

Evaluating Different Weeds for Phytoremediation Potential Available in Tannery Polluted Area by Conducting Pot and Hydroponic Experiments

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ABSTRACT

The pot experiments were conducted to determine and compare the toxic effects of Chromium, Copper, Cadmium, Nickel and Lead on different parameters like shoot length, number of branches and area of leaf on different wild weed *Cannabis sativa*, *Solanum nigrum* and *Chenopodium album*. The investigated amounts of metal were in the range of 7 different concentrations i.e. 5ppm, 10ppm, 50ppm, 100ppm, 200ppm, 300ppm and 350ppm. The average toxicity increases with increase in the concentration of metals but in certain cases variations were observed in toxicity parameters. The morphological response in *Cannabis sativa* showed that most of the changes on the morphological characteristics were observed at 100 ppm. The shoot length, leaf area and number of branches decrease at 100 ppm and above. The maximum variations as compared to other metals were shown in copper stress condition. In *Chenopodium album* all the metals except lead show morphological variation with increase in metal concentration. The morphological toxicity increases with increase in metal concentration. The overall pollen fertility analysis shows that metal exposure leads to the development of sterile pollens. This shows the relative toxic effect of metals on the pollen fertility. In the hydroponic experiments, the Chromium metal exposure on the weed *Cannabis sativa* (C) and (P) for 15 days shows decrease in the amount of Chromium in the medium detected through diphenylcarbazide method, which shows the hyper accumulation of chromium by these weeds.

Key words: *S. nigrum*, *Cannabis sativa*, *Chinopodium album*, Relative toxicity, Standardization concentration, ppm, Hyperaccumulators, Pollen fertility.

INTRODUCTION

Current state of environment is degrading on day to day basis because of increased anthropogenic activities and further disposal of wastes generated to land and rivers leading to major pollution of soil and groundwater. The industrial practices also lead to the release of various heavy metals into the soil (Mattigod and Page 1983). Pollution may be defined as the adverse effect caused due to disruption of equilibrium of an ecosystem, which further results in adverse effects on the health of organisms. The major sources of heavy metals are the practices done by the tannery

industries in Indian sub continent. During the pre-tanning processes, a large amount of metal is released into the environment. Some species of plants have the ability to accumulate heavy metals into their body parts such as roots, stem and leaves. Such plants are termed as hyper accumulators and are considered under green technology which is cost effective and ecofriendly known as phytoremediation. The extraction and inactivation of heavy metals in the soil can be done by this energy efficient technique known as phytoremediation. Phytoremediation is an emerging technology, which provides promising results in the reduction of pollution (Madhuri *et al.* 2014).

An integrated multidisciplinary approach to cleanup the contaminated soils, phytoremediation combines the disciplines of plant physiology, soil microbiology and soil chemistry (Cunningham and Ow 1996). The development of phytoextraction technique came from the discovery of variety of wild weeds, often endemic to naturally mineralized soils that concentrate high amounts of essential and nonessential heavy metals. *Rorippaglobosashows* Cd hyperaccumulation as shown in the work of Yuebing *et al.* 2007. Phytovolatilization is the process in which the water soluble and volatile contaminants are taken up by the plant and through the process of transpiration contaminants are released into the atmosphere (Madhuri *et al.* 2014). The modified volatile product produced by the degradation of initial contaminants is less toxic as shown in transformation of toxic selenium to less toxic dimethyl selenide gas (Chaudhary *et al.* 1998). Rhizofiltration is a cost-competitive technology in the treatment of surface water or groundwater containing low, but significant concentrations of heavy metals such as Cr, Pb, and Zn (Raskin and Ensley 2000). Hydroponic technique is also being used to accumulate and concentrate the metals in their various body parts especially roots (Flathman and Lanza 1998; Salt *et al.* 1995; Dushenkov *et al.* 1995; Zhu *et al.* 1999b). Phytodegradation which is also known as phyto-transformation is a process in which, the breakdown of contaminants occurs by plants through metabolic processes within the plant through plant root symbiotic associations (McGrath and Zhao 2003).

MATERIAL AND METHOD

Field site, analysis of soil and weeds

In this study, we investigated 3 weeds i.e. *Cannabis sativa*, *Chenopodium album* and *Solanum nigrum* collected from the "Kala Sanghiya Drainage", near Kapurthala. The area is continuously polluted by the heavy metals coming from the leather industries. The water of the drainage is continuously used up by the farmers for the irrigation purposes. This research includes the metal stress of different concentration on the weeds under observation in the natural conditions by taking five different metals i.e. Chromium(Cr), Copper(Cu), Cadmium(Cd), Nickel(Ni) and Lead(Pb). The aim of this study is to assess those weeds which show

least variations in their morphological characteristics under different metal stress conditions in Pot experiments and Hydroponic experiments. We also analyzed the pollen fertility of the weeds under different metal concentration.

Field Demarcation and collection of weed samples

Demarcation of area was done around Kala Sanghiya drainage. The area was demarcated as Polluted area(P) as Gazipur and Control areas (C) as Phiali. *Cannabis sativa*, *Chenopodium album* and *Solanum nigrum* were collected near the field of Kala Sanghiya drainage demarcated as (P) and the control samples from the same is collected from the other side of the road demarcates as(C).

Seed drying and Sapling of plants

After the sample collection, the seeds were dried under natural conditions for about 15-20 days. Saplings were prepared in the botanical garden of Lovely Professional University, (Chehru) near Phagwara. Hundred seeds were sown in the soil to germinate; out of them only forty uniform plants were allowed to grow in each pot, at a uniform distance. Seedlings were prepared after 3-4 weeks and height was approximately 2-3 cm. Sampling were prepared in around 2 months.

Preparation of salt concentration and homogenization

Air-dried soil of 2.5 kg was sieved through a 4 mm sieve so that no solid particles are left behind. The soil should be clean from the coarse particles. The clean soil were treated with different metal concentration i.e. standardization concentration of all the five metals at 5ppm, 10ppm, 50ppm, 100ppm, 200ppm, 300ppm and 350ppm and for comparison an unamended (control) was taken. Five different metal salts chromium chloride, copper sulphate, cadmium chloride, nickel sulphate and lead nitrate were used. They all are water soluble salts, readily dissolves in water (distilled). 50ml of water was used to dissolve the salts at different standardized concentration.

Pot Experiments

Plastic pots of 10 cm in height and 15 cm in diameter were used. Pots containing 250 grams of soil were taken and were supplemented with

homogenized mixture of salt. For each weed having 5 metals and 7 concentrations were used. Pots were placed in net house shaded with transparent polythene sheet, to protect from rainwater leaching. Plants were grown under natural light and ambient temperature in order to keep all plants under conditions as similar as possible.

Pollen fertility analysis

Mature state plants were selected for pollen fertility experiments. Anthers were collected and preserved in Carnoy's fixator for 24 hrs and then transferred to 90% ethanol. Carnoy's fixator was used at 6:3:1 proportion having the composition: Ethanol 600 ml, Chloroform 300ml, Acetic acid 100 ml. Glyceracetamine is a dye to stain the fertile pollens was used in 1:1 proportion having the composition: Glycerine 10 ml, Acetocarmine 10ml. The prepared slide was gently covered with cover slip. The slide was left for half an hour. Further it was observed under Light microscope at 100X. The slide was divided in 4 parts and pollens were counted and classified as sterile and non sterile.

Hydroponic experiment

Media preparation

MS media (nutrient medium) is used (Murashige and Skoog medium) by dissolving 2.652 grams/1000ml distilled water.

Chromium metal was used at additional concentrations.

50 ml glass tubes were taken and poured 50ml prepared MS media into it. Metal salt was added into the media. Chromium chloride was used for the experimental purpose and its salts concentration was added at 5 ppm, 10 ppm, 50 ppm, 100 ppm, 200 ppm, 300 ppm and 350 ppm. Further, Diphenylcarbazide method was used to calculate the optical density at different prepared concentrations (Shigematsu *et al.* 1977).

RESULT AND DISCUSSION

The table 1 shows the change in the morphological characteristics of the weed *Cannabis sativa* under the various metal stressed conditions. The *Cannabis sativa* shows morphological changes as the concentration of

metal increases. This shows that *Cannabis sativa* is the hyper accumulator of Chromium metal which shows changes in their morphological character with increase in metal concentration. In the copper metal stressed conditions, a great extent of variation were observed in the weed. At 100ppm metal exerts stress on the weed, the leaves area, shoot length and no. of branches decreases to large extent and the total biomass of the weed decreases with large variation in their morphological characteristic. As in the readings above, the Cadmium metal exposure to the plant do not exerts any change in the growth of plant but at 100 ppm metal exerts stress on the weed and there is a great extent of variation in the shoot length, leaf area and no. of branches of the weed. The Nickel exposure of *cannabis sativa* at 5 ppm shows a great variation in their shoot length, leaf area and no. of branches shows that Nickel exerts a large stress on the biomass of the plant, but at 50 ppm the shoot length increases as compared to 5 ppm, which shows that the weed can tolerate the stress up to 50 ppm and again metal stress shows variation in the morphological characteristics. The weed under Lead stress conditions shows morphological variations, weed shows least variation at 5 ppm, but as the metal concentration increases the weed shows morphological changes. In the given readings, Lead metal can exert stress maximum at 350 ppm and weed can tolerate the metal stress condition without showing much variation till 50 ppm.

The figure 1 (a) shows that from five different heavy metals, Copper metal stress effects most to the shoot length of *Cannabis sativa*. It means maximum variation occurs in the morphological characteristic of *Cannabis sativa* at copper stressed conditions. Least variations in the morphology are observed at cadmium exposure.

In the figure 1 (b), the analysis of leaf area in the various metals stressed conditions shows that under copper exposure there is a great extent of variations observed from 5ppm to 350ppm by comparing the data, it was analyzed that the weed exert stress in copper exposure and shows variations in their leaf area as the concentration of metal increases. In cadmium and chromium exposure, appropriate results are shown i.e.

Table 1: Morphological analysis of *Cannabis sativa* under different metal exposure in pot experiments

S.C	Morphological Analysis (Cr)			Morphological Analysis (Cu)			Morphological Analysis (Cd)			Morphological Analysis (Ni)			Morphological Analysis (Pb)		
	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B
control	10cm	5.29cm2	13	10cm	5.29cm2	13	10cm	5.29cm2	13	10cm	5.29cm2	13	10cm	5.29cm2	13
5ppm	9.8cm	4.93cm2	13	9.0cm	4.39cm2	7	10cm	4.0cm2	10	6.0cm	3.0cm2	8	9.0cm	4.90	6
10ppm	8.0cm	5.36cm2	12	8.5cm	3.47cm2	6	10cm	4.0cm2	10	5.8cm	3.0cm2	8	8.0cm	3.0cm2	4
50ppm	7.0cm	3.8cm2	9	7.0cm	4.66cm2	4	9.0cm	3.8cm2	8	7.0cm	3.0cm2	7	8.0cm	3.45cm2	4
100ppm	6.8cm	4.54cm2	10	3.8cm	1.73cm2	4	8.0cm	3.6cm2	9	6.0cm	4.0cm2	5	7.0cm	1.5cm2	3
200ppm	6.4cm	3.24cm2	11	3.2cm	1.79cm2	3	6.0cm	3.2cm2	7	5.0cm	3.0cm2	5	6.0cm	1.5cm2	4
300ppm	6.0cm	2.55cm2	5	3.5cm	1.23cm2	3	6.0cm	2.8cm2	6	5.2cm	2.9cm2	5	7.0cm	3.45cm2	3
350ppm	5.3cm	2.85cm2	3	3.3cm	1.40cm2	3	6.0cm	2.8cm2	6	4.9cm	2.5cm2	8	5.0cm	3.36cm2	5

(S.C:- Standardization Concentration), (S.L:- Shoot Length), (L.A:- Leaf Area), (N.O.B:- Number of Branches)

Table 2: Morphological analysis of *Chenopodium album* under different metal exposure in pot experiments

S.C	Morphological Analysis (Cr)			Morphological Analysis (Cu)			Morphological Analysis (Cd)			Morphological Analysis (Ni)			Morphological Analysis (Pb)		
	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B
control	10cm	4.5cm2	15	10cm	4.5cm2	15	10cm	4.5cm2	15	10cm	4.5cm2	15	10cm	4.5cm2	15
5ppm	10cm	4.35cm2	15	8.5cm	2.73cm2	13	9.0cm	2.8cm2	10	9.0cm	3.5cm2	13	10cm	2.6cm2	12
10ppm	9.0cm	2.88cm2	17	8.2cm	2.8cm2	13	9.0cm	3.0cm2	10	10cm	3.2cm2	12	9.0cm	1.5cm2	10
50ppm	13cm	5cm2	16	8.0cm	1.5cm2	12	8.0cm	2.4cm2	8	9.0cm	2.5cm2	14	8.8cm	1.6cm2	7
100ppm	12cm	4.0cm2	12	7.6cm	1.5cm2	10	10.0cm	2.0cm2	9	11cm	1.3cm2	13	8.3cm	1.7cm2	9
200ppm	9.7cm	2.3cm2	10	7.2cm	1.2cm2	11	11.0cm	2.3cm2	8	5.0cm	1.2cm2	7	7.0cm	1.9cm2	7
300ppm	8.0cm	3.0cm2	9	7.0cm	1.5cm2	13	10.0cm	2.0cm2	7	7.0cm	1.0cm2	7	8.0cm	1.3cm2	8
350ppm	7.0cm	2.5cm2	7	6.4cm	1.6cm2	12	9.0cm	2.4cm2	9	5.0cm	1.3cm2	8	6.0cm	1.5cm2	6

Table 3: Morphological analysis of *Solanum nigrum* under different metal exposure in pot experiments

S.C	Morphological Analysis (Cr)			Morphological Analysis (Cu)			Morphological Analysis (Cd)			Morphological Analysis (Ni)			Morphological Analysis (Pb)		
	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B	S.L.	L.A	N.O.B
control	7.0cm	4.2cm ²	6	7.0cm	4.2cm ²	6	7.0cm	4.2cm ²	6	7.0cm	4.2cm ²	6	7.0cm	4.2cm ²	6
5ppm	7.0cm	4.0cm ²	5	7.0cm	4.0cm ²	5	5.0cm	2.8cm ²	4	6.9cm	4.0cm ²	5	8.0cm	2.5cm ²	8
10ppm	6.0cm	2.0cm ²	5	6.0cm	2.0cm ²	5	6.0cm	2.75cm ²	5	6.4cm	3.9cm ²	6	7.0cm	2.3cm ²	7
50ppm	5.0cm	1.5cm ²	6	5.8cm	1.5cm ²	6	6.0cm	2.5cm ²	5	6.0cm	3.6cm ²	4	6.9cm	2.2cm ²	6
100ppm	6.0cm	2.0cm ²	5	5.0cm	2.0cm ²	5	5.5cm	1.5cm ²	6	5.8cm	2.5cm ²	4	6.0cm	2.0cm ²	6
200ppm	7.0cm	1.5cm ²	5	4.5cm	1.5cm ²	5	5.0cm	1.8cm ²	6	5.4cm	2.4cm ²	3	5.0cm	1.7cm ²	5
300ppm	6.0cm	1.3cm ²	6	4.2cm	1.3cm ²	6	6.0cm	2.5cm ²	5	5.2cm	2.0cm ²	2	4.7cm	1.4cm ²	6
350ppm	5.0cm	2.0cm ²	5	4.0cm	2.0cm ²	5	5.0cm	1.5cm ²	4	5.0cm	2.0cm ²	3	4.2cm	1.3cm ²	6

maximum growth at control and decrease in leaf area from 5ppm to 350ppm. In the nickel metal stress conditions, the growth become static shows the metal have no adverse effect on the leaf area of *Cannabis sativa*.

The exposure of various metals on the *Cannabis sativa* also adversely affects the number of branches. The maximum variation again occurs in copper stressed conditions. The maximum decrease occurs in number of branches at 200ppm. In the Chromium stressed conditions, great variations occur from control to 350 ppm. At 200 ppm, the number of leaves gain increase means certain environmental factors and hormones release at this particular metal concentration. In nickel stressed state, 350 ppm favors the growth of the number of leaves as shown in the above figure 1 (c).

The *Chenopodium album* weeds with metal exposure of lead. Up to 10 ppm metal does not exert any stress on the weed but at 50 ppm due to the metal stress the shoot length, leaf area and number of branches increases, it means certain hormones and other environmental factors are present which supports the morphological growth of the plant and weed get adapted in the metal stress conditions but again at 200 ppm the morphological growth decreases, which means weed is less adapted at high metal concentration and shows large morphological variations. The *Chenopodium album* under copper metal stress conditions, the shoot length decreases with increase in metal concentration, there is a diverse change in the leaf area as the metal concentration increases, but not much effect on the number of branches. It means, there are certain hormones and environmental factors which favour the growth. At the metal cadmium stress condition, as the concentration of metal increases, changes occurs in the morphology of the weed., overall the cadmium metal do not exert stress on the shoot of the weed, but leaf area and number of branches decreases as the metal concentration increases. In the Nickel stress condition, the plant do not show much variation in the shoot up to 50ppm, but at 100ppm the shoot length increases, it means weed is adapted up to 100ppm and show normal growth, but at 200ppm there is a adverse effect of metal concentration on the weed. High metal concentration at 200 to 350ppm exerts large stress

Table 4: Pollen Fertility Analysis in *Cannabis sativa* exposure to different metal concentrations

S.C	Pollen Fertility Analysis(Cr)				Pollen Fertility Analysis (Cu)				Pollen Fertility Analysis(Cd)			
	Fertile pollens	Total pollens	Pollen fertility	Average	Fertile pollens	Total pollens	Pollen fertility	Average	Fertile pollens	Total pollens	Pollen fertility	Average
control	2306	2416	95.45%	95.03%	2306	2416	95.45%	95.03%	2306	2416	95.45%	95.03%
5ppm	1878	1985	94.61%	N.A.O	1878	1985	94.61%	N.A.O	1878	1985	94.61%	N.A.O
10ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
50ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	2206	1898	1982	92.79%
100ppm	1839	1995	92.18%	92.18%	1750	1897	92.25%	92.37%	N.A.O	2080	2256	92.20%
200ppm	1767	1917	92.18%	90.27%	1588	1717	92.49%	90.53%	2103	2294	91.67%	91.28%
300ppm	1940	2157	89.94%	N.A.O	1637	1791	91.40%	N.A.O	1846	2031	90.89%	N.A.O
350ppm	1916	2115	90.59%	N.A.O	1568	1749	89.65%	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O

(S.C:- Standardization Concentration, N.A.O:- No Anther Observed)

on the weed (shoot length, leaf area and number of branches). In the lead stress conditions, due to increase in the metal concentration the morphological characteristic of the weed shows great variation. The shoot length, leaf area and no. of branches decrease with increase in the concentration of lead metal (Table 2).

In the figure 2 (a), in all the metal exposure the maximum effect is shown by the nickel metal exposure on the shoot length of the weed at 350ppm. The minimum effect is shown by the cadmium metal which shows that the toxic effect of metal on the weed is less and the weed accumulates large amount of cadmium metal without showing stress on the morphological characteristics. This shows the weed is adaptable to the metal stress environment. At 50 and 100 ppm in chromium metal exposure, enhancement of shoot length shows these conditions are favorable for the plant to grow.

The maximum stress on the leaf area was shown in nickel stress conditions as compared to other metals. The leaf area decreases to a large extent in chromium stress conditions, at 50 ppm leaf area increases shows that this is the most favorable condition for the plant to grow at maximum level. Overall favorable growth in leaf area was observed in chromium metal. This shows the weed is adaptable to these particular conditions [Figure 2 (b)].

The maximum stress on the number of branches was observed in lead stressed conditions as shown in the figure 2 (c). Chromium metal stress showed appropriate results from control to 350 ppm. Maximum number of branches at control and minimum at 350ppm. No such effect of copper metal was observed in the weed. In cadmium metal exposure, at 5ppm exposure shows a great extent of morphological variations.

The *S. nigrum*, when exposed to metal stress condition at different standardization concentration, the variation occurs in their morphological characteristics, in the given readings up to 100 ppm the variations occurs in the morphology (decrease in S.L, Leaf area and number of branches), but at 200ppm again the shoot of the

plant increases but the leaf area is decreased to large extent, metal stress do not affect the number of branches. It means at 200ppm the weed is adapted to stress tolerant conditions. And at 300 and 350ppm least variations occur in the morphology, overall *S. nigrum* is adapted to Chromium metal stress. In the Copper stress conditions, as the concentration of metal increases, no variations occur in the shoot length and number of branches. But variation occurs in leaf area, which

shows that certain factors are present in the leaf which effect the morphology of the weed, due to increase in metal concentration but overall least variation occurs and weed is adapted to copper stress conditions. In the Cadmium stress conditions, least variation occurs from 5ppm to 350ppm in the shoot length and number of branches. But large variations occur in the leaf area, which shows that the toxic metal effect is observed in leaves only, with increase in the metal concentration. In the Nickel

Table 5: Pollen Fertility Analysis in *Chenopodium album* exposure to different metal concentrations

S.C	Pollen Fertility Analysis(Pb)				Pollen Fertility Analysis (Cu)			
	Fertile pollens	Total pollens	Pollen fertility	average	Fertile pollens	Total pollens	Pollen fertility	average
control	1439	1516	94.92%	93.62%	1439	15169	4.92%	93.62%
	1466	1588	92.32%		1466	1588	92.32%	
5ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
10ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
50ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
100ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
200ppm	1591	1746	93.12%	91.54%	1677	1797	93.32%	92.58%
	1566	1703	91.96%		1698	1839	91.84%	
300ppm	1720	1888	90.10%	90.70%	1868	2058	90.77%	90.76%
	1688	1849	91.29%		1933	2130	90.75%	
350ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O

Table 6: Pollen Fertility Analysis in *Solanum nigrum* by exposure to different metal concentration

S.C	Pollen Fertility Analysis(Cu)				Pollen Fertility Analysis (Ni)			
	Fertile pollens	Total pollens	Pollen fertility	average	Fertile pollens	Total pollens	Pollen fertility	average
control	1371	1451	94.49%	94.48%	1371	1451	94.49%	94.48%
	1211	1282	94.46%		1211	1282	94.46%	
5ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
10ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
50ppm	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O	N.A.O
100ppm	1353	1458	92.80%	91.95%	N.A.O	N.A.O	N.A.O	N.A.O
	1299	1426	91.09%		N.A.O	N.A.O	N.A.O	
200ppm	1296	1421	91.20%	91.34%	N.A.O	N.A.O	N.A.O	N.A.O
	1406	1537	91.48%		N.A.O	N.A.O	N.A.O	
300ppm	N.A.O	N.A.O	N.A.O	N.A.O	1577	1746	90.32%	90.75%
	N.A.O	N.A.O	N.A.O		1548	1698	91.17%	
350ppm	N.A.O	N.A.O	N.A.O	N.A.O	1504	1701	88.42%	89.59%
	N.A.O	N.A.O	N.A.O		1425	1570	90.76%	

stress condition, increase in metal concentration does not affect much on the morphological characteristic of plant. At 50ppm the shoot length as compared to control increases, means weed is adapted at 50ppm and again at 100ppm, metal stress conditions decreases the shoot length. Leaf area of the plant decreases, with increase in metal concentration but no metal affect is observed on the number of branches. In the Lead metal stress conditions, as the metal concentration increases,

the shoot length and leaf area decreases. But no variation occurs in the number of branches. So in metal stress conditions increase in metal concentration causes a great variation in the morphological characteristics (Table 3).

In the figure 3 (a), the maximum stress on the morphology of plant was exerted by copper metal. With increase in metal concentration, the shoot length of the plant decreases and becomes

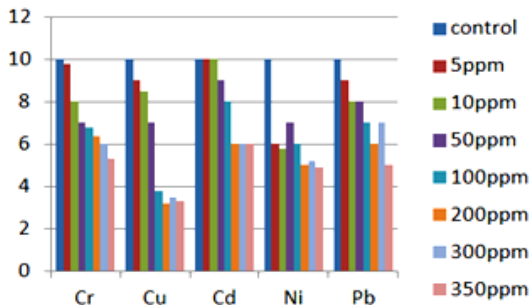


Fig. 1 (a): The figure shows the change in the shoot length of *Cannabis sativa* by the exposure of heavy metals

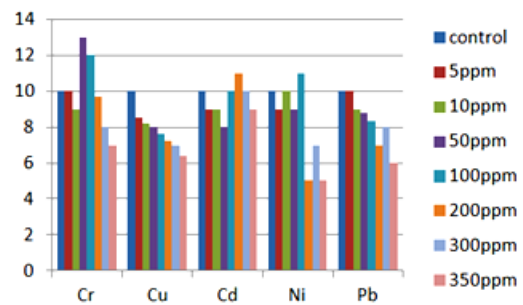


Fig. 2 (a): The figure shows the change in the shoot length of *Chenopodium album* by the exposure of heavy metals

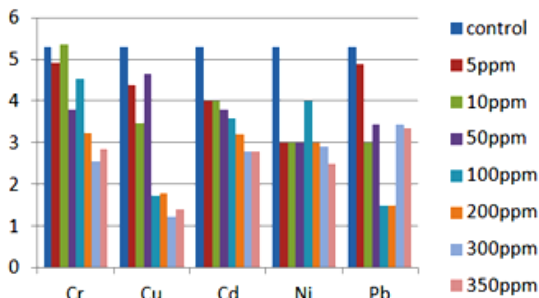


Fig. 1 (b): The figure shows the change in the leaf area of *Cannabis sativa* by the exposure of heavy metals

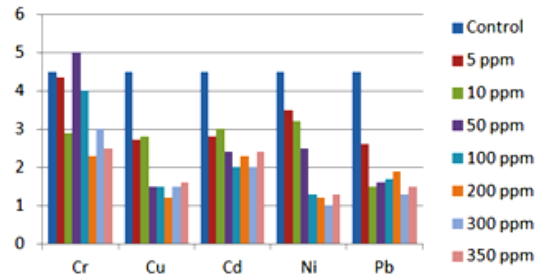


Fig. 2 (b): The figure shows the change in the leaf area of *Chenopodium album* by the exposure of heavy metals

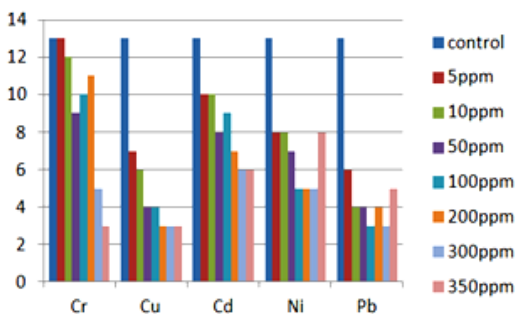


Fig. 1 (c): The figure shows the change in the number of branches of *Cannabis sativa* by the exposure of heavy metals

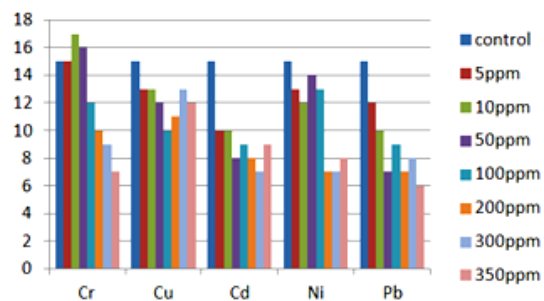


Fig. 2 (c): The figure shows the change in the number of branches of *Chenopodium album* by the exposure of heavy metal

minimum at 350ppm. Similar results were shown by the nickel metal but the metal doesnot affect so much on the morphology as compared to copper metal. At certain concentration, of the metal exposure, increase of shoot length was observed, which shows these conditions are favorable for the plant growth.

Different metal exposure on the leaf area shows that in nickel and lead stressed conditions, increase in metal concentration, decreases the biomass of the plant as shown in the figure 3 (b).

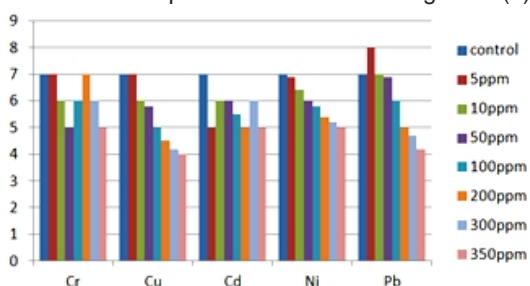


Fig. 3(a): The figure shows the change in the number of branches of *Chenopodium album* by the exposure of heavy metals

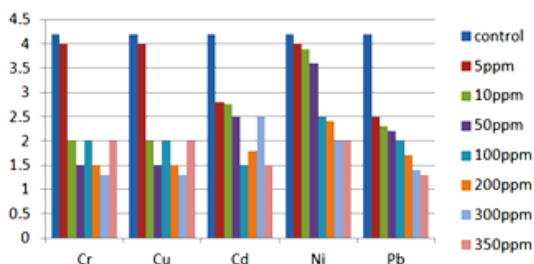


Fig. 3(b): The figure shows the change in the leaf area of *Solanum nigrum* by the exposure of heavy metals

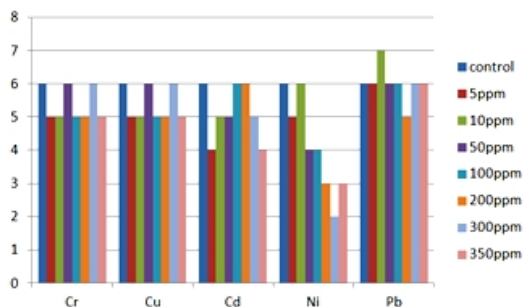


Fig. 3(c): The figure shows the change in the no. of branches of *Solanum nigrum* by the exposure of heavy metals

Maximum stress was observed in the lead stressed conditions. In both the chromium and copper exposure, decrease in leaf area was observed at 300ppm and then increase at 350 ppm. It means in both the exposures, certain hormones and growth factors were released at 350ppm [Figure 3 (b)].

The maximum variations in the morphological characteristics were observed in nickel stressed conditions. No such variations are observed in the other metal, which shows that the plant are adaptable in that particular conditions and

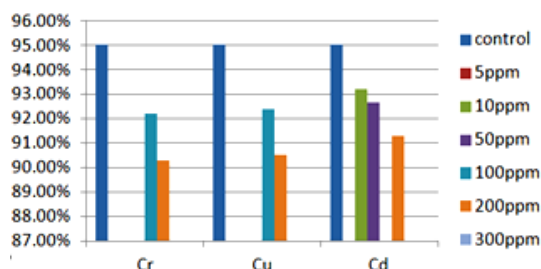


Fig. 4 : The effect of different metal concentration on the pollen fertility of *Cannabis sativa*

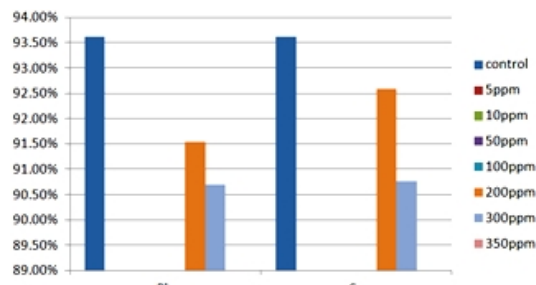


Fig. 5 : The effect of different metal concentration on the pollen fertility of *Chenopodium album*

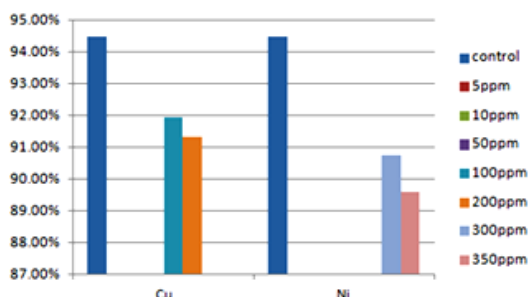


Fig. 6: The effect of different metal concentration on the pollen fertility of *Solanum nigrum*

show normal morphological growth [Figure 3(c)].

In the figure 4, pollen fertility analysis of *Cannabis sativa*, concluded with the outcome that the exposure of 100 ppm copper and 100 ppm chromium show approximately similar levels of pollen fertility levels, i.e. 92.37% and 92.18% and high pollen fertility was observed in case of cadmium exposure at 200 ppm i.e. 91.28% as compared to the chromium and copper metal exposure. The overall analysis showed that the metal exposure

can affect the pollen fertility rate due to metal toxic effect on the plant.

In the figure 5, the pollen fertility was observed in *Chenopodium album* exposed to lead and copper metal at different concentrations of metal. It was observed that metal exposure at 200 ppm in case of copper, pollen fertility was 92.58% as compared to the lead exposure, where it was observed that pollen fertility was 91.54%. At 300 ppm metal exposure to *Chenopodium album*

Hydroponic experiment

***Cannabis sativa* (Control)**



Fig. 7: Day 1(*Cannabis sativa* (C))

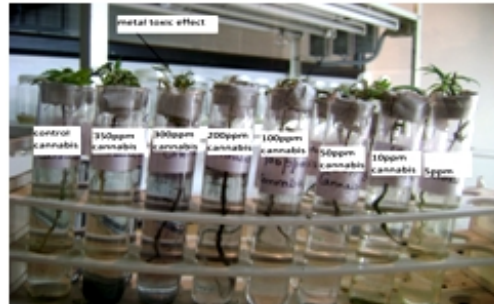


Fig. 8: Day 15(*Cannabis sativa*(C))

***Cannabis sativa* (Polluted)**



Fig. 9: Day 1(*Cannabis sativa* (P))



Fig.10:Day 15 (*Cannabis sativa* (P))

Optical Density(*Cannabis sativa*)

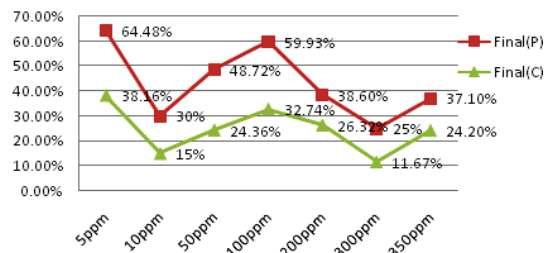


Fig. 11: The figure depicts the amount of chromium absorbed by the *Cannabis sativa* Polluted (P) and Control (C)

showed equal effect on pollen fertility i.e. 90%.

In the figure 6, it was observed that metal concentration affects the pollen fertility of the weed *Solanum nigrum*. The data analysis concluded that upto 100 ppm, no affect on anther were observed but at 100 ppm copper exposure to plant affects the pollen fertility as compared to control, where it was 94.48% and at 100 ppm exposure of copper, it was 91.95%. The nickel exposure to plant effects adversely at higher concentration exposure, and no affect on anthers were observed at 100 ppm. At

200 ppm, the pollen fertility in copper exposure was 91.34% but no variations as compared to control were observed in nickel exposure. No anthers were observed at 300 and 350 ppm in copper exposure but the data analysis observed pollen fertility in nickel exposure at 300 and 350 ppm which was 90.75% and 89.59%

In the Hydroponic experiment (Figure 7), amount of chromium absorbed by *Cannabis sativa* in control and polluted samples were investigated. The ability of the plants taken from the polluted areas have more ability to accumulate chromium as compared with the Control. The graph depicts that the polluted plants can accumulate chromium upto as compared with the Control plant, which accumulate only 32.74% of chromium from 50 ppm of active chromium available in media. The overall analysis depicts that in 350 ppm, the polluted plants accumulated 37.10% of chromium as compared with the Control plants which accumulate only 24.20% of chromium. The whole analysis concludes that the *Cannabis sativa* of polluted area are good hyperaccumulator of chromium metal as compared with the Control plant taken from normal agricultural land.

CONCLUSION

Agricultural practices are must for mankind and essential for the development of human race. Soil has to remain sustainable for agriculture purposes, it becomes essential to remediate the soil from the toxic heavy metals. Sustainable soil reservoir is very important for the continuum of living organisms. This particular study focuses on

phytoremediation of soil from heavy metals through wild weed varieties. Four parameters are assessed during the study as shoot length, leaf area and number of branches and pollen fertility of *Cannabis sativa*, *Chenopodium album*, and *Solanum nigrum*. Increase in toxicological parameters along with certain level of variations was observed with increase of metal concentration. Like in *S. nigrum* copper metal exposure leads to decrease in the leaf area but shoot length and number of branches are least affected. It means that the weed is adapted for certain metal exposure levels. Lead metal exposure in all the weeds shows maximum toxic effects with increase in metal concentration. The best possible observations were obtained up to 50ppm in case of *cannabis sativa* in all the metal exposure. The pollen fertility analysis in all the weeds decreases at higher concentration of metal. The pollen fertility decreases to highest levels at 350ppm. In the hydroponic experiments, maximum toxic effect of heavy metals was seen in *Cannabis sativa*(P) as compared to *Cannabis sativa*(C). Chemical composition of nutrient solution, pH also decreases in polluted samples of *Cannabis sativa* as compared to control. The current set of experiments establish the basic data for carrying out metal based bioremediation protocols in various metal polluted industrial waste water with the help of wild weeds undertaken in this study. All of the weeds undertaken in the current study are capable of sufficient level of bioaccumulation and still they are capable of maintaining their growth rates and reproduction levels. This analysis needs further work to optimize the full capability of these specific weed strains.

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