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RESEARCH ARTICLE

IMPACT OF HEAVY METALS ON GROWTH AND METABOLISM OF *VIGNA RADIATA*

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ABSTRACT

The effects of arsenic, chromium, manganese, molybdenum and nickel on the growth, chlorophyll, sugar and protein content were investigated in 7 days-old seedlings (*Vigna radiate* (L). Wilczek) with various concentration (5, 10, 25, 50 and 100 mg/l). Heavy metal induces a number of physiological and biochemical changes, such as growth, total chlorophyll content, sugar and protein content declined progressively with increasing concentrations of heavy metals compared with the control plants.

INTRODUCTION

Pollution can be classified in to air, water, soil, solid waste and radioactive pollution, on the basis of the medium in which the pollutants are present. Among these, the problem of water pollution is getting greater dimension day by day in India. Water pollution is defined as the addition of any thing to water which alters the natural quality. Water is mostly polluted by the industrial wastewaters released from various industries (Kudesia, 2000). Heavy metals are the main constituents of many industrial effluents. The industrial, agricultural and municipal wastes are the key sources of these toxic heavy metals in the wastewater (Kirupalakshmi *et al.,* 2004). Heavy metals like manganese, iron, copper, zinc and molybdenum are essential for plant life, but are required in very small or trace amounts as in other living beings. The other heavy metals *viz.,* chromium, nickel, cadmium, mercury, lead etc are not or as much essential for plants or animals.

One of the major concerns is the accumulation of heavy metals in edible parts of the crops creating hazards to animal and human health. Hence it would be worthwhile to undertake a study on the effects of heavy metals on the agricultural crops. Arsenic (As) is one of the most noxious heavy metals, having the atomic weight 74.92, atomic volume 13.08 and relative density 5.73, which causes very serious health hazards to humans (Singh and Jaiwal, 2003). Arsenic is also considered as 'slow killer' which causes blision on the palm of hands and soles of the feet which can eventually turn to gangrenous cancers (Rai and Pal, 2002). Chromium also plays a major role in polluting the environment. Chromium is released from chemical fertilizer, animal wastes, and sewage sludge and by different industrial processes such as electroplating, leather tanning, paint, textile and wood. Chromium is a metallic element with bluish white tinge. It is very hard in nature, having the atomic number 24, atomic weight 52.01, atomic volume 7.3 and relative density 7.14 (Mertz and Schwartz, 1995). Manganese is an essential trace element, toxic at higher concentrations. It has the atomic weight 54.938, atomic volume 7.4 and relative density 7.39. It is also essential for plants. Burning of fossil fuels is the main source of Mn in the environment.

Molybdenum (Mo) that can become a pollutant if it gets accumulated beyond the required level in the soil (Sharma, 1995). Molybdenum must be present in the plants, so that nitrites, nitrates, can be metabolized into amino acid and proteins. It possesses an atomic weight of 95.94, an atomic volume of 9.4 and a relative density of 10.2. Nickel is a silvery metal that forms about 0.008% of the earth's crust, having an atomic weight of 58.71, an atomic volume of 6.7 and a relative density of 8.8. Nickel is one of the most toxic heavy metal and is known to interfere with the quality of atmosphere after its release from various sources. Nickel is produced during production of stainless steel, storage batteries, spark plugs, magnets and machinery. High concentration of nickel has shown toxic effects on plants (Hewitt, 1953).

MATERIALS AND METHODS

The present investigation has been carried out to the effect of different concentrations of heavy metals such as arsenic, chromium, manganese, molybdenum and nickel on seed germination, seedling growth and biochemical studies of greengram (*Vigna radiata* (L.) Wilczek) var. Vamban 1.

Preparation of heavy metals solutions

A known weight of sodium arcenate (2.66g) for arsenic, potassium dichromate (2.96g) for chromium, manganous sulphate (2.92g) for manganese, ammonium molybolate (1.8g) for molybdenum and Nickel sulphate (2.71g) for the source of Nickel was weighed and dissolved in 1000 ml of distilled water separately. From this stock solution and different concentration (5, 10, 25, 50 and 100mg/l) was prepared and used for germination studies.

Germination studies

The seeds were surface sterilized with 0.1% mercuric chloride solution for 2 minutes and washed thoroughly with tap water and then by distilled water for 30 minutes. The seeds were placed equispacially in sterilized petriplates lined with filter paper treated with various concentrations of different heavy metal solutions. The seeds were irrigated with distilled water was treated as control. After 7 days from sowing, the total germination percentage was calculated. Ten seedlings from each replicate were selected for recording the morphological parameters such as length of root and shoot and their dry weight.

Biochemical studies

Chlorophyll

The amount of chlorophyll present in the leaf was estimated by the method of Arnon (1949). 0.5 gram of fresh leaf material was ground with mortar and pestle with 10 ml of 80 per cent acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved and utilized for chlorophyll estimation.

Estimation of protein

The protein content of samples was estimated by Lowry method (Lowry *et al.,* 1951). To the test sample 5 ml of alkaline solution (alkaline sodium carbonate: copper sulphate and sodium potassium tartarate) was added mixed thoroughly and allowed to stand at room temperature for 10 minutes. Folin-Phenol reagent (0.5 ml) was added and kept at room temperature for 30 min. After 30 minutes the OD was measured. Bovine serum albumin as used as standard.

Estimation of total sugar

Plant samples were treated with 80 per cent boiling ethanol for taking extractions (5ml extract representing 1g of tissue). Five readings for each sample were taken. One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1 ml of distilled water and 1 ml of 1N sulphuric acid were added and incubated at 49˚C for 30 minutes. The solution was neutralized with 1N sodium hydroxide using methyl red indicator. One ml of Nelson's reagent was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ration (Reagent A: 25g sodium carbonate, 25g sodium potassium tartarate, 20g sodium bicarbonate and 200g anhydrous sodium sulphate in 1000ml: Reagent B: 15g cupric sulphate in 100ml of distilled water with e drops of concentrated sulphuric acid) was added. The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arsenomolybdate reagent (25g ammonium molybdate, 21ml concentrated sulphuric acid, 5g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37˚C in a water bath for 48 hours) was added. The solution was thoroughly mixed and diluted to 25 ml and measured at 495 nm in a spectrophotometer. The reducing sugar contents of unknown samples were calculated from glucose standard.

RESULTS

In the present study, seed germination percentage decreased gradually at higher concentration level when compared with control (Table 1). The seedling growth parameters like root length and shoot length were high in control plants (Table 2).However, there was a progressive decline in root length and shoot length with increase of heavy metal concentrations (As, Cr, Mn, Mo and Ni). The fresh weight and dry weight of the seedlings decreased at extreme level of concentration when compared with control plants (Table 3).The effect of various concentrations of heavy metals on the biochemical constituents of green gram at seedling stage was analysed and recorded (Table 4). The photosynthetic pigments such as chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents of green gram seedlings was decreased with increasing level of heavy metal concentrations. The total sugar and protein content is high in control plant and it is gradually decreased with increase in heavy metal concentrations (Table 5).

DISCUSSION

Heavy metals are one of the most important groups of pollutants of aquatic environment, which originate from domestic sewage, industrial effluents and agricultural run off etc. Addition of heavy metals (As, Al, Cr, Mn, Mo, Ni, Cu, Pb, etc.) into the environment causes toxic and carcinogenic effects on flora and fauna and create great ecological crisis at the global level (Prakash *et al.,* 2004).Germination, the critical phase in the life cycle of a crop plant, is subjected to numerous environmental stresses. Any disturbance in the environment in which the seed germinate, affects the germination and ultimately the growth, dry weight and yield of crops (Dixit *et al.*, 1986). Seed germination has been used bymany workers to monitor water pollution responses. Several growth parameters such as percentage of germination, seedling survival and seedling weight have been taken as criteria to assess the plant responses to specific pollution (Shanmughavel, 1993). The control plants exhibited maximum germination percentage when compared with all other concentrations of heavy metals. Similar trend was also reported in wheat (Sharma and Mehrotra, 1993; Sharma, 1995; Panda and Patra, 1997) due to heavy metal. The reduction in germination percentage of greengram at higher concentrations may be attributed to the interference of heavy metal ions during germination metabolism (Chaugh and Sawhney, 1996 and Sankarganesh, 2008). Similar inhibition of root and shoot length were recorded by Samantry, 2002, Samantry and Deo, 2004, Hsu and Kao, 2003. .

Table 1. Effect of heavy metals on germination percentage of greengram (*Vigna radiata* (L.) Wilczek)

Heavy metal concentrations (mg/l)	Types of heavy metals				
	Arsenic	Chromium	Manganese	Molybdenum	Nickel
Control	95.0±4.75	95.0±4.75	95.0±4.75	95.0±4.75	95.0±4.75
5	81.2±4.06	85.2±4.26	90.0±4.50	85.0±4.25	85.0±4.25
10	73.0±3.65	74.0±3.70	84.0±4.20	80.0±4.00	75.0±3.75
25	62.0±3.10	64.0±3.20	73.0±3.65	75.5±3.77	65.3±3.26
50	45.0±2.25	45.0±2.25	60.0±3.00	60.0±3.00	55.0±2.75
100	35.0±1.75	42.0±2.10	50.0±2.50	45.0±2.25	40.0±2.00

± Standard deviation
No germination was recorded beyond 100 mg/l

Table 2. Effect of heavy metals on root length and shoot length (cm/seedling) development of greengram (*Vigna radiata* (L.) Wilczek)

Heavy metal concentrations (mg/l)	Types of heavy metals									
	Arsenic		Chromium		Manganese		Molybdenum		Nickel	
	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length	Root length	Shoot length
Control	8.70 ± 0.435	19.00 ± 0.95	8.70 ± 0.435	19.00 ± 0.95	8.70 ± 0.435	19.00 ± 0.95	8.70 ± 0.435	19.00 ± 0.95	8.70 ± 0.435	19.00 ± 0.95
5	5.34 ± 0.267	12.24 ± 0.612	5.50 ± 0.275	14.74 ± 0.737	6.00 ± 0.3	17.20 ± 0.86	5.80 ± 0.29	17.00 ± 0.85	5.50 ± 0.275	16.34 ± 0.817
10	4.20 ± 0.210	11.04 ± 0.552	4.28 ± 0.214	11.70 ± 0.585	5.50 ± 0.275	16.50 ± 0.825	5.06 ± 0.253	16.50 ± 0.825	4.60 ± 0.230	15.28 ± 0.764
25	3.04 ± 0.152	9.04 ± 0.452	3.40 ± 0.170	11.00 ± 0.55	4.74 ± 0.237	14.64 ± 0.732	4.14 ± 0.207	15.00 ± 0.75	3.64 ± 0.182	11.6 ± 0.58
50	2.20 ± 0.110	7.18 ± 0.359	2.40 ± 0.120	8.04 ± 0.402	4.50 ± 0.225	12.40 ± 0.62	3.96 ± 0.198	9.16 ± 0.458	3.10 ± 0.155	10.40 ± 0.52
100	1.84 ± 0.092	4.20 ± 0.21	2.30 ± 0.115	4.48 ± 0.224	2.94 ± 0.147	6.84 ± 0.342	2.76 ± 0.138	6.54 ± 0.327	2.70 ± 0.135	6.70 ± 0.335

± Standard deviation
No germination was recorded beyond 100 mg/l.

Table 3. Effect of heavy metals on fresh weight and dry weight (g/seedling) of greengram (*Vigna radiata* (L.) Wilczek)

Heavy metal concentrations (mg/l)	Types of heavy metals									
	Arsenic		Chromium		Manganese		Molybdenum		Nickel	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Control	0.618 ± 0.0309	0.064 ± 0.0032	0.618 ± 0.0309	0.064 ± 0.0032	0.618 ± 0.0309	0.064 ± 0.0032	0.618 ± 0.0309	0.064 ± 0.0032	0.618 ± 0.0309	0.064 ± 0.0032
5	0.520 ± 0.026	0.036 ± 0.0018	0.560 ± 0.028	0.044 ± 0.0022	0.570 ± 0.0285	0.056 ± 0.0028	0.544 ± 0.0272	0.052 ± 0.0026	0.540 ± 0.027	0.050 ± 0.0025
10	0.464 ± 0.0232	0.034 ± 0.0017	0.540 ± 0.027	0.036 ± 0.0018	0.520 ± 0.026	0.052 ± 0.0026	0.502 ± 0.0251	0.050 ± 0.0025	0.484 ± 0.0242	0.040 ± 0.002
25	0.292 ± 0.0146	0.026 ± 0.0013	0.310 ± 0.0155	0.030 ± 0.0015	0.344 ± 0.0172	0.034 ± 0.0017	0.330 ± 0.0165	0.034 ± 0.0017	0.320 ± 0.016	0.032 ± 0.0016
50	0.236 ± 0.0118	0.016 ± 0.0008	0.240 ± 0.012	0.022 ± 0.0011	0.286 ± 0.0143	0.030 ± 0.0015	0.264 ± 0.0132	0.028 ± 0.0014	0.256 ± 0.0128	0.026 ± 0.0013
100	0.210 ± 0.0105	0.012 ± 0.0006	0.220 ± 0.011	0.018 ± 0.0009	0.260 ± 0.013	0.022 ± 0.0011	0.244 ± 0.0122	0.020 ± 0.001	0.232 ± 0.0116	0.018 ± 0.0009

± Standard deviation
No germination was recorded beyond 100 mg/l.

Table 4. Effect of heavy metals on chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents (mg/g fr. wt.) of greengram (*Vigna radiata* (L.) Wilczek) seedlings

Con. (mg/l)	Types of heavy metals														
	Arsenic			Chromium			Manganese			Molybdenum			Nickel		
	Chl 'a'	Chl 'b'	Total chl	Chl 'a'	Chl 'b'	Total chll	Chl 'a'	Chl 'b'	Total chl	Chl 'a'	Chl 'b'	Total chl	Chl 'a'	Chl 'b'	Total chlorophyll
Control	0.01057 ± 0.00053	0.00862 ± 0.00043	0.0192 ± 0.00096	0.01057 ± 0.00053	0.00862 ± 0.00043	0.01919 ± 0.00096	0.01057 ± 0.00053	0.00862 ± 0.00043	0.0192 ± 0.00096	0.01057 ± 0.00053	0.00862 ± 0.00043	0.01919 ± 0.00096	0.01057 ± 0.00053	0.00862 ± 0.00043	0.0192 ± 0.00096
5	0.00792 ± 0.0004	0.00605 ± 0.00030	0.01398 ± 0.0007	0.00866 ± 0.00043	0.00612 ± 0.00031	0.01478 ± 0.00074	0.00901 ± 0.00045	0.0067 ± 0.00034	0.01568 ± 0.00078	0.0090 ± 0.00045	0.0069 ± 0.00035	0.0159 ± 0.0008	0.0088 ± 0.00044	0.00646 ± 0.00032	0.01526 ± 0.00076
10	0.00747 ± 0.00037	0.0049 ± 0.00025	0.01236 ± 0.00062	0.00856 ± 0.00043	0.00463 ± 0.000232	0.0132 ± 0.00066	0.00905 ± 0.00045	0.0055 ± 0.00028	0.0151 ± 0.00076	0.00905 ± 0.00045	0.0061 ± 0.00031	0.01456 ± 0.00073	0.00865 ± 0.00043	0.0053 ± 0.00027	0.01397 ± 0.0007
25	0.00706 ± 0.00035	0.00267 ± 0.00013	0.00973 ± 0.00049	0.00789 ± 0.00039	0.00305 ± 0.000153	0.01094 ± 0.00055	0.00884 ± 0.00044	0.00397 ± 0.000199	0.01358 ± 0.00068	0.00834 ± 0.00042	0.0047 ± 0.00024	0.01231 ± 0.00062	0.00825 ± 0.00041	0.00372 ± 0.00019	0.01197 ± 0.0006
50	0.00617 ± 0.00031	0.00268 ± 0.00013	0.0089 ± 0.00045	0.0069 ± 0.00035	0.00301 ± 0.00015	0.0095 ± 0.00048	0.00821 ± 0.0004	0.00378 ± 0.00019	0.01162 ± 0.00058	0.00701 ± 0.00035	0.0038 ± 0.00019	0.01079 ± 0.00054	0.00689 ± 0.00034	0.0033 ± 0.00017	0.01015 ± 0.00051
100	0.005281 ± 0.00026	0.00274 ± 0.00013	0.0080 ± 0.0004	0.0055 ± 0.00028	0.00281 ± 0.00014	0.0085 ± 0.00043	0.0059 ± 0.0003	0.00374 ± 0.00019	0.0103 ± 0.00052	0.00653 ± 0.0003	0.0034 ± 0.00017	0.00966 ± 0.00048	0.00583 ± 0.00029	0.0032 ± 0.00016	0.00917 ± 0.00046

± Standard deviation; Con. = Concentration; Chl. = chlorophyll;

A significant reduction in root and shoot length of *Vigna radiata* was recorded due to heavy metal treatments. The reduction in root and shoot length is possibly due to the accumulation of heavy metals in the plant tissues and its interaction with the minerals (Sundaramoorthy and Sankarganesh, 2007). The fresh and dry weights showed a similar trend in case of seedling length. The decrease in fresh and dry mass of root and shoot were mainly due to the inhibition of water uptake and enlargement of cells. Higher uptake of heavy metals by roots resulted reduced the fresh and dry weight of seedling (Sharma and Mehrotra, 1993). Similar reduction of fresh and dry weight under various metal were observed in rice with cadmium (Hsu and Kao, 2003).

Reduction in germination and subsequent seedling growth of the crop due to heavy metal treatments ultimately led to impairment in various biochemical constituents. The reduction in chlorophyll content was recorded due to heavy metal stress. The decrease in chlorophyll content at higher concentrations may be due to interference of heavy metals with pigment metabolism (Sankarganesh, 2008). The total sugar content is high in control plant and it is gradually decreased with increase in heavy metal concentrations. Similar changes were recorded in greengram (Mahadeswaraswamy and Therasa, 1983), soybean (Sidharthan and Lakshmanachary, 1996) and blackgram (Lakshmi and Sundaramoorthy, 2003). It may be due to the imbalance, which might eventually lead to the depletion of carbohydrate reserve (Murata *et al.*, 1969).

The higher concentrations of heavy metal significantly reduced the protein contents in plants. There was a gradual decline in protein content with a progressive increase metal concentration. During transport of heavy metals into the plants the sulphydryl group of protein may be reduced causing deleterious effect in the normal protein form (Rai *et al.*, 1992). To state finally, the selected heavy metals (arsenic, chromium, manganese, molybdenum and nickel) treated plants showed the greater variation in morphological and biochemical studies. Since these heavy metals are toxic to germination studies, it is suggested that the industrial effluents containing heavy metals should be treated properly and diluted suitably, before they are discharged into the near by water bodies.

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