to achieve homogenized contamination. The pots without addition of any heavy metal were considered as control. A total of 5 pots were kept for each vegetable treatment. 20 healthy seeds of each teat species - *Spinacia oleracea* and *Amaranthus caudatus* were sown in each experimental set-up for both doses of metals. The plants were raised using normal practice under field conditions. In germinated seedlings growth parameters (root length, shoot length, number of leaves, fresh weight of root, fresh weight of stem and fresh weight of leaf, dry weight of root, dry weight of stem and dry weight of leaf) were measured. For this, three plants were uprooted randomly form each pot after every ten days interval and brought to the laboratory. In lab, plants were thoroughly washed using tap water to remove adhering soil and then plant parameters were measured in fresh sample. To record dry biomass of all plant parts the plant samples were dried in hot air oven at 80^0C and then biomass was measured dry-weight basis.

Determination of physico-chemical properties of soil: Soil parameters (pH, electrical conductivity, organic C, calcium, magnesium, nitrate, phosphate and sulphate) were measured before and after experimentation in all potting soils. The pH and electrical conductivity was measured in aqueous solution using a digital pH meter (Equiptronics, India) and EC meter (Systronics-304), respectively. The organic C was measured using methodology as described by Walkley-Black[10]. Phosphate, sulphate and nitrate were measured spectrophotometrically. Exchangeable cations (Ca^{+2}, Mg^{+2}) were analyzed using the ammonium acetate extraction method at pH 7.0 as described by Hendershot et al[11]. For heavy metals, 1g soil sample was digested in acid mixture and measured using Atomic Absorption Spectrophotometer (AAS, ECIL-4141).

Statistical analysis: Based on the data obtained from the experiment, the result presented are the mean ± standard deviation (SD) gained from at least three replicate samples using SPSS (17.0) Statistical software. For the tabular evaluation of result Micro-soft Excel (MS Excel 2007) was used.

Results and Discussion

Effects of metals on vegetative growth of Spinacia oleracea: Table-1 represents the growth of *Spinacia oleracea* against different doses of heavy metals. The root length, stem elongation and leaf numbers showed increment in all set-ups (both control and experiment) over the time. The maximum growth occurred during 20-30 days of experimentation in all set-ups. Both metals (Cu and Cd) inhibited the root and stem elongation at high concentration (i.e., 250 ppm). Cd was more toxic to plants as compared to Cu. The leaf number was not much affected by Cu and Cd in experimentations. Both metals significantly caused reduction in the fresh weight of root and stem in young plants. The experimental duration (exposure duration) caused significant impact on plant growth parameters. The dry weight of leaves in young plants was more affected by Cu while Cd had severe impact in old plants. The toxic effect of Cd was more prominent in plants than Cu. Root showed more sensitivity against heavy metals followed by stem and leaf. Studies had revealed that heavy metals cause adverse affects on plant growth, which further lead to decrease plant yield and inhibition of enzymatic activities[12-13]. Some parameters like biomass are very sensitive towards heavy metals in higher plants[14]. The adverse effect was visible in 20 days old plants and it that showed significant correlation with concentration. The toxic effect of Cu on root growth was more in 60 days old plants and that of Cd was seen after 20 days of growth. Both metals caused significant effects on stem growth in old plants. The root growth was more sensitive to the toxicity end point than shoot growth. Low concentration of Cd reduces root growth without affecting leaves, and moderately higher concentrations austerely inhibit root development and accumulate in leaves[15]. The organ sensitivity was in the order root>stem>leaf. Increase in accumulation of Cd in plants tends to reduction in formation of new cells which leads to reduction in shoot and root lengths[16]. Bhanderi et al[17] reported the effects of $CdCl_2$ on growth of medicinal plants *Cassia tora, Cassia occidentalis* and *Plantago ovata*. The vegetative and reproductive growth was reduced by Cd application. The root elongation was most sensitive process. Result thus, suggested a significant impact of Cu and Cd in plants. As Cu acts as micro-nutrient in plant and have significant positive impact on plant growth if supplied at low dose. But at high level it becomes toxic to plant. The similar trend was observed in this study which suggests that dose of metal is a sensitive phenomena in terms of toxicity of any chemical substance.

Table-1
The effect of heavy metals (Cu and Cd) application on the plant root, stem length (cm/plant) and leaf number (no/plant) of *Spinacia oleracea* and *Amaranthus caudatus*

Contamination		*Spinacia oleracea*			*Amaranthus caudatus*		
		Root length	Stem length	Leaf number	Root length	Stem length	Leaf number
Control	10 d	1.16 ± 0.57	4.50 ± 0.20	2 ± 0	1.53 ± 0.25	4.10 ± 0.30	2 ± 0
	20 d	2.50 ± 0.50	6.00 ± 0.10	4 ± 0	2.53 ± 0.05	4.96 ± 0.15	4 ± 0
	30 d	3.70 ± 0.20	6.50 ± 0.40	5 ± 0	2.80 ± 0.10	6.70 ± 0.10	6 ± 0
	40 d	5.00 ± 0.30	6.80 ± 0.10	5 ± 0	5.73 ± 0.15	7.13 ± 0.05	6 ± 0
	50 d	5.23 ± 0.11	7.03 ± 0.05	5 ± 0	7.83 ± 0.05	8.03 ± 0.15	6 ± 0
	60 d	7.50 ± 0.10	8.00 ± 0.10	5 ± 0	10.46 ± 0.4	9.40 ± 0.26	7.66 ± 0.57
$CuCl_2$ 50	10 d	1.10 ± 0.00	4.26 ± 0.11	2 ± 0	0.96 ± 0.05	3.90 ± 0.10	2 ± 0
	20 d	1.33 ± 0.15	5.60 ± 0.10	4 ± 0	2.30 ± 0.10	3.93 ± 0.25	3.66 ± 0.57
	30 d	3.66 ± 0.05	5.53 ± 0.20	5 ± 0	2.50 ± 0.10	5.96 ± 0.15	4 ± 0
	40 d	4.60 ± 0.20	5.70 ± 0.30	5 ± 0	5.00 ± 0.20	6.53 ± 0.25	6 ± 0
	50 d	4.70 ± 0.10	5.90 ± 0.10	5 ± 0	6.60 ± 0.26	6.90 ± 0.20	6 ± 0
	60 d	5.60 ± 0.10	6.03 ± 2.00	5 ± 0	7.53 ± 0.35	8.30 ± 0.20	8 ± 0
$CuCl_2$ 250	10 d	0.96 ± 0.05	4.10 ± 0.17	2 ± 0	0.90 ± 0.20	3.60 ± 0.10	2 ± 0
	20 d	1.33 ± 0.05	5.40 ± 0.30	4 ± 0	2.26 ± 0.15	4.93 ± 0.15	4 ± 0
	30 d	3.50 ± 0.10	5.46 ± 0.35	5 ± 0	2.36 ± 0.05	5.53 ± 0.15	4.33 ± 0.57
	40 d	4.00 ± 0.10	5.60 ± 0.10	5 ± 0	4.70 ± 0.20	5.06 ± 0.05	5.66 ± 0.57
	50 d	4.20 ± 0.10	5.80 ± 0.30	5 ± 0	5.80 ± 0.10	5.60 ± 0.26	6 ± 0
	60 d	4.50 ± 0.10	6.03 ± 0.06	5 ± 0	7.00 ± 0.20	7.90 ± 0.20	6.66 ± 0.57
$CdCl_2$ 50	10 d	0.80 ± 0.20	3.20 ± 0.10	2 ± 0	0.73 ± 0.05	3.10 ± 0.20	2 ± 0
	20 d	1.26 ± 0.05	4.76 ± 0.05	4 ± 0	1.00 ± 0.10	2.90 ± 0.00	4 ± 0
	30 d	2.33 ± 0.06	4.86 ± 0.06	5 ± 0	1.46 ± 0.15	4.33 ± 0.25	4 ± 0
	40 d	3.00 ± 0.20	4.76 ± 0.05	5 ± 0	3.93 ± 0.15	4.46 ± 0.30	5 ± 0
	50 d	4.10 ± 0.20	4.90 ± 0.10	5 ± 0	4.00 ± 0.30	4.96 ± 0.15	5 ± 0
	60 d	4.16 ± 0.06	5.10 ± 0.10	5 ± 0	5.46 ± 0.35	7.13 ± 0.20	6 ± 0
$CdCl_2$ 250	10 d	0.60 ± 0.00	2.20 ± 0.10	1.66 ± 0.57	0.60 ± 0.10	2.16 ± 0.25	2 ± 0
	20 d	1.13 ± 0.05	1.73 ± 0.55	4 ± 0	0.80 ± 0.20	2.33 ± 0.25	3 ± 0
	30 d	1.80 ± 0.10	2.76 ± 0.58	4.33 ± 0.57	1.00 ± 0.30	3.43 ± 0.30	4 ± 0
	40 d	2.36 ± 0.25	4.53 ± 0.25	4.33 ± 0.57	2.86 ± 0.25	4.10 ± 0.20	5.33 ± 0.57
	50 d	2.70 ± 0.20	4.80 ± 0.20	4.66 ± 0.57	3.16 ± 0.15	4.46 ± 0.15	5 ± 0
	60 d	3.96 ± 0.06	5.03 ± 0.10	5 ± 0	3.16 ± 0.05	5.60 ± 0.20	5.33 ± 0.57

*Heavy metal concentrations were added in mg/kg of soil; ± Standard deviation of 3 replications; d: growth days

Table-2
The effects of heavy metals (Cu and Cd) application on the plant root, stem and leaf fresh and dry weight (gm/plant) of *Spinacia oleracea*

Contamination		*Spinacia oleracea*					
		Root FW	Root DW	Stem FW	Stem DW	Leaf FW	Leaf DW
Control	10 d	0.02 ± 0.00	0.01 ± 0.01	0.04 ± 0.01	0.02 ± 0.00	0.07 ± 0.01	0.06 ± 0.02
	20 d	0.05 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	0.11 ± 0.00	0.09 ± 0.00
	30 d	0.46 ± 0.05	0.36 ± 0.05	7.00 ± 0.20	6.50 ± 0.20	19.9 ± 0.26	17.63 ± 0.25
	40 d	2.90 ± 0.20	1.80 ± 0.10	12.16 ± 0.25	7.03 ± 0.25	26.63 ± 0.26	18.03 ± 0.15
	50 d	3.20 ± 0.10	2.20 ± 0.10	13.70 ± 0.10	8.00 ± 0.10	28.36 ± 0.25	24.26 ± 0.35
	60 d	5.40 ± 0.10	3.20 ± 0.10	16.33 ± 0.40	8.60 ± 0.15	32.33 ± 0.40	26.60 ± 0.36
$CuCl_2$ 50	10 d	0.02 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	0.05 ± 0.01
	20 d	0.04 ± 0.01	0.26 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.12 ± 0.01	0.09 ± 0.00
	30 d	0.20 ± 0.00	0.96 ± 0.00	5.00 ± 0.10	4.60 ± 0.10	16.76 ± 0.35	14.26 ± 0.05
	40 d	1.23 ± 0.05	0.76 ± 0.05	7.30 ± 0.10	5.16 ± 0.25	27.30 ± 0.40	15.26 ± 0.30
	50 d	1.46 ± 0.06	1.00 ± 0.10	7.63 ± 0.05	6.20 ± 0.10	28.20 ± 0.10	21.06 ± 0.30
	60 d	4.70 ± 0.10	2.03 ± 0.05	8.46 ± 0.20	6.96 ± 0.45	31.70 ± 0.20	23.03 ± 0.35
$CuCl_2$ 250	10 d	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.04 ± 0.01	0.03 ± 0.00
	20 d	0.04 ± 0.02	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.11 ± 0.02	0.08 ± 0.01
	30 d	0.10 ± 0.00	0.10 ± 0.00	3.10 ± 0.10	2.70 ± 0.20	16.56 ± 0.11	13.50 ± 0.10
	40 d	1.03 ± 0.06	0.60 ± 0.20	7.40 ± 0.20	3.20 ± 0.20	27.60 ± 0.10	19.40 ± 0.10
	50 d	1.23 ± 0.05	0.80 ± 0.05	7.56 ± 0.15	4.93 ± 0.15	28.00 ± 0.26	22.36 ± 0.35
	60 d	4.03 ± 0.05	1.66 ± 0.10	7.10 ± 0.10	3.20 ± 0.10	27.20 ± 0.10	14.16 ± 0.05
$CdCl_2$ 50	10 d	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.10	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
	20 d	0.03 ± 0.01	0.01 ± 0.00	0.03 ± 0.10	0.01 ± 0.00	0.04 ± 0.01	0.03 ± 0.00
	30 d	0.10 ± 0.01	0.02 ± 0.00	2.83 ± 0.05	2.40 ± 0.20	13.73 ± 0.47	11.66 ± 0.15
	40 d	0.30 ± 0.00	0.26 ± 0.11	3.90 ± 0.20	3.03 ± 0.15	16.20 ± 0.20	11.96 ± 0.15
	50 d	1.23 ± 0.05	0.80 ± 0.10	4.00 ± 0.30	4.23 ± 0.05	19.20 ± 0.10	15.03 ± 0.25
	60 d	3.16 ± 0.05	1.60 ± 0.10	4.80 ± 0.10	3.60 ± 0.20	22.36 ± 0.25	17.00 ± 0.30
$CdCl_2$ 250	10 d	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	20 d	0.02 ± 0.10	0.01 ± 0.00	0.01 ± 00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	30 d	0.10 ± 0.05	0.46 ± 0.00	2.10 ± 0.30	1.30 ± 0.15	11.46 ± 0.15	9.43 ± 0.25
	40 d	0.15± 0.05	0.80 ± 0.00	2.96 ± 0.57	1.63 ± 0.15	13.86 ± 0.25	10.30 ± 0.30
	50 d	1.16 ± 0.06	1.03 ± 0.05	3.63 ± 0.57	2.20 ± 0.10	15 .70 ± 0.10	13.46 ± 0.45
	60 d	2.70 ± 0.10	1.30 ± 0.10	4.13 ± 0.57	3.03 ± 0.15	19.20 ± 0.10	15.26 ± 0.15

*Heavy metal concentrations were added in mg/kg of soil; ± Standard deviation of 3 replications; d: growth days; FW: Fresh weight; DW: Dry weight

Effects on vegetative growth of Amaranthus caudatus: Table-3 described the results of Cu and Cd effect in growth parameters of *Amaranthus caudatus*. Over the time there was increment in root and stem length and leaf numbers in all set-ups. The rapid growth occurred between 20-30 days of exposure. The toxic effect of Cu on root growth was more prominent in old plants while in case of Cd the effect was seen during whole experimental duration. Both metals reduced the stem height with respect to increasing level of contamination in soils. Cu at higher concentrations reduced the leaf number in old plants. The high concentration of Cd caused reduction in leaf number as compared to control set-up. The leaf fresh weight was more affected by Cu and Cd in young plants. Similarly, the high concentrations of Cu and Cd have severe effects on leaf fresh weight of old plants. The dry weight of root, stem and leaf was lower in old plants than young ones in both metal contaminated soils. The low fresh weight indicates the adverse impact of heavy metal in plants[18]. Plant accumulates large portion of heavy metal in root followed by stem and leaf. The dry biomass of both roots and shoots was significantly reduced in Cd-treated plants compared to the control plants[19]. The toxic effect of Cu on root growth recorded more in old plants and that of Cd was observed throughout the growth period. Both the metals lowered the stem height of plants. Cu at higher concentrations lowered the leaf number in old plants. High concentration of Cd caused decrement in leaf number. The leaf was the least affected organ followed by stem and leaf. The toxic effect of Cd was more prominent in plant as compared to Cu. It is suggested that certain concentrations of Cd inhibits the plant growth while low concentration of Cu have significant positive impact on plant growth. The high Cu dose also has toxic impact in plants.

Table-3
The effects of heavy metals (Cu and Cd) application on the plant root, stem and leaf fresh and dry weight (gm/plant) of *Amaranthus caudatus*

Contamination		*Amaranthus caudatus*					
		Root FW	Root DW	Stem FW	Stem DW	Leaf FW	Leaf DW
Control	10 d	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
	20 d	0.02 ± 0.00	0.01 ± 0.00	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
	30 d	2.13 ± 0.05	1.83 ± 0.11	12.23 ± 0.60	8.20 ± 0.30	14.43 ± 0.50	8.16 ± 0.25
	40 d	7.10 ± 0.30	3.50 ± 0.30	16.23 ± 0.25	9.23 ± 0.15	18.20 ± 0.30	12.20 ± 0.20
	50 d	10.0 ± 0.30	5.26 ± 0.25	19.20 ± 0.20	10.66 ± 0.45	20.50 ± 0.30	13.23 ± 0.15
	60 d	15.26 ± 0.15	7.86 ± 0.35	21.30 ± 0.10	11.46 ± 0.25	28.40 ± 0.70	15.00 ± 0.20
$CuCl_2$ 50	10 d	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
	20 d	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
	30 d	1.46 ± 0.25	1.30 ± 0.20	8.13 ± 0.25	7.03 ± 0.15	9.53 ± 0.35	7.90 ± 0.20
	40 d	6.30 ± 0.20	2.73 ± 0.15	14.06 ± 0.25	7.33 ± 0.35	16.03 ± 0.25	8.20 ± 0.20
	50 d	8.70 ± 0.20	3.23 ± 0.15	15.70 ± 0.10	8.10 ± 0.20	18.66 ± 0.45	9.00 ± 0.10
	60 d	9.90 ± 0.20	4.56 ± 0.15	20.16 ± 0.25	9.50 ± 0.20	22.66 ± 0.55	10.40 ± 0.10
$CuCl_2$ 250	10 d	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.05	0.01 ± 0.00
	20 d	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
	30 d	1.16 ± 0.05	0.90 ± 0.20	6.93 ± 0.25	5.66 ± 0.15	9.03 ± 0.35	5.60 ± 0.20
	40 d	5.20 ± 0.10	2.10 ± 0.10	11.13 ± 0.25	6.20 ± 0.20	12.10 ± 0.10	6.60 ± 0.10
	50 d	7.60 ± 0.10	2.86 ± 0.25	13.66 ± 0.15	7.33 ± 0.15	13.90 ± 0.70	7.40 ± 0.20
	60 d	8.70 ± 0.20	4.03 ± 0.15	19.40 ± 0.20	7.90 ± 0.40	19.20 ± 0.50	9.00 ± 0.10
$CdCl_2$ 50	10 d	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	20 d	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
	30 d	0.23 ± 0.20	0.09 ± 0.00	4.00 ± 0.10	3.26 ± 0.20	3.00 ± 0.40	2.13 ± 0.15
	40 d	1.30 ± 0.20	1.70 ± 0.30	6.03 ± 0.25	3.03 ± 0.25	6.86 ± 0.15	3.60 ± 0.10
	50 d	2.30 ± 0.20	2.20 ± 0.10	7.56 ± 0.15	4.23 ± 0.15	10.30 ± 0.10	5.40 ± 0.10
	60 d	7.30 ± 0.20	3.50 ± 0.30	9.80 ± 0.30	5.03 ± 0.25	13.70 ± 0.50	6.50 ± 0.10
$CdCl_2$ 250	10 d	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.05	0.01 ± 0.00
	20 d	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.05	0.01 ± 0.00
	30 d	0.10 ± 0.00	0.09 ± 0.01	2.13 ± 0.05	1.30 ± 0.20	2.43 ± 0.15	1.50 ± 0.10
	40 d	0.13 ± 0.05	0.05 ± 0.20	2.70 ± 0.20	1.60 ± 0.20	2.40 ± 0.30	1.60 ± 0.00
	50 d	0.96 ± 0.05	0.93 ± 0.05	4.23 ± 0.25	2.23 ± 0.15	4.20 ± 0.40	2.26 ± 0.15
	60 d	1.30 ± 0.10	0.96 ± 0.05	5.73 ± 0.35	3.30 ± 0.10	5.63 ± 0.25	3.20 ± 0.10

*Heavy metal concentrations were added in mg/kg of soil; ± Standard deviation of 3 replications; d: growth days; FW: Fresh weight; DW: Dry weight

Effects on soil physico-chemical parameters: The results of soil physico-chemical analysis are presented in table-4. Before starting experiment the soil pH was 7.31 ± 0.02, which falls well in the optimum range (5.5-8.0), considered satisfactory for crops growth. The electrical conductivity measured which is a measurement of presence of total anions and cataions in soil was 437 ± 14 (mho/cm). The organic carbon (%) in pot soil was 1.24 ± 0.03. The organic matter presents in soil holds soil open allows infiltration and percolation of air and water, and may hold more moisture than its weight. Values of Ca^{+2}, Mg^{+2}, Cl, Cu and Cd were 18 ± 2, 38.33 ± 2.5, 26.66 ± 3.5, 21.56 ± 0.85, 1.82 ± 0.10, respectively in test soil which were well suitable for the maximum plant growth. The copper content in most plants grown in natural soils occurred between 2 and 20 ppm but here in this study it was slightly higher (21.56 ± 0.85 ppm). Plants roots are frequently higher in Cu concentration because Cu is strongly bound to soils it is very immobile. Phosphorus is another key macronutrient required for plant growth measured to 38 ± 1ppm. Phosphorus is necessary for creating a balance between the other plant nutrients and maintaining the normal plant growth. Essential plant growth indicating nutrient nitrate was in the range of 55 ± 2 ppm followed by sulphate which was 29.66 ± 3.5 ppm. The major source of nitrogen in soil due to is bacteria and cynobacteria which are used to fix atmospheric nitrogen, precipitation, surface and ground water drainage. All sulphate from organic matter released in the form of H_2S, may be converted in to elemental sulphur or sulphates which depends upon pH.

Table-4
Basic properties of soil used for present study

Parameters	Soil content
pH	7.31 ± 0.02
EC (mho/cm)	437 ± 14
Organic carbon (%)	1.24 ± 0.03
Ca^{+2} (ppm)	18 ± 2
Mg^{+2} (ppm)	38.33 ± 2.5
Cl (ppm)	26.66 ± 3.5
Cu (ppm)	21.56 ± 0.85
Cd (ppm)	1.82 ± 0.10
Nitrate (ppm)	55 ± 2
Phosphate(ppm)	38 ± 1
Sulphate (ppm)	29.66 ± 3.5

*± Standard deviation of 3 replications

After experimentation, there was significant change in soil physico-chemical properties. The soil pH was significantly affected by metal contamination. The change in pH indicates the soil mineralization process which is results in formations of organic acids and other acidic intermediate compounds. Except to few set-ups pH showed sight decrement net. The properties of soils with *Spinacia oleracea* set-up showed different the ranges of organic C (0.76 – 1.07 %), Ca^{+2} (13.33 – 18 ppm), Mg^{+2} (38.67 – 48 ppm), Cu (90 – 12.2 ppm), Cd (1.68 – 24.7 ppm) and nitrate (40 – 48 ppm). The soil organic C was high in control set-up (1.20 %) than treatment. The high ranges of few important parameters in treated soil indicate the metal interference in soil mineralization process. While in set-up with *Amaranthus caudatus* the soil parameters were recorded as: pH (7.13 – 7.40), EC (421 – 490 mho/cm), organic C (0.72 – 1.07 %), Ca^{+2} (11.66 – 18.66 ppm), Mg^{+2} (34.44 – 43.33 ppm), Cu (33 – 65.66.2 ppm), Cd (1.57 – 28.2 ppm), nitrate (19 – 31.3ppm) and sulphate (21.33 – 34.33 ppm). It is clear from data set that metal inoculation in soils caused significant changes in soil properties. The decrease in organic carbon may indicate the harmful effect on microbes involved in soil organic matter mineralization. The soil mineralization and content of a particular nutrient/element in soils depends upon a variety of factors: soil organic matter, soil porosity, soil water holding capacity, plant up-take rate, leaching potential of a metal, interaction of soil chemicals etc. The Calcium and magnesium level in soil was disturbed by different concentrations of Cu and Cd. The soil phosphate level showed direct relationship with metal load. In general sometime the occurrence of metal in soil affects the sulpahte level in soil. For e.g. 50 ppm of Cu increased phosphate but same concentration of Cd, lowered it. Cd 250ppm caused the maximum depletion in soil phosphate level. As heavy metals were added in the form of chloride salts, there was rise in chloride level in soils and it was directly proportional to the concentration of metal concentration in soils. The higher phosphate level in the soil indicates that heavy metal decreased the phosphate uptake but less amount of phosphate in the soil contaminated with heavy metal suggests that for maintaining the growth, plant may absorb more amount of phosphate. The sulphate and nitrate content in soil was disturbed. The retardation in growth was due to decrease in organic carbon content. The above data indicates that retardation in plant growth may be due to disturbance in chemical properties and mineral ion balance.

Table-5
Chemical analysis of soil after germination of vegetables (60days)

Contamination	pH	EC (mho/cm)	OC (%)	Ca^{+2} (ppm)	Mg^{+2} (ppm)	Cl (ppm)	Cu (ppm)	Cd (ppm)	Nitrate (ppm)	Phosphate (ppm)	Sulphate (ppm)
Spinacia oleracea germinated soil											
Control	7.23 ± 0.09	431 ± 8	1.20 ± 0.1	17 ± 3	37.33± 3.5	20 ± 3	18.23± 0.6	1.59± 0.3	38.66 ± 3.5	28 ± 2	25 ± 2
$CuCl_2$ 50	7.87 ± 0.06	487 ± 15.5	1.07 ± 0.03	13.33± 2.5	38.66± 2.5	34.66 ± 0.5	29.30± 3.4	1.75± 0.8	41.66 ± 3.5	30 ± 3	22 ± 3
$CuCl_2$ 250	7.10 ± 0.14	396 ± 16.5	0.76 ± 0.1	15 ± 3	42.66± 1.5	65.33 ± 4	90.33± 3.5	1.68± 0.1	47 ± 5	22 ± 3	24.33 ± 3.5
$CdCl_2$ 50	7.28 ± 0.26	420 ± 19	1.01 ± 0.01	14 ± 1	41.33 ± 2.5	34.33 ± 0.5	17.26± 0.4	4.82± 0.7	40 ± 1	24 ± 3	23 ± 2
$CdCl_2$ 250	7.08 ± 0.04	466 ± 13.5	1.03 ± 0.1	18 ± 2	48 ± 6	62.33 ± 2.5	12.26± 0.5	24.70± 3.1	48.33 ± 2.5	19.33 ± 1.5	20 ± 2
Amaranthus caudatus germinated soil											
Control	7.32 ± 0.20	439 ± 18	1.14 ± 0.2	17.66 ± 1.5	25 ± 2.6	20 ± 2	17.6± 0.4	1.74± 0.04	46 ± 2	26 ± 1	24 ± 3
$CuCl_2$ 50	7.70 ± 0.10	421 ± 10	1.05 ± 0.05	11.66 ± 1.5	40.33 ± 1.5	33 ± 2	30.9± 3.9	1.57± 0.03	49 ± 3	31.33 ± 2.5	34.33 ± 3.5
$CuCl_2$ 250	7.13 ± 0.15	430 ± 15	0.72 ± 0.07	14.33 ± 3.5	39.33 ± 3.5	67 ± 2	87.46± 3.7	1.85± 0.01	37.66 ± 3.5	22.66 ± 1.5	23.33 ± 4.5
$CdCl_2$ 50	7.40 ± 0.08	474 ± 23	1.07 ± 0.1	14.66 ± 1.5	43.33 ± 0.5	34.33± 3	14.86± 0.4	6.65± 0.3	39.33 ± 2.5	26.66 ± 0.5	21.33 ± 2
$CdCl_2$ 250	7.19 ± 0.08	490 ± 5	0.77 ± 0.1	18.66 ± 1.5	41.66 ± 0.5	65.66 ± 1.5	13.33± 0.5	28.20± 1.3	36 ± 4	19 ± 1	22.66 ± 2.5

*± Standard deviation of 3 replications

Conclusion

On the basis of finding it is concluded that *Spinacia oleracea* and *Amaranthus caudatus* are sensitive towards Cu and Cd. The plant growth was inhibited by both the metals. The inhibitory effects were highly correlated with concentration of metal. From the growth study it was found that the heavy metal impact on growth was in the order of Cd>Cu. While heavy metal tolerance was in the range of Cu>Cd. *Amaranthus caudatus* was more sensitive towards metals than *Spinacia oleracea*. Root elongation can be selected as a simple parameter for evaluating the effect of essential and toxic metal on plant growth. Results suggest that heavy metal contents in soils have adverse impact on plant physiology and productivity. In case, if sewage water is used for irrigation purposes such plant family may accumulate a significant amount of metal and that may pose serious human health issues.

References

1. Ngayila N., Botineau M., Baudu M. and Basly J.P., Myriophyllum alterniflorum DC, Effect of low concentra-tions of copper and cadmium on somatic and photosynthetic endpoints: A chemometric approach, *Ecol. Indicat*., **9,** 307-312 (**2009)**

2. Liu D.H., Jiang W.S. and Gao X.Z., Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic, *Biol. Plant*, **47,** 79-83 (**2003)**

3. Adriano D.C., Trace Elements in the Terrestrial Environment, New York: *Springer*, (**2001)**

4. Marschner H, Mineral nutrition of higher plants, Academic Press London, *Academic press limited*, (**1995)**

5. Vig K., Megharaj M., Sethunathan N. and Naidu R., Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review, *Adv. Environ. Res*., **8,** 121-135 (**2003)**

6. Wang L., Zhou Q.X., Ding L.L. and Sun Y.B., Effect of cadmium toxicity on nitrogen metabolism in leaves of *Sola-num nigrum* L. as a newly found cadmium hyperaccumula-tor, *J. Hazard. Mater*, **154,** 818-825 (**2008)**

7. Pinto AP, Mota AM, de Varennes A and Pinto FC, Influence of organic matter on the uptake of cadmium, zinc, copper and iron by *Sorghum* plants, *Science of the Total Environment,* **326(1-3)**, 239-247 (**2004)**

8. Abedin M.J. and Meharg A.A., Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.), *Plant Soil*, **243(1)**, 57-66 (**2002)**

9. Yargholi B, Azimi AA, Baghvand A, Liaghat AM and Fardi GA, Investigation of Cadmium Absorption and Accumulation in Different of Some Vegetables, *American-Eurasian Journal of Agricultural and Environmental Sciences*, **3(3),** 357-364 (**2008)**

10. Walkley A. and Black I.A., An Examination of the Digestrates. Method for Determining Soil Organic Matter and Propose Modification of the Chronic Acid Titration Method, *Soil Sci.*, **37,** 29-38 (**1934)**

11. Hendershot W.H., Lalande H. and Duquette M, Ion exchange and exchangeable cations, In: Soil sampling and methods of analysis (Ed. Carter, M.R.), *Lewis Publishers*, Florida, USA, **b,** 167-176 (**1993)**

12. Zeng X., Li L. and Mei X., Heavy metals contents in Chinese vegetable plantation land soil and related sources analysis, *Agric. Sci. China*., **7(9),** 1115-1126 (**2008)**

13. Shafeeq A., Ali Z. and Muhammad S., Response of nickel pollution on physiological and biochemical attributes of wheat (*Triticum aestivum* L.) var. Bhakar-02, *Pak. J. Bot*., **44,** 111-116 (**2012)**

14. Arun K.S., Carlos C., Herminia L.Z. and Avudainaya-gam S., Chromium toxicity in plants, *Environ. Int*., **31,** 739-753 (**2005)**

15. Prasad M.N.V., Cadmium toxicity and tolerance in vascu-lar plants, Environm. *Experim. Bot*., **35,** 525-545 (**1995)**

16. Liu W.J., Zhu Y.G., Smith F.A. and Smith S.E., Do iron plaque and genotypes affect arsenate uptake and translocation by rice, *J. Exp. Bot*., **55,** 1707-1713 (**2004)**

17. Bhanderi PD, Trivedi LD and Vyas AV, A study on root bioassay as technique for the assessment Cd toxicity in medicinal plants, Seminar on Biotechnology: A tool of environmental research. Organized by ISRCDE, Ahmedabad, **1 (2004)**

18. Barcelo J and Poschenrieder CH, Plant water relations as affected by heavy metal stress A Review*, J. Plant Nutri*., **13,** 1-37 (**1990)**

19. Mysliwa-Kurdziel B, Prasad MNV and Stralka K, Photosynthesis in heavy metals stress plants, In: Prasad MNV (ed) Heavy metal stress in plants, 3rd edn, *Springer, Berlin*, 182-200 **(2004)**