Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2675

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (2):** 43-55 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Reducing Destructive Environmental Impacts of Sungun Copper Mine Effluents with using of Phytoremediation Processes

Amir Jafarpour[1], Jafar Abdollahi Sharif[2] and Alireza Eivazi[3*]
[1]Graduated Student of Mining Engineering, Urmia University, Urmia, Iran
[2]Associate Professor of Faculty Engineering of Urmia University, Urmia, Iran
[3]Assistant Professor of Seed and Plant Improvement Research Department, West Azerbaijan Agricultural and Natural Resources Research And Education Center, AREEO, Urmia, Iran
*Corresponding Author E-mail: j.a.sharif@urmia.ac.ir

Received: 12.03.2017 | Revised: 25.03.2017 | Accepted: 26.03.2017

ABSTRACT

The effluent of Sungun copper mine concentration has destructive effects on Arasbaran forests in the north of Iran. Therefore two separately factorial experiments based on completely randomized design were carried out with different concentrations of irrigated pulp and pulpy soil of this mine effluent. Three crops including wheat, bean and corn were used as first factor in two experiments. Six levels of second factor were different concentrations of tickner pulp used at irrigated and soil experiments with 20%, 40%, 60%, 80%, 100% concentrations and 0% as control. Results showed that there were significant differences for traits of root length, root weight, aerial organs weight, stem diameter, leaf area, stem length and leaf number ($p \leq 0.05$). With in crops, aerial organs weight of bean seedlings grown in irrigated pulp (2009.7mg) was more than soil pulp (1535mg). Bean 60days seedlings according to the amount of three indices including GMP, MP and HAR were tolerant in all concentrations of irrigated and soil pulps at two experiments. Also wheat seedling was identified as the most susceptible plant. Within plants bean had the most variation percentages accumulations of lead (440%), chromium (440%) and copper (473%) and wheat with high values of cadmium (483%) and manganese (499%) also, nickel (280%) more allocated to corn. Roots had more ion accumulations than aerial organs. Therefore it was suggested that bean saw in copper mines for removing copper due to this plant had the most accumulation of copper within evaluated plants and introduced as hyper phytoremediation crop.

Key words: *Bean, Corn, Effluents, Heavy metals, Mine, Wheat*

INTRODUCTION

Nowadays, adhering environmental aspects of mining projects is an important part of the sustainable development goals. Management of the waste and tailing manufacturing processes is the problem that exists in the mining and industrial units. The Sungun copper complex in the north west of Iran is not an exception and discharge of heavy metal ions in to the Arasbaran forests could have adverse on environmental, political and social effects and subsequently international consequences.

Cite this article: Jafarpour, A., Sharif, J.A. and Eivazi, A., Reducing Destructive Environmental Impacts of Sungun Copper Mine Effluents with using of Phytoremediation Processes, *Int. J. Pure App. Biosci.* **5(2):** 43-55 (2017). doi: http://dx.doi.org/10.18782/2320-7051.2675

According to the discharge 1204 m^3/h tailings in the waste dam of the Sungun copper mine review the disposal system of Sungun copper plant tailings is essential. Cleanup methods of soil and water contaminated with heavy metals by the phytoremediation processes are utmost importance. These methods are highly efficient due to being economic, simply run, lack of residual secondary, no change in physical, chemical and biological properties of soils, water and the environment surrounding the mine, having a positive approach to natural and public acceptance. Using different methods of phytoremediation can be suitable for environmental improvement of the mine plant tailings. Abiyarifard[1] evaluated the effects of chromium and silicon ions on growth and grain yield of triticale under greenhouse conditions. Results showed that the highest level of heavy metals in grains (12.84) and shoots observed in 300mg chromium and silicon treatments. Nayak *et al*[17]., demonstrated that phytoremediation of hexavalent chromium by common wheat seedlings (*Triticum aestivum* L.) was low cost and effective soil treatment for bioremediation. Also, Khan et *al*[14]., investigated the potential of *arbuscular mycorrhizal* fungi in phytoremediation of heavy metals and effects on grain yield of wheat crop. Data showed no increase in grain and shoot yield by fungi inoculation with Zn, Cu, Fe, Mn at different levels but increased root yield, plant height, spike length and 1000-kernel weight as compared with control. In addition to phytoremediation of heavy metals, Gouda *et al*[7]., (2016) studied on bioremediation of sandy soil contaminated with petroleum hydrocarbons. The remediation methods that were tested include phytoremediation using alfalfa, bioremediation using *Pseudomonase putida*. After 90 days experiments, these two treatments were able to reduce more than 98% of contamination. Influence of heavy metals of Cu, Ni, Zn, Hg, Cr, Pb and Cd on seed germination and early seedling growth in oil crop *Eruca sativa* was evaluated by Zhi *et al*[25]., under laboratory conditions. Among ions, only Ni at more than 1mM concentrations decreased seed germination. Werle *et al*[23]., studied the phytoremediation as an effective method to remove heavy metals from contaminated areas. They discussed the techniques employed to identify the evolved products and presents the product evolution patterns and yields. Kanwal *et al*[11]., investigated the effects of *Arbuscular mycorrhizal* fungi on wheat growth, physiology, nutrition and cadmium uptake under increasing cadmium stress. *Arbuscular* mycorrhizal fungi improved the capability of reactive oxygen species (ROS) and reduced cadmium concentration in wheat. Hence, this fungus in combination with wheat was suitable for reduction of cadmium toxicity and also showed a potential role in phytostabilization of soil moderately polluted with cadmium. Hamzah *et al*[9]., in evaluating phytoremediation of cadmium-contaminated agricultural lands with using of indigenous plants showed that reduction of cadmium were *Vetiveria Zizanioides* (71.2%), *Eleusine indica L.* (58.9%), *Ageratum conyzoides L.* (52.2%), *Euphorbia hirta* (51.8%) and *Chromolaena odorata* (22.1%). Singh and Singh[21], analyzed the phytoremediation processes with a sustainable approach for restoration of metal contaminated sites. They pointed out phytoremediation is a technology that involve using of plants to remediate contaminated areas with organic and inorganic pollutants for commercial purposes. The physical, chemical and phytoremediation techniques for removing heavy metals were studied by Sharma *et al*[20]. They reported that microbes which are used for the removal of heavy metal from the water bodies include bacteria, fungi, algae and yeast. Some important antioxidants such as flavonoids, pectin and phytic acid are also used for the elimination of the heavy metals. Yasin *et al*[24]., investigated the effect of selenate fertilization and *YAM2* bacterium inoculation on Se uptake and plant growth of wheat. Bacterial inoculation significantly enhanced Se concentration in wheat kernels (167%) and stems (252%) compared to un-inoculated plants. Based on results of researchers, the main purpose of this research is removing of pollutants in tailings of Sungun copper concentrations with sowing wheat, corn and bean crops.

MATERIAL AND METHODS

Sampling of the Sungun mine effluent

The Sungun copper mine is located in East Azerbaijan province of Iran in Varzeqan county, 75km north-west of provincial town of Ahar (Figure 1). It is the most important geological and industrial feature in the area and is the largest open-pit copper mine in Iran. The ore is processed directly at a concentration plant at the mine.

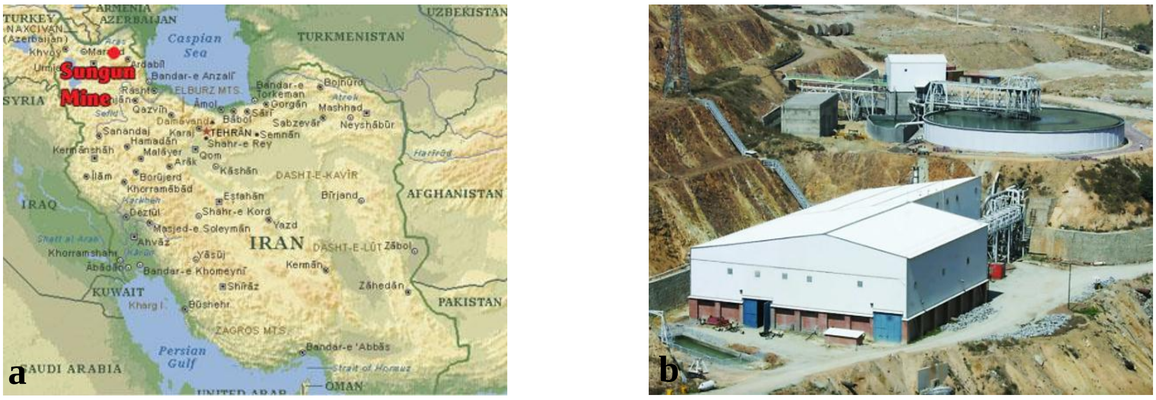

Fig. 1: Location (a) and schematic (b) copper mine tailing tickner of Sungun

Three times tickner pulp effluent were sampled with volume equivalent 500 liters and stored. Samples were analyzed and solid particles are 50% and discharged in Sungun mine tailings dam (Table 1).

Table 1: Chemical properties of elements and compounds of Sungun copper mine tickner effluent in different samples

Sample	SiO_2 (%)	S (%)	Al_2O_3(%)	CuO (%)	Mo (%)	Fe (%)	Cu (%)
sampling I	66.27	2.72	15.00	0.033	0.007	2.65	0.087
sampling II	64.56	2.65	15.22	0.024	0.006	2.88	0.079
sampling III	50.75	2.06	14.91	0.029	0.007	3.11	0.088

Experimental design

Seeds of wheat, corn and bean were selected based on climatic and growth conditions and geographic region of mine as plant materials. Two separately factorial experiments based on completely randomized design were carried out with five replications and different concentrations of irrigated pulp and pulpy soil of Sungun copper mine effluent. Three species of plants including wheat, bean and corn were used as first factor in two experiments. Six levels of second factor were different concentrations of tickner pulp used at irrigated and soil experiments with 20%, 40%, 60%, 80%, 100% concentrations and 0% as control. Two different growth conditions of pulpy soil and irrigated with above concentrations were used at separately experiments.

Experiment I

Seeds of wheat, corn and bean which are irrigated with water containing different concentrations of 0%, 20%, 40%, 60%, 80% and 100% pulp were grown in pots at five replicates. Dimension of pots were 25cm height 30cm diagonal. Pots filled with fertile garden soil. Irrigation water with above concentrations provided in separate containers and pots treated with them. Meteorological parameters such as temperature (25˚C day, 15˚C night), humidity (75-85%) and day length (14-10h) in greenhouses were kept constant. During growth stage when soil pots became dry with 100^{CC} of irrigated pulp attained to field capacity. After 60days of growing stage from each pot 10 seedlings were separated to roots and aerial organs then washed and dried. Morpho-physiological traits including root length, root weight, aerial organs weight, stem diameter, leaf area, stem length and leaf number were measured. Samples were packed in separate bags. Then put them in the oven at 72°C for 24 hours for drying and ready for measuring heavy metals accumulation with atomic absorption spectrometry. It should be noted that after drying samples were weighted again.

Experiment II

Pulpy soil experiment was carried out as well as irrigated with above different concentrations of pulp. Ten seeds of wheat, corn and bean separately sown in pots with mix of garden fertile soil and 0%, 20%, 40%, 60%, 80% and 100% pulp with five replications. Pots irrigated with free of heavy metals. Dimension of pots were 25cm height 30cm diagonal. After 60 days growing stage morpho-physiological traits and heavy metals accumulations were measured in roots and aerial organs of seedlings.

Atomic absorption spectrometry

Plant samples at each experiment were ground. For removing the organic materials and other non-metallic substances, samples pulverized for 24h in a furnace at temperature were 600°C. Then immediately after removal of charred on the grill, giving them diluted solution of nitric acid were added. The possible reactions prevent the evaporation of substances in samples. After that, dilute solution of sulfuric acid was added. The resulting solution, was poured on balloons and then bring the volume, the experiments were poured glass and were ready for testing atomic absorption spectrometry. Standard solutions for measuring heavy metals of copper, iron, lead, cadmium, nickel, chromium and manganese be prepared and devices to be calibrated.

Statistical analysis

Analysis of variance for traits which was recorded under green house conditions was done separately for each experiment irrigated and pulpy soil with Mstat-C soft-war. Means were compared with Duncan's multiple range tests at 0.05 probability level. Also, t-student test was used for comparing two experiments. Stress tolerance indices were calculated as below formulas:

$GMP=\sqrt{(Ys)(Yp)}$, Fernandez[5],

$SSI=1-[Ys-Yp]/SI$ and $SI=1-[Ys/Yp]$, Fischer and Maurer[6],

$STI=(Ys)(Yp)/(Yp)^2$, Fernandez[5],

$TOL=Yp-Ys$, Rosielle and Hamblin[19],

$HAR=2(Yp.Ys)/(Ys+Yp)$, Kristin *et al*[15].,

$MP=(Yp+Ys)/2$, Rosiele and Hamblin[19],

Yp and Ys were aerial organ weight at control and stress conditions, respectively.

RESULTS AND DISCUSSION

Results analysis of variance for 60 days seedlings of wheat, corn and bean with different concentrations of irrigated and soil pulps experiments showed that there were significant differences for traits of root length, root weight, aerial organs weight, stem diameter, leaf area, stem length and leaf number.

Wheat

Root length of wheat at irrigated with 20% pulp (16.9cm) had the minimum value and for concentrations of 40%, 60% and 80% were the same group (Table 2). Root weight at irrigated with 40% pulp was the highest amount (90.4mg). Low roots weight of wheat seedling at control treatment that had the highest root length was due to the low density roots. It seems that concentrations of pulp stimulate root growth of wheat seedlings. The affect of pulp on root growth was more effective than aerial organs. The lowest amount of stem diameter was in 20% treatment (1.0mm). Leaf area reduced with increasing pulp percentage due to irrigation with different concentrations of dissolved metals which seedlings faced under stress. Trend of changes was similar for aerial organs length, weight of aerial organs and stem diameter at different pulp concentrations. Studies report that wheat accumulates heavy metals in their tissues at higher concentrations and therefore reduce its growth and development[12].

At different concentrations of pulpy soil experiment all traits at control treatment had the highest values (Table 2). Changes trend in root length and root weight is reverse which means that the effect of different concentrations of pulp on weight of seedling roots far more than the length of its roots. In terms of aerial organs weight of wheat that grown in soil with different concentrations of pulp was classified in three a, b and c categories. It is obvious that variation trend decreased in aerial organs weight and stem diameter. Although this process varies more sharply in the weight of aerial organs. Decreasing of aerial organs was at all concentrations of heavy metals also reported by Karunyal *et al*[13].

Table 2: Mean comparisons of wheat traits at different concentrations of irrigated and soil pulps

Treatment		Root length (cm)	Root weight (mg)	Aerial weight (mg)	Stem diameter (mm)	Leaf area (cm^2)	Stem length (cm)	Leaf number
Concentration of irrigated pulp (%)	Control	33.0^{a}	24.4^{c}	233.0^{a}	1.8^{a}	7.6^{a}	26.7^{a}	2.8^{a}
	20	16.9^{c}	71.8^{b}	136.0^{b}	1.0^{c}	6.7^{a}	22.3^{b}	2.0^{b}
	40	19.1^{bc}	90.4^{a}	150.0^{b}	1.1^{bc}	6.3^{bc}	23.2^{b}	1.8^{b}
	60	19.7^{bc}	76.4^{ab}	146.0^{b}	1.1^{bc}	5.0^{bc}	23.5^{b}	2.0^{b}
	80	20.6^{bc}	65.2^{b}	136.0^{b}	1.2^{b}	4.6^{c}	22.7^{b}	2.0^{b}
	100	22.0^{b}	67.2^{b}	150.0^{b}	1.2^{b}	6.4^{ab}	22.7^{b}	2.0^{b}
Concentration of Soil pulp (%)	Control	33.0^{a}	24.4^{b}	237.0^{a}	1.8^{a}	7.6^{a}	26.7^{a}	3.0^{ab}
	20	26.6^{b}	87.4^{a}	211.6^{b}	1.6^{ab}	8.4^{a}	26.2^{a}	3.4^{a}
	40	26.8^{b}	86.2^{a}	132.0^{c}	1.4^{bc}	5.4^{b}	22.9^{ab}	3.0^{ab}
	60	27.6^{b}	85.8^{a}	136.4^{c}	1.4^{bc}	5.0^{b}	21.4^{b}	2.6^{bc}
	80	26.5^{b}	87.0^{a}	136.4^{c}	1.4^{bc}	5.9^{b}	21.9^{b}	2.2^{c}
	100	25.3^{b}	88.6^{a}	139.2^{c}	1.2^{c}	5.7^{b}	21.7^{b}	2.0^{c}
Means with the same letter(s) don't have significant differences with multiple range test at 0.05 probability level.								

Bean

Root length declined with increasing metal stress (Table 3). Root weight at control treatment was the maximum value (1238mg) and at 20 and 40% pulp irrigation located in the second group (1062 and 1022mg, respectively). At 20% pulp irrigation aerial organs weight was the maximum value (2372mg). Root weight has negative relations with aerial organs weight. Stem diameter of all concentrations had the lower values than control. Leaf area continued downward trend up to irrigated with 60% pulp (12.3cm^2) and then had ascending trend due to compatible versus concentrations. Changes trend in aerial organs weight and length were almost identical and these traits in 20% pulp water irrigation had suddenly increased and then was almost constant at higher amounts of pulp concentrations.

Maximum root length of bean was for treatment which grown in soil with 40% concentration of pulp (28.5cm) (Table 3). Changes in root length are greater than its weight. Control and soil with 40% concentration pulp in aerial organs weight were the most and least values 2026.2mg and 1256.2mg, respectively. Stem diameter of treatments were located in three groups. Changes trend in stem diameter were less than aerial organs weight. Heavy metals in crops cause to generate toxic free radicals *i.e.* reactive oxygen species (ROS) produce oxidative stress[4]. ROS react with biomolecules like lipids, proteins, pigments, nucleic acid cause lipid peroxidation, harm to cell membranes and inhibit enzymatic activity resulting in disruption of cell functioning and finally reduce or inhibit plant growth and development as shown in our experiments.

Table 3: Mean comparisons of bean traits at different concentrations of irrigated and soil pulps

Treatment		Root length (cm)	Root weight (mg)	Aerial weight (mg)	Stem diameter (mm)	Leaf area (cm^2)	Stem length (cm)	Leaf number
Concentration of irrigated pulp (%)	Control	26.1^{a}	1238.0^{a}	1912.0^{bc}	5.0^{a}	28.1^{a}	25.0^{d}	4.2^{ab}
	20	22.0^{ab}	1062.0^{ab}	2372.0^{a}	4.1^{c}	22.0^{b}	43.3^{a}	4.6^{ab}
	40	21.3^{b}	1022.0^{ab}	$21.28.0^{ab}$	4.2^{bc}	18.8^{bc}	42.7^{ab}	4.8^{a}
	60	18.1^{b}	860.0^{b}	1944.0^{bc}	4.4^{bc}	12.3^{e}	42.5^{ab}	4.4^{ab}
	80	18.9^{bc}	896.0^{b}	1864.0^{bc}	4.3^{bc}	13.9^{de}	37.9^{bc}	4.2^{ab}
	100	16.4^{c}	914.0^{b}	1838.0^{c}	4.5^{b}	17.0^{cd}	34.9^{c}	4.0^{b}
Concentration of Soil pulp (%)	Control	26.3^{bc}	1236.0^{a}	2026.2^{a}	5.2^{b}	25.7^{ab}	26.0^{a}	4.6^{a}
	20	25.6^{bc}	890.2^{b}	1418.2^{c}	4.3^{c}	23.5^{c}	22.9^{b}	4.4^{a}
	40	28.5^{a}	734.6^{d}	1256.2^{e}	4.1^{c}	26.1^{a}	21.4^{c}	4.6^{a}
	60	27.2^{ab}	907.8^{b}	1367.6^{d}	5.6^{a}	25.2^{ab}	20.1^{d}	4.4^{a}
	80	26.1^{bc}	849.6^{c}	1412.8^{c}	5.1^{b}	22.4^{d}	20.2^{d}	3.4^{b}
	100	25.0^{c}	856.2^{c}	1732.0^{b}	4.4^{c}	24.7^{b}	19.8^{d}	3.0^{b}
Means with the same letter(s) don't have significant differences with multiple range tests at 0.05 probability level.								

Corn

Root length and weight of corn reduced at all concentrations of irrigated water pulp in comparison control treatment (Table 4). Also, leaf area and aerial organs weight at control treatment located in the first group and other treatments were in the second group. Stem diameter was maximum value (2.5mm) at control treatment and 20% pulp irrigation had the minimum value (1.0mm). Changes trend was similar in aerial organs length and weight therefore seedlings after adapting to environmental conditions had an upward trend with a very slight slope. The maximum leaf number of seedlings was related to control treatment (4.4) and the minimum value allocated to 20% and 40% pulp irrigation (2.2). A sudden drop was shown in leaf number of seedlings at transition from control to irrigate with 20% pulp. This process caused by metal stress that with passing time and increasing concentration, crop achieved its compatibility to environmental conditions again.

In pulpy soil experiment, except of stem diameter, all traits at control treatments were the highest amount. In 20% pulpy soil had maximum value (3.3mm) for stem diameter and then its changes almost constant at different concentrations. In oil crop of *Eruca sativa* only Ni at higher concentrations (≥1mM) significantly decreased seed germination. Heavy metals of Cu, Hg, Cr, Pb and Cd decreased the root length at first and then shoot length, fresh seedling weight and seed germination was always the last to be influenced[25]. Also in our experiments some traits more affected and others less.

Table 4: Mean comparisons of corn traits at different concentrations of irrigated and soil pulps

Table 4- Mean comparisons of corn traits at different concentrations of irrigated and soil pulps.								
Treatment		Root length (cm)	Root weight (mg)	Aerial weight (mg)	Stem diameter (mm)	Leaf area (cm^2)	Stem length (cm)	Leaf number
Concentration of irrigated pulp (%)	Control	32.0^{a}	483.6^{a}	663.0^{a}	2.5^{a}	25.8^{a}	34.8^{a}	4.4^{a}
	20	19.7^{b}	296.0^{b}	448.0^{b}	1.0^{c}	9.7^{b}	19.8^{c}	2.2^{c}
	40	20.2^{b}	302.0^{b}	484.0^{ab}	1.1^{bc}	10.0^{b}	19.7^{c}	2.2^{c}
	60	22.4^{b}	316.0^{b}	480.0^{ab}	1.1^{bc}	10.8^{b}	24.2^{bc}	2.8^{b}
	80	22.8^{b}	342.0^{ab}	442.0^{b}	1.2^{b}	12.3^{b}	29.3^{ab}	2.6^{bc}
	100	22.4^{b}	376.0^{ab}	454.0^{b}	1.2^{b}	12.2^{b}	28.3^{ac}	3.0^{b}
Concentration of Soil pulp (%)	Control	32.2^{a}	483.6^{a}	624.4^{a}	2.5^{b}	25.8^{ab}	36.0^{a}	4.6^{a}
	20	30.7^{ab}	400.8^{b}	478.0^{bc}	3.3^{a}	27.4^{a}	34.2^{b}	4.8^{a}
	40	27.2^{bc}	416.2^{ab}	475.4^{bc}	2.9^{ab}	21.1^{c}	28.7^{c}	3.8^{b}
	60	26.2^{bc}	411.0^{ab}	504.0^{b}	2.9^{ab}	19.8^{bc}	26.8^{d}	3.6^{b}
	80	25.8^{bc}	374.4^{b}	439.2^{d}	2.5^{b}	22.3^{bc}	26.7^{d}	3.6^{b}
	100	224.9^{c}	383.6^{b}	454.0^{cd}	2.5^{b}	23.6^{ac}	25.5^{d}	3.8^{b}
Means with the same letter(s) don't have significant differences with multiple range tests at 0.05 probability level.								

Plant growth conditions

Comparisons of plant growth conditions showed that there was statistically significant difference between aerial organs weight of bean seedlings (Table 5). Means of bean seedlings grown in irrigated pulp (2009.7mg) was more than pulpy soil (1535mg). Seedlings grown in the fertile soil which irrigated with different concentrations of pulp had less adverse effect on growth of bean. Therefore fertile soil eliminates bad effects of irrigated pulps.

Table 5: Mean comparisons of aerial organs weight of wheat, corn and bean under different growth conditions

Growth conditions	Wheat (mg)	Corn (mg)	Bean (mg)
Irrigated pulp experiment	158.5	495.2	2009.7
Pulpy soil experiment	165.4	495.8	1535.5
t-value	-0.6188^{ns}	-0.0221^{ns}	0.9903**
Probability level	0.5385	0.9824	0.000
ns and **: not significant and significant at 0.05 probability levels, respectively.			

Stress tolerance indices

Whatever amounts of STI, GMP, MP and HAR were more; therefore seedlings would be tolerated to heavy metal stress. In contrast if TOL value was low that indicated high tolerance of seedlings.

Bean 60days seedlings according to the amount of five indices of STI, GMP, TOL, MP and HAR were tolerant in all concentrations of irrigated water pulp (Table 6). Also, this crop grown in soil with different concentrations of pulp had the highest values for three indices and tolerant. Negative values of TOL for some concentrations showed that heavy metals have been improved performance of bean. Then metal stress of irrigated water containing pulp was very convenient to grow bean. Also wheat seedlings based on indices of STI, GMP, MP and HAR were identified as the most sensitive to irrigation with five different concentrations of pulp. Reports represented that wheat at seedling and germination growth stage was susceptible to environmental influences including heavy metals[10]. The corn plant had highest amount in the different concentrations just based on TOL index. Zhi *et al*[25]., in their experiment indicated that Eruca oil crop is tolerant to Cu, Hg, Cr, Cd and highly tolerant to Pb, Ni, Zn and can be developed as an industrial oil crop for phytoremediation of soils contaminated by heavy metals.

Bean crop as ideal plant for phytoremediation had the ability to accumulate high heavy metals content, tolerate high salt concentration, having fast growth rate, higher biomass production, easily harvestable from roots and translocation metals to their above ground parts efficiently.

Table 6: Tolerance indices of wheat, bean and corn under different concentrations of irrigated and soil pulps

concentration		STI		GMP		TOL		MP		HAR	
		Irrigated	Soil	Irrigated	Soil	Irrigated	Soil	Irrigated	Soil	Irrigated	Soil
Wheat	20	0.58	0.89	178	223	97	25	184	224	171	223
	40	0.64	0.55	186	176	83	105	191	184	182	169
	60	0.62	0.57	184	179	87	100	189	186	179	173
	80	0.58	0.57	178	179	97	100	184	186	171	173
	100	0.64	0.58	186	181	83	97	191	188	182	175
Bean	20	1.24	0.69	2129	1695	- 460	608	2142	1722	2117	1668
	40	1.11	0.61	2017	1595	- 216	770	2020	1641	2014	1550
	60	1.01	0.67	1927	1664	- 32	658	1928	1696	1927	1632
	80	0.97	0.69	1887	1691	48	613	1888	1719	1887	1664
	100	0.96	0.85	1874	1873	71	294	1875	1879	1874	1867
Corn	20	0.67	0.76	544	546	215	146	555	551	534	541
	40	0.73	0.76	566	544	179	149	573	549	559	539
	60	0.72	0.81	564	560	183	120	571	564	556	557
	80	0.66	0.70	541	523	221	185	552	531	530	515
	100	0.68	0.72	548	532	209	170	558	539	538	525

Nickel accumulation

Nickel accumulation in the roots of each wheat, corn and bean seedlings were more than aerial organs (Table 7). Wheat seedlings had the maximum accumulation of nickel at the root which irrigated with water containing 40% pulp (19mg). Also corn at this concentration showed the highest amount in the roots (16mg). But bean seedlings at irrigated pulp 20% were allocated the maximum accumulation (16mg). Nickel accumulation in aerial organs wheat, bean and corn with different concentrations of irrigated pulp were not statistically significant differences. It seems that these seedlings avoid accumulation of nickel in aerial organs. Sivakumar *et al*[22]., in removing heavy metals of tannery waste polluted soil reported that roots of *Hyptis suaveolens* accumulated more heavy metals than stem and leaves. This Result is similar with our experiments.

In the pulpy soil experiment, the maximum amount of nickel accumulation in the roots of wheat seedlings that grown in soil with 20% of pulp (18mg) and bean which grown in soil with 40% of pulp (18mg) were located in the similar statistical category. At all concentrations of pulp soil aerial organs of bean accumulated four fold of nickel than other crops. Therefore, for decreasing adverse effect of toxic nickel it is recommended sown bean at this areas. Bean by absorbing excessive nickel and store it in their vacuoles has increased own tolerance toward wheat and corn; but this trend was not observed in the wheat and corn at both stages growth conditions. Corn and wheat seedlings by preventing absorption of ions increased tolerance to adverse environmental conditions.

Lead accumulation

Maximum Lead accumulation observed in wheat roots at irrigated pulp 80% (20.4mg) (Table 7). In corn and bean seedlings maximum amounts with 16.3mg and 14.5mg were allocated at irrigated pulp 20% and 40%, respectively. Trend of accumulation in corn and wheat roots were almost identical. So that in irrigated with 20% pulp, a sudden increasing occurred and then drop was shown in the concentrations of 40% and 60%. Again at 80% and 100% has happened an increasing. It seems that the process of lead accumulation in bean seedlings was different.

Also, lead accumulation in aerial organs of wheat and corn increased with amplification of lead in the roots. Wheat seedling grown in 60% pulpy soil had the maximum lead accumulation in roots (14.9mg). Regardless of plant type and different concentrations of pulp, the accumulation of lead in experiment of irrigated pulp was higher than pulpy soil and changes range of it in irrigated pulp experiment was 20% more than pulp soil experiment. Lead accumulation in aerial organs was similar at both experiments.

Cadmium accumulation

The highest amounts of cadmium accumulations were shown in roots of wheat which irrigated 40% and 60% pulps 6.3mg and 6.6mg, respectively (Table 7). The maximum amount of cadmium accumulation in bean and corn were happened at the roots which irrigated 40% pulp 2.5mg and 5.6mg. Accumulation values of aerial organs of wheat and corn were more than bean. Maximum values of cadmium accumulations in aerial organs of wheat and corn seedlings were related to treatments which has the highest amounts of cadmium accumulation in their own roots.

Roots and aerial organs of wheat seedling which were grown in soil with 20% of pulp had the maximum values of the cadmium accumulations, 13.2mg and 10.8mg respectively. Other treatments had the same values of accumulations in roots and aerial organs. Also, Adiloglu[2], with Canola crop the same of our method removed cobalt from agricultural Tekirdag country soils of Turkey.

Table 7: Nickel, lead and cadmium accumulations in root and aerial organs of wheat, bean and corn under different pulp concentrations of irrigated and soil

Concentration (%)		Nickel accumulation (mg)				Lead accumulation (mg)				Cadmium accumulation (mg)			
		Irrigated pulp		Pulp soil		Irrigated pulp		Pulp soil		Irrigated pulp		Pulp soil	
		Root	Aerial organ	Root	Aerial organ	Root	Aerial organ	Root	Aerial organ	Root	Aerial organ	Root	Aerial organ
Wheat	control	10^{de}	0.8^{h}	10^{d}	0.8^{op}	0.9^{hl}	0.4^{l}	0.9^{m}	0.8^{m}	0.2^{fg}	0.2^{c}	1.4^{d}	0.1^{d}
	20	16^{ac}	0.7^{h}	18^{a}	0.2^{p}	18.4^{bd}	2.8^{il}	6.6^{eg}	0.3^{m}	1.1^{fg}	0.3^{c}	13.2^{a}	10.8^{ab}
	40	19^{a}	1.1^{gh}	12^{cd}	2.5^{lp}	0.5^{hl}	0.4^{l}	7.9^{e}	0.5^{m}	6.3^{c}	1.1^{fg}	8.4^{bc}	5.9^{c}
	60	17^{ab}	0.7^{h}	7^{ef}	1.3^{mp}	6.7^{gk}	0.4^{l}	14.9^{bc}	5.2^{fi}	6.6^{c}	4.1^{d}	0.1^{d}	0.6^{d}
	80	15^{ab}	1.3^{gh}	6^{ef}	2.7^{lp}	20.4^{a}	8.5^{fi}	5.4^{fh}	3.0^{il}	1.9^{eg}	1.2^{fg}	0.1^{d}	0.6^{d}
	100	17^{ab}	0.9^{h}	11^{cd}	5.8^{fi}	8.5^{fi}	3.5^{il}	10.4^{cd}	6.9^{eg}	0.7^{1}	2.0^{eg}	0.1^{d}	0.1^{d}
Bean	control	13^{cd}	3.8^{fh}	2^{jn}	1.5^{mp}	1.6^{hl}	1.1^{il}	2.3^{hk}	1.3^{km}	0.2^{fg}	0.1^{g}	0.1^{d}	0.1^{d}
	20	16^{ac}	4.1^{fh}	11^{cd}	8.0^{ef}	13.7^{df}	2.5^{jl}	7.1^{eg}	4.9^{gj}	1.0^{fg}	0.2^{g}	0.3^{d}	0.2^{d}
	40	14^{bd}	4.2^{fh}	18^{a}	5.5^{gj}	14.5^{ce}	2.8^{il}	15.7^{b}	3.5^{hk}	2.5^{df}	0.3^{g}	0.3^{d}	0.2^{d}
	60	12^{cd}	4.4^{fh}	13^{cd}	6.9^{eh}	11.4^{eg}	4.2^{hl}	7.6^{ef}	1.6^{km}	1.4^{fg}	0.6^{g}	0.4^{d}	0.4^{d}
	80	11^{ce}	5.0^{fh}	12^{cd}	3.0^{ko}	9.6^{eh}	3.1^{il}	11.9^{d}	3.6^{hk}	1.9^{eg}	$1.0f^{g}$	0.6^{d}	0.5^{d}
	100	10^{de}	2.5^{gh}	7^{eh}	3.4^{jm}	7.1^{gk}	1.2^{kl}	2.8^{jl}	2.3^{km}	0.6^{g}	0.5^{g}	0.1^{d}	0.5^{d}
Corn	control	1^{gh}	0.4^{h}	2^{jn}	0.4^{p}	1.3^{hl}	0.7^{l}	1.7^{km}	0.6^{m}	0.4^{g}	0.1^{g}	0.1^{d}	0.1^{d}
	20	10^{de}	1.4^{gh}	15^{b}	0.9^{op}	16.3^{cd}	1.4^{kl}	13.2^{cd}	0.6^{m}	0.8^{fg}	0.2^{g}	2.4^{d}	0.7^{d}
	40	16^{ac}	1.4^{gh}	13^{bc}	1.1^{np}	4.7^{hl}	0.9^{il}	14.8^{bc}	1.3^{km}	5.6^{cd}	3.7^{de}	1.6^{d}	1.1^{d}
	60	7^{ef}	1.3^{gh}	5^{gk}	0.5^{p}	1.9^{hl}	1.3^{il}	6.2^{eg}	2.1^{km}	4.2^{d}	1.8^{eg}	1.3^{d}	1.3^{d}
	80	7^{ef}	1.2^{gh}	7^{ef}	1.5^{mp}	9.9^{eh}	2.9^{eh}	8.1^{e}	1.3^{km}	0.7^{fg}	0.5^{g}	0.1^{d}	1.6^{d}
	100	3^{fh}	0.9^{h}	8^{ef}	0.9^{op}	7.3^{gi}	2.3^{jl}	5.2^{fi}	1.1^{km}	1.5^{fg}	2.9^{df}	0.1^{d}	0.1^{d}

Means with the same letter(s) don’t have significant differences with multiple range tests at 0.05 probability level.

Chromium accumulation

Chromium accumulation of seedlings which irrigated with different concentrations of pulp had roots of wheat, corn and bean maximum values than aerial organs (Table 8). The highest accumulation was shown in roots of wheat with irrigated 80% pulp (9.6mg) and treatments of 20%, 60% and 100% placed in second group. Roots of corn and bean irrigated with water 40% pulp had the highest values 8.3mg and 9.5mg, respectively. Al-Anbari *et al*[3]., reported that accumulation of chromium was high in roots of *Catharanthus roseus* than stem of it.

The maximum amount of chromium accumulation was observed in roots of wheat and corn that were grown in soil containing 20% pulp 7.6mg and 5.1mg, respectively. Also, trend of accumulation in roots of bean seedling has been constant at different concentrations. Amounts of chromium accumulations in roots of wheat, corn and bean in experiment which irrigated with different concentrations of pulp were more than pulp soil experiment. Al-Anbari *et al*[3]., used *Catharanthus roseus* for phytoremediating of chromium contaminated Iraqi soils. Chromium removal amounts was in August (93%), July (33%), June (12%) and May (7%) and they suggested that this plant has been effective for removing this metal from soil irrigated by waste water.

Manganese accumulation

Manganese accumulation in roots and aerial organs of wheat, corn and beans in seedlings irrigated with different concentrations of pulp were more than 2 to 4 fold than nickel, chromium, cadmium, and lead (Table 8). At all treatments amount of manganese accumulation in roots of wheat and corn which irrigated with 80% pulp was the highest values 178.0mg and 182.1mg, respectively. The highest amount of accumulation with in aerial organs was observed in corn seedlings. Trend of manganese accumulation in the roots of corn and wheat was similar. So that at the beginning with a gentle slope increases and then in irrigated 100% pulp has fallen eventually. It seems that wheat and corn seedlings had to adapt as a same method for environmental conditions. This trend was reverse for bean seedlings.

In pulpy soil experiment roots of wheat, corn and bean had more manganese accumulation than aerial organs. The maximum amounts were allocated to roots of wheat, corn and bean seedlings in soil containing 40% pulp with 276.6mg, 262.5mg and 286.9mg, respectively. The highest manganese accumulation levels were observed in aerial organs of wheat and corn grown in soil containing 40% pulp.

Copper accumulation

The copper accumulation in the roots of all wheat, corn and bean treatments was more than their aerial organs (Table 8). It is interesting that most of copper accumulation for each plant was shown in the treatments which were cultivated in the soil pulp. Also, in wheat roots it is prominent. Maximum values were allocated to root wheat with containing 60% pulp of irrigated water 91.8mg and soil 100% pulp 357.5mg.

Bean had high tolerance to metal stresses due to had more aerial organ weight than two other plants of wheat and corn.

Heavy metal up-taking in crops and transporting of it in soil profile and plant also was studied by Mehmandoost-Kotlar *et al*[16]. They demonstrated that the medium and strong waste water were unable to contaminate the cucumber fruits. However, despite a considerable amount of absorption by roots at first days of growth, heavy metal concentration decreased significantly in the last days of sampling. Therefore it seems that heavy metals were in low traces in economic organs and it could be consumed by human.

Table 8: *Chromium*, manganese and copper accumulations in root and aerial organs of wheat, bean and corn under different pulp concentrations of irrigated and soil

Concentration (%)		Chromium accumulation (mg)				Manganese accumulation (mg)				Copper accumulation (mg)			
		Irrigated pulp		Pulp soil		Irrigated pulp		Pulp soil		Irrigated pulp		Pulp soil	
		Root	Aerial organ	Root	Aerial organ	Root	Aerial organ	Root	Aerial organ	Root	Aerial organ	Root	Aerial organ
Wheat	control	1.3^{jk}	0.1^{jk}	0.5^{kl}	0.2^{l}	18.8^{p}	17.2^{p}	9.5^{r}	7.3^{r}	17.0^{no}	9.4^{o}	16.6^{o}	11.9^{o}
	20	8.8^{bd}	0.4^{jk}	7.6^{a}	0.3^{l}	129.1^{ef}	116.0	179.4^{g}	68.1^{oq}	72.0^{c}	37.1^{fl}	128.6^{hi}	27.7^{mo}
	40	4.7^{g}	1.0^{ik}	3.6^{e}	0.4^{kl}	132.4^{df}	81.6^{lm}	276.6^{b}	132.9^{hi}	75.5^{c}	28.9^{hn}	177.1^{fg}	38.5^{ko}
	60	8.4^{be}	1.2^{ik}	3.2^{ef}	0.8^{kl}	151.8^{bc}	75.3^{m}	188.9^{g}	78.9^{pq}	91.8^{ab}	27.6^{ho}	264.9^{bc}	64.4^{kl}
	80	9.6^{a}	1.6^{ik}	0.6^{kl}	0.5^{kl}	178.0^{a}	85.1^{km}	210.1^{de}	149.3^{h}	80.2^{bc}	25.4^{ko}	282.4^{b}	46.3^{ko}
	100	8.2^{be}	0.4^{k}	0.4^{kl}	0.4^{kl}	137.3^{ce}	108.7^{gj}	147.5^{h}	84.0^{mo}	78.4^{c}	24.6^{ko}	357.5^{a}	33.7^{lo}
Bean	control	1.0^{jk}	0.3^{jk}	1.1^{ik}	0.3^{l}	10.8^{p}	9.6^{p}	15.9^{r}	11.5^{r}	24.0^{lo}	14.2^{o}	22.8^{o}	16.3^{o}
	20	9.4^{b}	1.0^{jk}	3.3^{ef}	0.3l	151.6^{bc}	30.8^{op}	150.4^{h}	50.2^{qr}	46.6^{df}	23.5l^{o}	110.7^{i}	31.8^{lo}
	40	9.5^{b}	0.9^{jk}	3.7^{e}	0.5^{kl}	129.5^{ef}	31.7^{op}	286.9^{a}	54.3^{oq}	44.2^{dg}	30.2^{gn}	178.5^{fg}	30.2^{lo}
	60	8.5^{bd}	0.9^{jk}	2.6^{h}	2.4^{h}	39.5^{op}	44.7^{no}	198.8^{ef}	91.1^{ln}	45.4^{df}	30.3^{gn}	218.0^{de}	57.3^{km}
	80	8.5^{bd}	0.9^{jk}	3.5^{ef}	0.5^{kl}	124.4^{eh}	39.5^{no}	173.7^{g}	60.3^{pq}	46.6^{df}	34.9^{fm}	235.1^{c}	34.1^{lo}
	100	8.3^{bd}	0.7^{jk}	3.9^{ef}	1.5^{ik}	149.6^{bd}	36.4^{o}	157.6^{h}	66.8^{oq}	40.4^{ei}	21.3^{mo}	247.9^{cd}	45.1^{ko}
Corn	control	1.0^{jk}	0.1^{k}	0.4^{kl}	0.3^{l}	9.5^{p}	8.7^{p}	9.1^{r}	5.4	22.1^{lo}	10.0^{o}	18.0^{o}	15.0^{o}
	20	3.3^{gi}	0.2^{k}	5.1^{b}	0.4^{kl}	126.4^{eg}	14.8^{p}	109.6^{k}	88.5^{ln}	37.1^{fl}	13.7^{o}	118.0^{i}	59.5^{km}
	40	8.3^{be}	0.3^{jk}	3.4^{ef}	0.5^{kl}	160.6^{b}	119.0^{ei}	262.5^{bc}	221.6^{d}	47.0^{df}	15.5^{no}	129.9^{i}	25.7^{mo}
	60	4.0^{fg}	0.2^{k}	2.3^{gh}	0.5^{kl}	159.0^{b}	126.6^{eg}	248.4^{a}	220.7^{d}	41.7^{eh}	25.7^{io}	154.7^{gh}	69.7^{jk}
	80	7.1^{de}	0.7^{jk}	2.1^{h}	0.7^{jl}	182.1^{a}	137.0^{ce}	104.2^{kl}	98.3^{jk}	48.2^{df}	21.7^{mo}	228.6^{ce}	43.2k^{o}
	100	4.9^{fg}	0.2^{k}	2.0^{h}	0.6^{kl}	155.9^{bc}	100.6^{ik}	117.0^{ik}	101.0^{jk}	39.5^{ej}	16.5^{no}	250.9^{c}	17.6^{o}
Means with the same letter(s) don’t have significant differences with multiple range tests at 0.05 probability level.													

Variation percentage

Within plants bean had the most accumulations of lead, chromium and copper and wheat with high values of cadmium and manganese also, nickel more allocated to corn (Table 9). The success of heavy metal remediation process depends on those plants which can accumulates desired levels of metal concentration in their aerial organs (100-1000 folds) without any visible symptoms. Therefore in our selected crops, observed amount of accumulations were in many hundred folds and termed as hyperaccumulators crops. Different researchers reported that the indigenous hyperaccumulator plants could reduce the migration of heavy metals through the soil medium, also known as the phytostabilisation mechanism[18]. In another study by Hamzah *et al*[8]., also reported the potential of using indigenous hyperaccumulator plants to stabilize heavy metals and remediate contaminated soil to be used as agricultural soil again.

Table 9: Variation percentage of wheat, corn and bean seedling under metal stress conditions

Metal	Wheat	Corn	Bean
Nickel	212.71	280.06	219.76
Lead	418.94	320.88	440.74
Cadmium	483.42	410.06	359.48
Chromium	261.00	335.30	440.66
Manganese	499.24	431.46	357.88
Copper	320.11	418.77	473.43
Variation percentage=(mean under stress-mean non stress)/mean non stress			

CONCLUSION

A new method is presented for purification concentration of effluents and decreases of environmental damages with using of phytoremediation processes. Bean, wheat and corn have ability for absorb and store of heavy metals. Copper accumulation in pulpy soil experiment at both roots and aerial organs was more than irrigated pulp experiment in three seedlings of wheat, corn and bean. Bean seedlings had more ion accumulation in aerial organs than other seedling plants. It is suggested that bean was cultivated for reducing destructive environmental impacts of copper in Sungun mine due to had high values of accumulation.

Many new hyperaccumulator crops that have remediate abilities need to be discovered and identified, especially the plants that can contribute to social and economic development of local population, such as industrial species. The understanding of plants regarding metal uptake process and proper aerial organs disposal is still to be unveiled. Further, research is required to develop the plants with high growth rate, high total dry matter, improved heavy metal uptake, translocation and tolerance by using genetic engineering for the effective phytoremediation. The complexity of factors that control the efficiency of this technique, such as soil properties, plant species and climatic conditions, fact that more researchers need to be conducted.

Acknowledgment

Finance of this article supported by industrial university of Urmia, Iran.

REFERENCES

1. Abiyarifard, M., Phytoremediation and effects of chromium and silicon on triticale yield and growth. *Scinzer Journal of Agricultural and Biological Sciences*, **2(1):** 6-9 (2016).
2. Adiloğlu, S., Using phytoremediation with canola to remove cobalt from agricultural soils. *Polish Journal of Environmental Studies*, **25(6):** 2251-2254 (2016).
3. Al-Anbari, R., Al-Obaidy, A.H.M., and Al-Amari, T.J., Phytoremediation of chromium contaminated soils by using *Catharanthus roseus*. Mesopotamia

Environmental Journal. **2(4):** 32-40 (2016).

4. Dixit, V., Pandey, V. and Shyam, R., Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum Sativum*). *Journal of Experimental Botany*, **52:** 1101-1109 (2001).
5. Fernandez, G.C.J., Effective selection criteria for assessing plant stress tolerance. Proceeding of the 6th Symposium of Crop Science, Jun 17-19, Taiwan. p. 257 (1992).
6. Fischer, R.A. and Maurer, R., Drought resistance in spring wheat cultivars: I. Grain yield responses. *Australian Journal of Agricultural Research*, **29:** 897-912 (1978).
7. Gouda, A.H., El-Gendy, A.S., El-Razek, T.M.A., and El-Kassas, H.I., Evaluation of phytoremediation and bioremediation for sandy soil contaminated with petroleum hydrocarbons. *International Journal of Environmental Science and Development*, **7(7):** 490 493 (2016).
8. Hamzah, A., Kusuma, Z., Utomo, W.H., and Guritno, B., Siam weed (*Chromolaena odorata* L.) for phytoremediation of artisanal gold mine tailings. *Journal of Tropical Agriculture*, **50:** 1-2 (2012).
9. Hamzah, A., Hapsari, R.I. and Wisnubroto, E.I., Phytoremediation of cadmium-contaminated agricultural land using indigenous plants. *International Journal of Environmental and Agriculture Research*, **2:** 8-14 (2016).
10. Huang, J. W.W., Chen, J.J., Berti, W.R. and Chunningham, S.D., Phytoremediation of lead contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environmental Science Technology*, **31:** 800-805 (1997).
11. Kanwal, S., Bano, A. and Malik, R.N., Effects of *arbuscular mycorrhizal* fungi on wheat growth, physiology, nutrition and cadmium uptake under increasing cadmium stress. *International Journal of Agronomy and Agricultural Research*, **7:** 30-42 (2012).
12. Kanwal, S., Bano, A. and Malik, R.N., Role of *arbuscular mycorrhizal* fungi in phytoremediation of heavy metals and effects on growth and biochemical activities of wheat (*Triticum aestivum* L.) plants in Zn contaminated soils. *African Journal of Biotechnology*, **15(20):** 872-883 (2016).
13. Karunyal, S., Renuga, G. and Paliwal, K., Effects of tannery effluent on seed germination, leaf area, biomass and mineral content of some plants. *Bioresource Technology*, **47:** 215-218 (1994).
14. Khan, A., Sharif, M., Ali, A., Shah, S.N.M., Mian, I.A., Wahid, F., Jan, B., Adnan, M., Nawaz, S. and Ali, N., Potential of AM fungi in phytoremediation of heavy metals and effect on yield of wheat crop. *American Journal of Plant Sciences*, **5:** 1578-1586 (2014).
15. Kristin, A.S., Serna, R.R., Peraz, F.I., Enriques, B.C., Gallegos, J.A.A., Vallego, P.R., Wassimi, N. and Kelly, J.D., Improving common bean performance under drought stress. *Crop Science*, **37:** 43-50 (1997).
16. Mehmandoost Kotlar, A., Hashemi Monfared, S. A. and Azhdary Moghadam, M., Cadmium uptake by plant and transport in soil column under industrial Wastewater irrigation: A Case Study. *International Journal of Environmental Research*, **9(3):** 913-922 (2015).
17. Nayak, A.K., Jena, R.C., Jena, S., Bhol, R. and Patra, H.K., Phytoremediation of hexavalent chromium by *Triticum aestivum*. *Scientia Agriculturae*, **9(1):** 16-22 (2015).
18. Robinson, B., Banuelos G., Conesa, H.M., Evangelou, M.W.H. and Schulin, R., The phyomanagment of trace elements in soil. *Critical Reviews in Plant Science*, **28:** 240-266 (2009).
19. Rosielle, A.A., and Hamblin, J., Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Science*, **21:** 943-946 (1981).

20. Sharma, S., Rana, S., Thakkar, A., Baldi, A., Murthy, R.S.R. and Sharma, R.K., Physical, chemical and phytoremediation technique for removal of heavy metals. *Journal of Heavy Metal Toxicity and Diseases*, **1(2):** 1-15 (2016).
21. Singh, S. and Singh, A., Phytoremediation: a sustainable approach for restoration of metal contaminated sites. *International Journal of Science and Research*, **5:** 2171-2174 (2016).
22. Sivakumar, P., Kanagappan, M. and Das, S.S.M., Phytoremediation of tannery waste polluted soil using *Hyptis suaveolens*. *International Journal Pure Applied Bioscience,* **4(1):** 265-272 (2016).
23. Werle, S., Bisorca, D., Katelbach-Woźniak, A., Pogrzeba, M., Krzyżak, J., Ratman-Kłosińska, I. and Burnete, D., Phytoremediation as an effective method to remove heavy metals from contaminated area–TG/FT-IR analysis results of the gasification of heavy metal contaminated energy crops. *Journal of the Energy Institute*, **4:** 1-10 (2016).
24. Yasin, M., El-Mehdawi, A.F., Anwar, A., Pilon-Smits, E.A., and Faisal, M., Microbial-enhanced selenium and iron biofortification of wheat (*Triticum aestivum* L.)-applications in phytoremediation and biofortification. International Journal of phytoremediation. **17(4):** 341-347 (2015).
25. Zhi, Y., Deng, Z., Luo, M., Ding, W., Hu, Y., Deng, J., Li, Y., Zhao, Y., Zhang, X., Wu, W. and Huang, B., Influence of heavy metals on seed germination and early seedling growth in *Eruca sativa* Mill. *American Journal of Plant Sciences*, **6:** 582-590 (2015).