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Study on phytoremediation potential of *Withania somnifera* grown in soil contaminated with tannery effluent

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Abstract
Medicinal and aromatic plants are used worldwide as herbal remedies as an economical health care aid. They attain an important place in conservation of biodiversity. Environmental contamination due to heavy metals is a worldwide serious concern. These are trace elements which bioaccumulate and can easily pass into the food web. Various industrial wastes discharged on land and water without proper treatment are contaminating agricultural land and water bodies. Reclamation of such land is quite an expensive affair; in order to cope up with this situation heavy metal accumulating plants could be an effective, clean and cheap option. In the present study *Withania somnifera* (Solanaceae family) an evergreen shrub was used for the phytoremediation of manganese, iron, nickel, zinc, cadmium and chromium contaminated soils. Earthen pot experiment was performed. Pots were divided in two sets one treated as control and the other one was fully irrigated with tannery effluent. Heavy metals total accumulations in root, stem and leaf was calculated along with Bioconcentration factor, Translocation factor, biological concentration and accumulation values, total chlorophyll, carotenoids, phytochemical (total phenol) and secondary metabolite (flavonoids) was also calculated. Finally, the result shows that the plant species is an effective accumulator of these heavy metals and can help in phytoremediation.

Keywords: heavy metals, phytoremediation, translocation factor, *Withania somnifera*, secondary metabolites

Introduction

Pollution of the environment, a problem since the industrialization era, is elucidated by contamination of agricultural soils with toxic heavy metals. Heavy metal contamination of soils has been a major environmental problem in world in the last few decades. The high concentrations of heavy metal induce adverse effects on the plant productivity, food quality, and environmental health. Metal contamination as a consequence of mining operations, untreated industrial discharge, smelting activities or pesticides and sewage sludge uses causes significant damages on surrounding flora, fauna and human (Kumar *et al*. 2017 and Pandey *et al*. 2014) [16, 22]. The oxidative stress in living cells and biological macromolecules is chiefly due to binding of heavy metals to the DNA and nuclear proteins (Flora *et al*. 2008) [9]. Tannery wastewater contains large amount of chemical compounds including toxic substances. The tanning activity is an essential process for the leather industry and most tanneries in the world (about 90%) use chromium salts to provide better leather flexibility, better water resistance and a high shrinkage temperature (Alfredo *et al*., 2007) [3] and high concentration of Cr salt especially, Cr(VI) have carcinogenic, mutagenic and teratogenic effects on humans, many plants, animals, and bacteria inhabiting aquatic environments (Naik *et al*., 2007) [19]

There is a need for innovative technologies to remediate contaminated lands, although there are many technologies but some are a way too costlier and methods like vapour extraction,enzymatic degradation and solidification either produce secondary pollutants or make soil unfit for agriculture by reducing its fertility (Sivarajasekar, 2014, Sivarajasekar *et al*. 2017d, e and Alpaslan and Yukselen 2002) [28, 4]. Bioremediation has acquired attention over the recent decades, as a rising, clean and eco-friendly approach that employs the natural abilities of living organisms to amend the polluted lands (Vijayalakshmi *et al*. 2018). In general bioremediation can be classified into two types as in- situ or ex- situ. While in the in- situ bioremediation, the contaminants are treated directly at the site, in ex- situ bioremediation the contaminants are collected from the site and are treated elsewhere (Paz-Alberto and Sigua 2013; Ramachandran *et al*. 2013) [23, 24]. Phytoremediation is defined as the technique involving the use of green plants, to remediate, remove, immobilize, or transform environmental contaminants such as heavy metals, organic compound and other elements in natural resources (Dan Salt,1995) [2]. The plants used for Phytoremediation must be fast growing and have the ability to accumulate large quantities of metal contaminants in their shoot tissue.

Withania somnifera (popularly known as Ashwagandha or Winter Cherry) is an important medicinal plant that belongs to the family Solanaceae. It is also known as 'Indian Ginseng' in traditional Indian system of medicine (Singh *et al*. 2001) [27] and widely used as an herbal tonic and health food in Vedas. The different parts of the plant are generally used as anti-inflammatory, anticancer, anti-stress and immune-modulator, and helpful in central nervous system, endocrine and cardiovascular activities (Alam *et al*. 2011) [2]. Many researchers have found that the essential oil from medicinal and aromatic plants is free from the risk of heavy metals accumulation from plant biomass (Khajanchi, L. *et al* 2013 and Zheljazkov, V. D, 2006) [34]. Growing medicinal and aromatic plants for land reclamation could be very economical. However, the metal uptake process and accumulation varies from plant to plant, it depends on the concentration and solubility of available metals in soil and the plant species growing on those soils. The present investigation was therefore undertaken to determine the potential of *W. somnifera* as a bioaccumulator in reclamation of a land mainly polluted due to discharge of tannery effluent. This will also be very helpful way in adding to the economy of any nation by exporting the essential oil from this medicinal plant.

Materials and method

Withania somnifera plants were grown under glasshouse conditions in earthen pots. The earthen pots used were washed and dried and then high quality plastic sheets were kept inside covering the whole inside portion and soil was filled in it for further experiments. Treatment was supplied everyday in the evening. Whenever there was high evapotranspiration tap water was supplied to prevent it. For pot culture studies alluvial soil of Lucknow was collected in bulk from area having uniform surface and texture. Before collection it was ensured that the selected area had received no manure and fertilizer. The collected soil was thoroughly mixed with nutrient solution and left for seven days. The nutrient solution comprises of NPK and micronutrient solution. Nitrogen, phosphorus and potassium (NPK) were added in the ratio 120: 60: 60 as basal dose for soil. The micronutrient solution used for soil preparation consists of 1ppm Borax solution, 0.5ppm Ammonium molybdate, 5ppm $CuS0_4.5H_20$, 10ppm $MnSO_4.4H_2O$, 10ppm $FeSO_4.7H_2O$, 9.5ppm $ZnSO_4.7H_2O$. Effluent samples were collected from the outlet of the Superhouse Limited Tannery No.1, Kanpur-Lucknow road, Unnao, Uttar Pradesh. Pots were divided in two sets of ten pots each, one set was treated as control (N) and other was irrigated only with tannery effluent (T). After 60 days growth period leaves of plants were assayed for heavy metals concentration along with translocation factor, chlorophylls (Chl) a and b, carotenoid, total phenolic content as well as for total flavonoids content.

Soil was thoroughly sieved to fine powder by the help of a mesh and air dried for 48 hrs. Sample was prepared by digesting soil in wet acid HNO3: HClO4 (10:1) digests and metal concentration was determined by atomic absorption spectrophotometer (Perkin Elmer A Analyst 300). Tannery water sample was filtered by Whattmans filter paper and it was heated continuously on hot plate near to dryness and then sample was prepared in wet acid HNO3: HClO4 (10: 1) digests and further metal concentration was determined by atomic absorption spectrophotometer (Perkin Elmer A Analyst 300). Plants were separated into roots, stem and leaves, and total biomass was determined by oven drying (70°C) the samples. The tissue metal concentration in roots, stem and leaves was determined by atomic absorption spectrophotometer (Perkin Elmer A Analyst 300) in wet acid HNO3: HClO4 (10: 1) digests. Chlorophyll (a + b) and Carotenoids were extracted with 80% acetone and measured spectrophotometrically (Perkin Elmer UV/VIS Lambda Bio 20) as described by method of Lichtenthaler, (1987) [17].

Quantification of phytoremediation efficiency - The concentration, transfer and accumulation of metals from soil to roots and shoots was evaluated in terms of Biological Concentration Factor (BCF), Translocation Factor (TF) and Bioaccumulation coefficient (BAC).

Biological Concentration Factor

(BCF) was calculated as metal concentration ratio of plant roots to soil (Yoon *et al*., 2006) [33]. The Bioconcentration Factor (BCF) of metals was used to determine the quantity of heavy metal absorbed by the plant from the soil. This is an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil (Ghosh and Singh, 2005a). The plants which have a greater value of BCF have a greater potential for phytostabilization.

BCF = metal concentration in plant roots / metal concentration in soil.

Translocation factor (TF)

The efficiency of phytoremediation can be quantified by calculating translocation factor. The TF expresses the capacity of a plant to store the MTE in its upper part. This is defined as the ratio of metal concentration in the upper part to that in the roots (Chakroun *et al*., 2010) [7]. The translocation factor indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots. It is calculated as follows (Padmavathiamma and Li, 2007) [27].

$$\text{Translocation Factor (TF)} = \frac{C(\text{shoot})}{C1\ (\text{root})}$$

Where, C is the shoot concentration of the metal in plant shoots and C1 is the root concentration of the metal in plant roots. A greater value of translocation factor indicates the translocation of the metal from root to above ground part (Jamil *et al*. 2009) [11]. According to Yoon *et al*. (2006) [33], only plant species with TF greater than 1 have the potential to be used for phytoextraction.

Bioaccumulation coefficient

It is defined as the ratio of metal concentration in plant shoots to the soil. All the plants that have a value of BAC greater than one are suitable for phytoremediation.

BAC = metal concentration in plant shoot / metal concentration in soil.

Total phenols were determined in alcohol soluble fractions by the method of Swain and Hillis, (1959) [30]. From the alcohol soluble material pigments were removed with the help of lead acetate and potassium oxalate. Suitable aliquots were drawn from the filtrate in 50 ml test tubes and made to 7.5 ml volume with glass distilled water. The solution was then treated with 0.5 ml Folin's reagent and 1 ml of super saturated solution of sodium carbonate. After 1 hour, the colour intensity was measured at 725nm. Flavonoids in leaves were determined by Aluminium- Chloride Colorimetric Assay for Total Flavonoid Content method. Finely chopped leaves were ground in mortar pestle in 80% methanol. The extract was ultrasonicated and then centrifuged for 5 min. To the aliquot double distilled water was added further 10% NaNO2, 10%AlCl3 and 1M NaOH was added and the whole mixture was shaken thoroughly and the absorbance was read at 510nm by preparing a standard curve against Quercetin.

All measurement was made on samples drawn in triplicate and the data were statistically analysed (ANOVA) for significance (LSD at P= 0.05). The data in figures are presented as bar diagram of mean values ± standard error (SE, n=3).

Result and Discussion

Soil serves as a crucial growth substrate for growth and development of living organisms. Presence of deleterious substances or contaminants may inhibit the quality of soil matrix. During the experimental process various physical parameters along with heavy metal concentration were analyzed for tap water, soil used during the experiment and tannery waste water. Effluent was slightly alkaline in nature having grayish black color and a foul smell. Highest concentration of chromium was found in tannery waste water among all other trace elements followed by cadmium, nickel, iron, zinc and manganese being the least in quantity (Table 1). Presence of chromium and chromium salts in tannery waste is previously observed by many researchers. Use of sludge especially released by tannery industries for irrigation can cause heavy metal accumulation like Cd, Zn, Cr, Ni, Pb and Mn in surface soils, which can further leach into the groundwater and finally enters the food chain and hence it not only effects the plants but also poses risks to human health (Murugesan *et al*., 2008).

Table 1: Physico-chemical parameters of tannery effluent, tap water and soil used in experiment

Parameters	Tannery waste water	Soil	Water
pH	8.6	6.5	7.0
Colour	Grayish black	-	-
Smell	Foul smell	-	-
Fe	8.49	5.73	ND
Mn	6.03	2.12	ND
Cd	11.49	2.00	ND
Zn	47.70	23.57	ND
Ni	14.79	6.76	ND
Cr	128.67	8.49	ND

Except pH, colour and smell all values are in mg l^{-1}. ND: not detected

Ashwagandha exposed to the tannery effluent showed few morphological changes. The plant height was slightly reduced by nearly 8% when compared to the control. The leaf area was only reduced to 4% in plants irrigated only with tannery effluent. There was not much ill-effect of the effluent on growth of *W. somnifera*. This shows it can grow well in such condition for the purpose of remediation. This result is in conformity with Gaur (2018) for *Catharanthus roseus* grown under different heavy metal stress. The total dry matter yield nearly reduced by 8% in treated plants as compared to the control. Similar results were observed by Arifa *et al*., 2013 in sunflower. Rusan *et al*., 2007 [25]; Kilicel and Dag, 2006 and Hewitt and Keller, 2003 also reported the same effects of wastewater on the biomass of maize, soybean and wheat plant the dry matter yield of root was observed to higher in treated plants than compared to the control ones (Table-2).

Table 2: Effect of heavy metals on dry matter yield in *Withania*

Plant part	Treatment		LSD (P=0.05)
	N	T	
	Dry matter yield: g plant^{-1}		
Leaves	2.60±0.30	2.14±0.42	0.012
Stem	1.56±0.29	1.40±0.47	0.020
Root	1.98±0.40	2.09±0.21	0.016
Whole plant	6.14±0.99	5.63±1.10	0.120

Maximum accumulation of metals followed the order Zn > Ni > Cr > Fe > Cd > Mn in treated plants. Manganese was translocated in a slow manner throughout the period of experimentation. Highest accumulation of Mn was recorded in roots than leaves and stem in both control and treated (Table 3). The results of Mn accumulation and TF below than 1 in *W. somnifera* revealed that the rate of translocation were meagre in case of control plants. (Table 4). Ashwagandha grown in tannery waste amended soil accumulated zinc maximum in leaves (38.27 µg/g dry wt.) than in root and stem. The results revealed that Ashwagandha is a good accumulator of Zinc (Table 3 and Table 4). In case of Cr and Fe maximum accumulation of metals was observed in roots (more than 50%) than in stem and leaves, where Cr was observed about 8.99 µg/g dry wt. in roots of treated plants and iron was reported 7.36 µg/g dry wt. in roots (Table 3). The results indicated that *W. somnifera* is good accumulator of Cr and Fe however, TF for Fe in treated plants is less than 1, it may be due to metallic interaction (Table 4). While in Cd translocation to leaves was observed to be quiet less in control plants, however, it was found to be more pronounced in treated plants. Accumulation of Ni was observed to be higher in treated ones than compared to the control plants. The root and stem of both the levels were very profound in accumulation and translocation of Ni, which reveal that maximum quantity of Ni is translocated up to the leaves indicating ashwagandha to be suitable phytoremediator of Ni (Table 3). Except Fe, almost all five metals have TF above 1, which makes *Withania* suitable for further phytoextraction (Table 4). The results obtained were in conformity with Subhashini and Swamy, (2016) for *Catharanthus roseus* grown in heavy metal contaminated soil.

Table 3: The tissue metal concentrations of various metals in *W. Somnifera*

Treatment	Plant part	**Tissue metal concentration** µg g^{-1}dry wt.					
		Fe	M n	Cd	Zn	Ni	Cr
Control	Leaves	4.06±1.14	1.68±1.16	0.81±0.02	22.51±1.16	4.21±0.08	3.39±0.67
	Stem	0.27±0.28	0.94±1.53	1.13±0.29	19.07±1.56	5.02±1.56	2.98±1.40
	Root	4.44±1.54	2.65±0.28	1.65±0.09	30.21±1.73	11.03±2.04	6.23±0.33
T3	Leaves	2.12±1.01	1.90±1.23	2.01±0.89	38.27±1.97	9.51±1.44	6.30±0.67
	Stem	1.33±0.06	1.03±2.10	2.12±0.04	24.41±1.83	14.82±1.69	7.06±0.92
	Root	7.36±1.03	2.99±0.24	2.76±0.62	35.68±1.91	12.03±1.01	8.99±1.33

Table 4: (A) Translocation factor, (B) Biological concentration factor and (C) Bioaccumulation Coefficient of *W. somnifera*fordifferent metal

(A) Metals	Treatment	
	N	T
	Translocation Factor	
Fe	0.975	0.468
Mn	0.988	1.176
Cd	1.175	1.496

Zn	1.376	1.756
Ni	0.836	2.022
Cr	1.022	1.486

(B) Metals	**Treatment**	
	N	**T**
	Bio concentration Factor	
Fe	0.774	1.284
M n	1.235	1.410
Cd	0.825	1.380
Zn	1.281	1.513
Ni	1.631	1.780
Cr	0.733	1.05

(C) Metals	**Treatment**	
	N	**T**
	Bioaccumulation Coefficient	
Fe	0.755	0.602
Mn	1.235	1.382
Cd	0.970	2.065
Zn	1.764	2.659
Ni	1.365	3.597
Cr	0.750	1.541

The three phytoremediation parameters calculated for *W. somnifera* inorder to phytoremediate the heavy metals revealed here that the TF < 1 for Fe in control plants as well as the treated ones; may be due to presence of other heavy metals in the substratum as a result of which Fe could not be freely translocated. The bioaccumulation coefficient was less than 1 for both control and treated plants and bioconcentration factor was observed to be less than 1 in control plants but it was greater than 1 when compared to the treated plants. These results indicate that despite presence of other heavy metals *W. somnifera* is capable for phytostabilization, although the translocation was not so precise. (Fig. 1 and Table 4). In case of Mn, all three parameters were found to be more than 1 in both control and treated level. BCF value was close to 1 in control plants. The results are clearly indicating the translocation of Mn is quiet well from roots to above ground parts, making it a suitable species for phytoremediation. (Fig. 1).

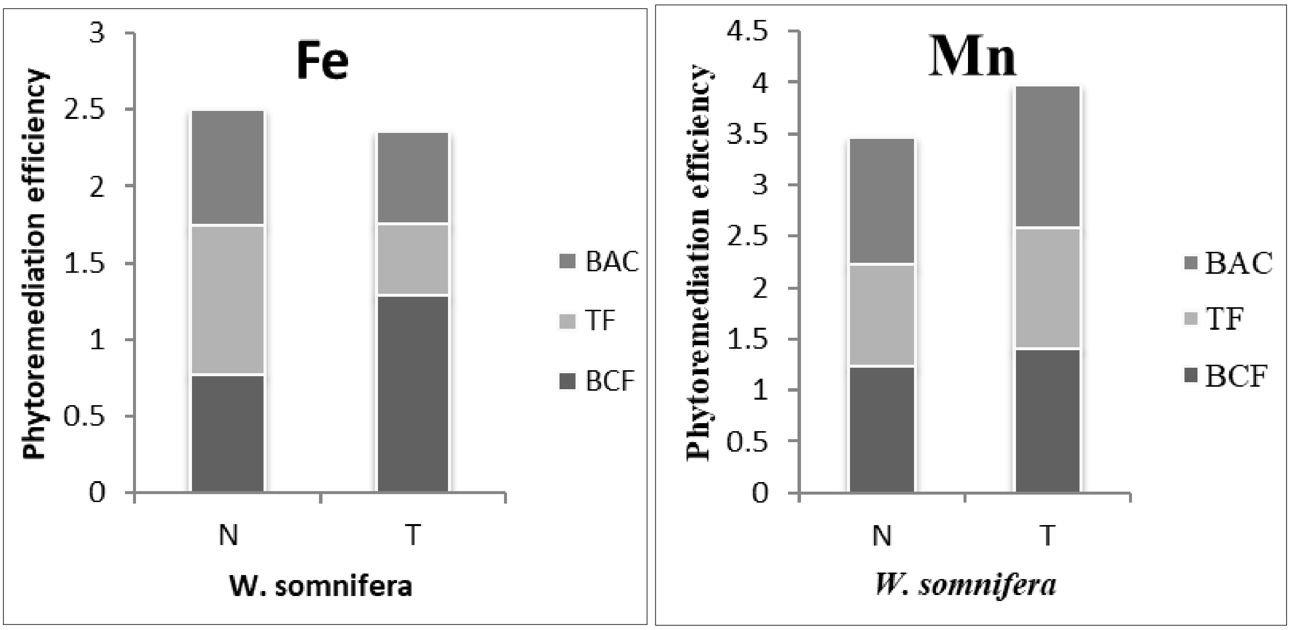

Fig 1: Phytoremediation efficiency of *W. somnifera* for iron and manganese grown in control plants (N) as well as plants grown in soil irrigated with tannery effluent (T).

Withania is suitable for phytoextraction of cadmium indicated by value of TF > 1 for both control (1.175) and treated (1.496) plants. Other parameters BAC and BCF were found to be less than 1 in control plants but these values were greater than 1 in treated plants indicating that *W. somnifera* is suitable for phytoremediation of soil polluted by release of tannery effluent. (Fig. 2). (Zhang *et al*., 2002). Zn was phytoaccumulated quiet well by *Withania* since the TF value > 1. Here the values of BAC and BCF were also noted to be greater than 1. (Fig. 2). It proves *Withania* is suitable for phytoremediation and phytoextraction of zinc. (Table 4).

The treated plants have TF > 1 i.e, 2.022 in the case of Ni, however, the control plants have TF < 1. (Table 4). BCF and BAC value was also noted to be greater than 1 in control as well as treated plants for Ni indicating *Withania* a potential hyperaccumulator. (Fig. 3). In the case of chromium, value of TF > 1, both other parameters BAC and BCF was less than 1 in control plants, whereas it was reported that both BAC and BCF value in treated plant was greater than 1, which clearly indicate that *Withania* has potential for both phytostabilization and phytoextraction and also it is a potential species for phytoremediation. (Yoon *et al*. 2006) [33] (Fig. 3 and Table 4).

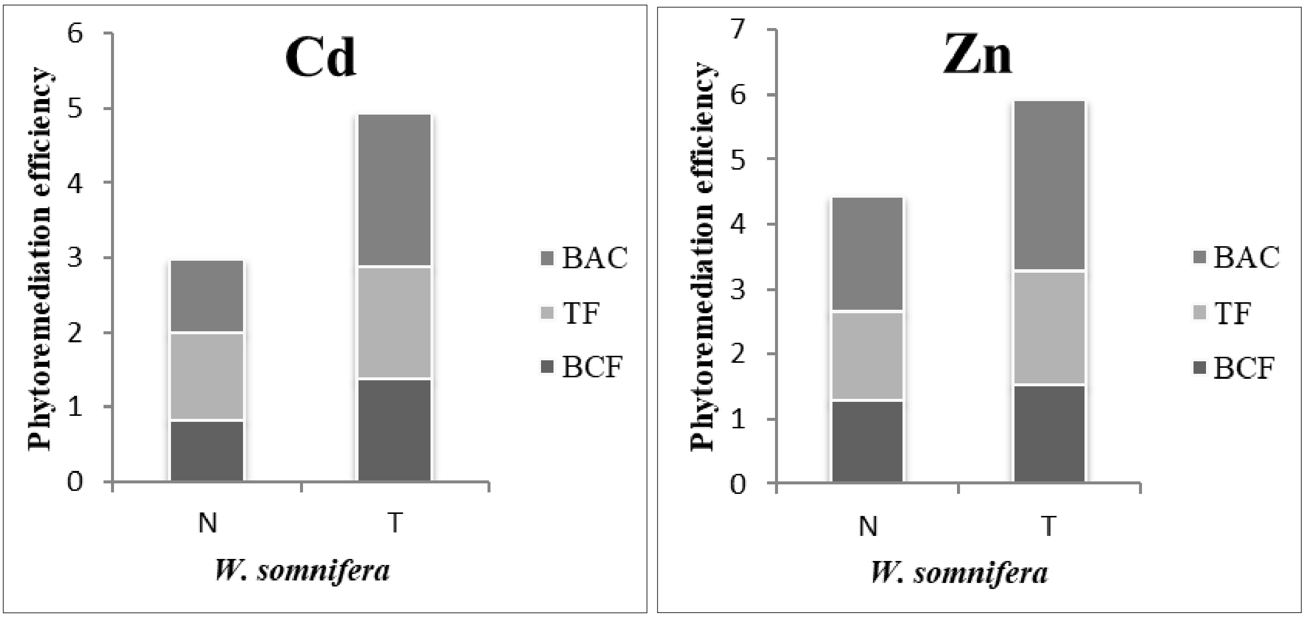

Fig 2: Phytoremediation efficiency of *W. somnifera* for cadmium and zinc grown in control plants (N) as well as plants grown in soil irrigated with tannery effluent (T).

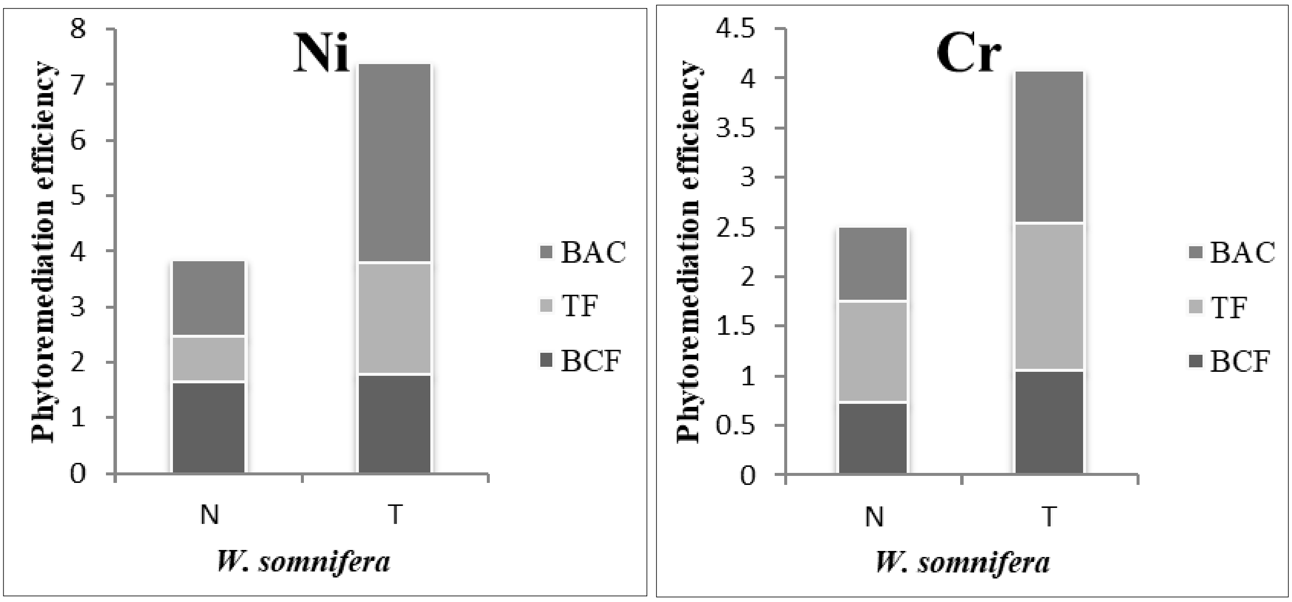

Fig 3: Phytoremediation efficiency of *W. somnifera* for nickel and chromium in control plants (N) as well as plants grown in soil irrigated with tannery effluent (T).

Presence of Cr in tannery effluent negatively impacts the plants and its toxic effects can lead to generation of reactive oxygen species (ROS) which is ultimately responsible for oxidative stress in plants. Foliar content of chlorophyll pigments including chlorophyll a, chlorophyll b and total chlorophyll were assayed. As compared to control chlorophyll (chl a, b and total) concentration in the leaves of plants grown in pots irrigated with tannery effluent decreased, (Fig 1) it could be due to Cr toxicity which affects photosynthesis (Gupta *et al*, 2011) [14]. Increase in content of Cr may hamper biosynthesis of chlorophyll and deterioration of chlorophyll content (Lushchak, 2011, Sharma, A *et al*, 2019 and Valko, M., 2006) [18, 26, 33]. Carotenoid act as important metabolites in protecting the cellular system from the cytotoxic effects of reactive oxygen species (Agarwal *et al*., 2006) by quenching the singlet oxygen. A decrease in carotenoid content was also observed in treated *Withania* plants, and this is indicative of oxidative stress (Fig 1).

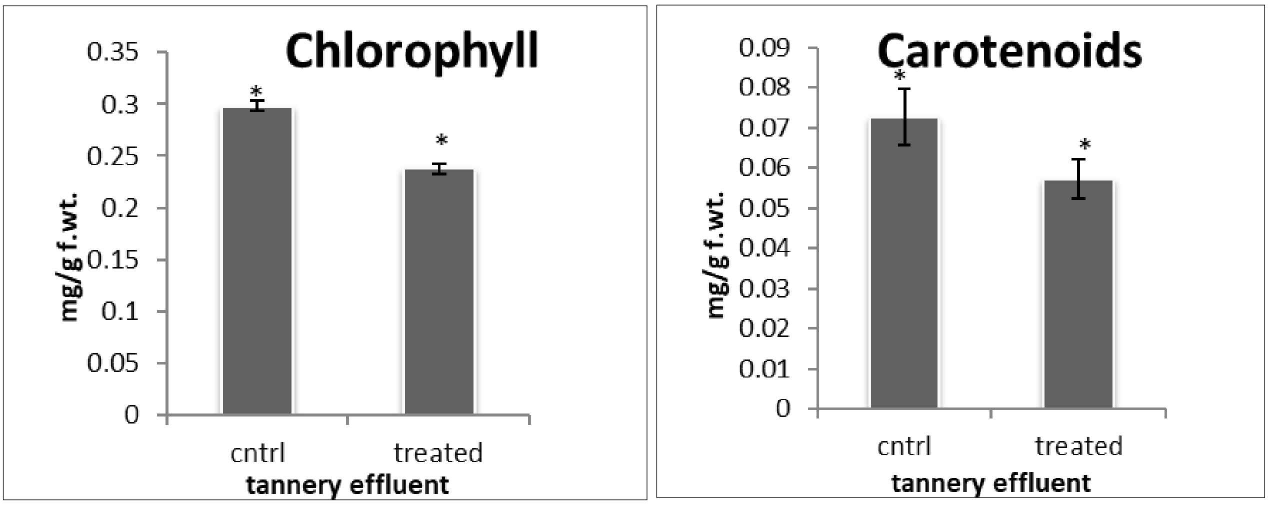

Fig 4: Effect of effluent treatment on concentration of chlorophyll and carotenoids in the leaves of *W. somnifera*. Bars indicates ± S.E. of three independent values. * indicates significant differences compared to control at P=0.05.

Phenols are phytochemicals that account for most of the antioxidant activity in plants and plant products (Okpuzar, 2009) [20]. Flavonoids are structurally diverse secondary metabolites or naturally occurring phenolic compound which have many functions including plant development and defence mechanism. Both phenolic content and flavonoids decreased in treated plants which is possible indication of heavy metal stress in the alteration of phenolic compound, as observed by Kisa D, *et al* (2016) [15] for *Zea mays* under heavy metals stress. Increase or decrease in these low molecular anti-oxidants depends on the severity of stress.

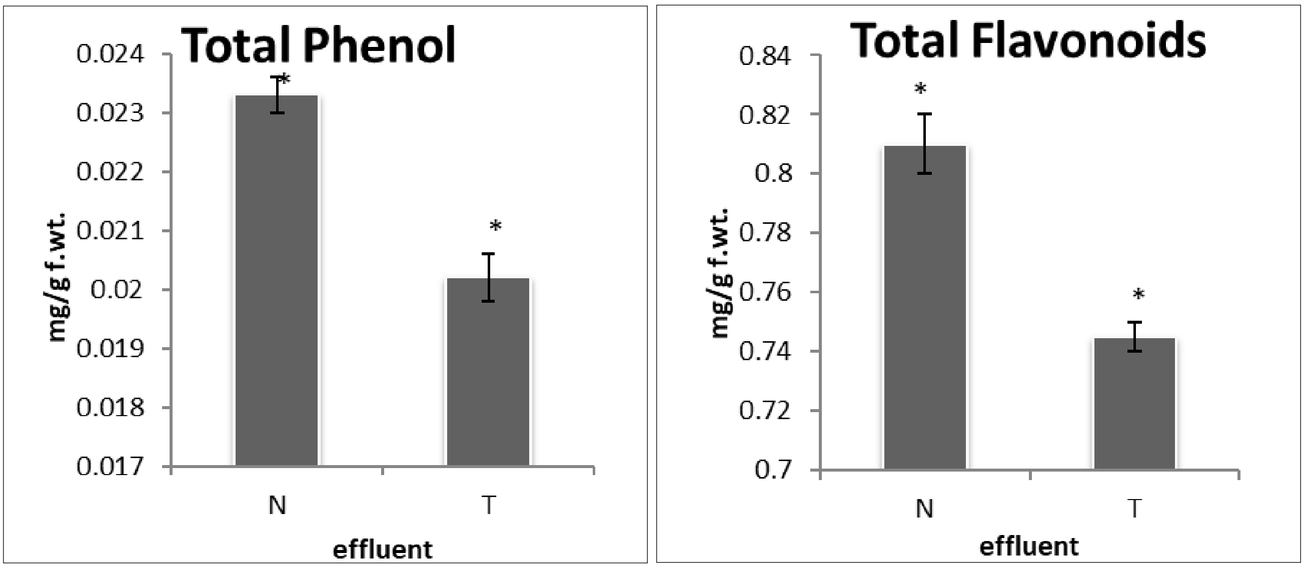

Fig 5: Effect of effluent treatment on concentration of total phenolic concentration and total flavonoid content in the leaves of *W. somnifera*. Bars indicates ± S.E. of three independent values. * indicates significant differences compared to control at P=0.05.

Phytoremediation is clean and green technology in reclaiming contaminated sites. Use of medicinal plants for this purpose could add benefits as these non edible plants having high aroma are not destructed by animals and so there is less chance to pass the accumulated heavy metals in food web. In the present study, the results shows that *Withania somnifera* was good accumulator of manganese, iron, nickel, zinc, cadmium and chromium contaminated soils. It can well tolerate these heavy metals and can be used as a potential hyperaccumulator in tannery sludge contaminated lands.

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