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Removal of Selected Heavy Metals from Pharmaceutical Effluent by *Aloe vera* L.

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ABSTRACT

Due to vast probable contaminations from pharmaceutical effluent, the main purposes of this study were to decrease the heavy metal toxic levels from waste water of pharmaceutical research and educational laboratories. The potential ability of *A. Vera* for cleaning up contaminated educational sites soil and the probable capability of this plant to phytoextract different metals (Chrome (III) and (VI) , Nickel, Copper, Lead and Cadmium) were studied. The initial concentration of heavy metals/metalloid in the plants and effluents were analyzed before introduction into the studied soil samples. After 10 days of treatment up to 60 days in every ten days, final concentration of heavy metals/metalloid in effluent samples and plants were analyzed using Atomic Absorption Spectroscopy. Results showed that *A. Vera* transition factors for all heavy metals in treated soil were higher than one and *A. Vera* can up-take lead and Cadmium after 20 days ($p<0.01$) more than other studied metals. A highly significant, although low, positive correlation ($r= 0.52$, $p=0.01$, $n=200$) was found between Lead and Cadmium and chrome (III) of the greenhouse –grown *A. Vera* , compared to a non-significant and much lower correlation between the three other heavy metals: Nickel, Cr(VI) and Copper . The phytoremediation of lead and Cadmium trend by this plant indicates that *A.vera* cultivated in the soil can be consider as a suitable hyper-accumulator by its relatively large ratio of biomass concentration of the contaminant to soil concentration. The present investigation shows that the *A. Vera* is effective and inexpensive adsorbent for the removal of Pb ,Cd, Ni, Cu, Cr (III) and (VI) from contaminated soil by heavy metals.

Key words: Phytoremediation**,** Heavy metals, *Aloe Vera L.*, Pharmaceutical effluent.

INTRODUCTION

Pharmaceuticals are a large and diverse group of compounds designed to prevent, cure, and treat disease, and improve health[1-2]. Significant fractions of the parent compound are excreted in un-metabolized form or as metabolites (active or inactive) into raw sewage and wastewater treatment systems. Municipal sewage treatment plant effluents are discharged to water bodies or reused for irrigation, and bio-solids produced are reused in agriculture as soil amendment or disposed to landfill. Pharmaceuticals enter the environment from a myriad of scattered points[3]. The main sources of contamination include pharmaceutical production plants, WWTPs, hospitals, landfills and even graveyards[4-5]. The most investigated route of entry of pharmaceuticals into the environment is that from municipal WWTPs. Human excretion of unchanged or slightly transformed Active pharmaceutical ingredients (APIs) conjugated to polar molecules such as glucoronide enters the WWTP where these conjugates may then be cleaved, releasing the original API into the environment[6]. Activated sludge WWTPs has received particular attention[7-8]. A limited number of studies also found pharmaceuticals in drinking water[9] and hospital wastewater[10]. Pharmaceutical wastewater streams containing heavy metals are produced from different laboratories such as Toxicology and Food Toxicology Labs. Some examinations and chemical test and processes generate significant quantities of wastewaters containing heavy metals (such as cadmium, zinc, lead, chromium, nickel, copper, silver and titanium) from a variety of analyses tests. Heavy metals cause serious health effects, including reduced growth and development, cancer, organ damage, nervous system damage, and in extreme cases, death. Exposure to some metals, such as mercury and lead, may also cause development of autoimmunity, in which a person's immune system attacks its own cells[11] .The conventional processes for removing heavy metals from wastewater include many processes such as chemical precipitation, flotation, adsorption, ion exchange, and electrochemical deposition. Chemical precipitation is the most widely used for heavy metal removal from inorganic effluent. Lime and limestone are the most commonly employed precipitant agents due to their availability and low-cost in most countries[12-13]. Lime precipitation can be employed to effectively treat inorganic effluent with a metal concentration of higher than 1000 mg/ L. Other advantages of using lime precipitation include the simplicity of the process, inexpensive equipment requirement, and convenient and safe operations. However, chemical precipitation requires a large amount of chemicals to reduce metals to an acceptable level for discharge. Other drawbacks are its excessive sludge production that requires further treatment, slow metal precipitation, poor settling, the aggregation of metal precipitates, and the long-term environmental impacts of sludge disposal[13]. Recently, adsorption has become one of the alternative treatment techniques for wastewater laden with heavy metals. The efficacy of various plants in eliminating different heavy metal contaminants, particularly Lead and Cadmium is a major concern nowadays due to the vast soil pollution in many countries around the world especially Iran[14-20]. Basically, adsorption is a mass transfer process by which a substance is transferred from the liquid phase to the surface of a solid, and becomes bound by physical and/ or chemical interactions[21]. Various low-cost adsorbents, derived from agricultural waste, industrial by-product, natural material, or modified biopolymers, have been recently developed and applied for the removal of heavy metals from metal-contaminated wastewater. In general, there are three main steps involved in pollutant sorption onto solid sorbent: (i) the transport of the pollutant from the bulk solution to the sorbent surface; (ii) adsorption on the particle surface; and (iii) transport within the sorbent particle. Technical applicability and cost-effectiveness are the key factors that play major roles in the selection of the most suitable adsorbent to treat inorganic effluent. Phytoremediation is environmental-friendly, cost-effective and natural green biotechnology for the removing xenobiotic, including toxic metals, from the environment using some species of the plants. The land in Iran is very fertile, providing favorable conditions for various medicinal plants to grow. Since ancient times, local people in every part of Iran have grown *Aloe Vera* in their home gardens, using the leaves of *A. Vera* to aid in healing wounds. *A. Vera* is cultivated in many places in Iran but not in wide range. About 90% of the plants are estimated to come from wild harvest [17, 22-23]. *A. Vera* has been on this earth for millennia and it is a member of the Liliaceae family. The plant Aloe *vera* Linne, also known as Aloe barbadensis Miller, and Aloe vulgaris Lamarck [24]. As *A. Vera* can easily grow in Iran climates and due to vast probable contaminations from pharmaceutical effluent , the main purposes of this study were to decrease the heavy metal toxic levels from waste water of pharmaceutical research and educational laboratories determine the potential ability of *A. Vera* for cleaning up contaminated educational sites soil and the probable capability of this plant to phytoextract different metals (Chrome , Nickel, Copper, Lead and Cadmium) and also determine metal transfer factors from soil (TFS) of *A. Vera* in order to ascertain its phytoremediation potential.

EXPERIMENTAL

Effluent samples were collected between 1 April to october 5th, 2015. Sample collection containers (1 L, amber glass) were washed in hot water, rinsed three times with distilled water, rinsed three times with acetone, and then baked in a heated oven at 250ºC for a minimum of four hours. A 24-hour composite sample (500 mL of effluent) was collected by WWTP operators from each WWTP, using their own equipment, and 100 2 mL of a solution containing 5.0 g/L of Na2EDTA and 25 mg/L of ascorbic acid was added at the time of collection. The samples were shipped overnight on wet ice, and stored at 4°C until extraction. Because of the large number of sampling sites and chemical analytes, it was logistically too difficult and expensive to collect and analyze field blanks as well as duplicates from each location. Field blanks were collected from 20% of the sampling sites, with the field blanks being prepared from laboratory distilled water that was transferred into sampling containers and preserved at the time of collection. Duplicates were collected and analyzed for 10% of the sample sites.

Waste water Effluent

Effluents from five educational and research laboratories in pharmaceutical sciences branch, Azad university in Tehran, including, Food Science and Technology research (Effluent 1&2), Toxicology (Effluent 3,4), Analytical chemistry(Effluent 5) were used in this study. Effluent 1 and Effluent 2 were from the same laboratory but were collected on separate occasions with a 3 week time interval. Although Effluent 1 and Effluent 2 come from the same WWTP, they were treated as 2 different effluents due to the variability of their characteristics. This difference is attributed to the significant experiments which occurred following the first sampling event. After collection, the effluent was immediately transported to the research laboratory for analysis. Physico-chemical parameters such as pH, Electrical Conductivity, Total Solids, Total Dissolved Solids, Total hardness, Chloride, Sulphate, Dissolved oxygen, , Calcium, Sodium, Cadmium, Lead, Zinc, Copper, Chrome, Manganese ,Iron and Potassium were analyzed as per the standard methods[26-27].

The initial concentration of heavy metals/ metalloid in the plants and effluents were analyzed before introduction into the studied soil samples. After 10days of treatment up to 60 days in every ten days, final concentration of heavy metals/metalloid in effluent samples and plants were analyzed using Atomic Absorption Spectroscopy. The samples were analyzed by an Atomic Absorption Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals and using at least five standard solutions for each metal. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines [28-30]. The efficiency of plant in accumulating heavy metals in their areal parts such as leaf, stem and also root were calculated using bio-concentration factor.

Analysis of Samples

A. Vera (one month old plants) were grown in a local nursery until transplant into the research study. For metal analysis 2g of shoots along with leaves and roots of 50 numbers of plants sample were taken separately in every ten day in this study during 60 days. 8ml of concentrated nitric acid (65%) and 1 ml of per chloric acid (70%) was added and 1 ml hydrogen peroxide (17, 26). Application of concentrated HNO_3 along with thirty percent hydrogen peroxide H_2O_2 (Merck) for mineralization of samples to the complete digestion of samples (15-16, 31) following Environmental Protection Agency (EPA) Method 3052 was done. All glassware and plastic containers used were washed with liquid soap, rinsed with water, soaked in 10% volume/volume nitric acid at least overnight, and rinsed abundantly in deionized water and dried in such a manner to ensure that any contamination does not occur. Five-point calibration curves (five standards and one blank) were constructed for each analyte. The calibration curve correlation coefficient was examined to ensure an $r^2 \leq 0.998$ before the start of the sample analysis. The digested samples were diluted with 10% HNO_3 and brought up to 50 mL and analyzed by a graphite furnace atomic absorption spectrophotometry, (GFAAS). The measurements were performed using a Perkin Elmer Pin A Acle 900 T atomic absorption (AA) spectrophotometer and using at least five standard solutions for each metal. All recoveries of the metals studied were greater than 95%Digestion followed by the measurement of total concentrations of Cd using Atomic Absorption Spectrophotometer using an air-acetylene flame for heavy metals: Pb, Cd, Co, Cu, Cr, Ni and Cu. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines Physical and chemical properties and concentrations of heavy metals (Chrome, lead, Cadmium, Copper, Cobalt and Nickel) in soils before and after planting *A. Vera* and also after the growth period (in every 10 days) measured[21-26]. In order to assess amount of heavy metals transfer from soil to plant (shoot and root), translocation factor was determined by dividing metal concentration at shoot by its concentration at root[27-29]. Different parts of plant samples (roots and leaves) were separated and washed and digested by wet method according the standard protocol for measuring Cadmium and Lead. The uptake rate is given by the following equation[17, 32, 21].

$$U = (TSCF)\ (T)\ (C) \quad (1)$$

Where

U = uptake rate of contaminant, mg/day

TSCF = transpiration stream concentration factor, dimensionless

T = transpiration rate of vegetation, L/day

C = aqueous phase concentration in soil water or groundwater, mg/L.

The ratios were higher than one it was considered as suitability of plant at that condition for use in phytoremediation. The enrichment factor (EF) has been calculated to derive the degree of soil contamination and heavy metal accumulation in soil and in plants growing on contaminated site with respect to soil and plants growing on uncontaminated soil [12,14,17,31-32]. Metal contents were detected by Atomic Absorption Spectrophotometer by wet digestion method in Research Laboratory in Pharmaceutical Sciences Branch University.

Estimation of Heavy Metals in Effluents

The heavy metals/metalloid in the effluent samples such as Chrome, Nickel, Copper, lead, cadmium and Iron were analyzed before and after treatment by AAS after digestion of plant materials by AOAC method[28-32]. The plant samples were washed in deionized water dried (24 hrs at 80°C) immediately to stabilize the tissue and stop enzymatic reactions. After drying, samples were ground to pass a 1.0mm screen using the appropriate Wiley Mill. After grinding, the sample were thoroughly mixed and a 5- to 8-g aliquot withdrawn for analyses and storage[33]. Weighed 0.5 to 1.0 g of dried (80°C) plant material that has been ground (0.5 to 1.0 mm) and thoroughly homogenized and place in a tall-form beaker or digestion tube. Added 5.0 ml concentrated HNO_3 (65 %) and cover beaker with watch glass or place a funnel in the mouth of digestion tube and allow to stand overnight or until frothing subsides. Place covered beaker on hot plate or digestion tube into block digester and heat at 125°C for 1 hour. Removed the digestion tube and allowed cooling. Added 1 to 2 ml 30% H_2O_2 and digest at the same temperature. Repeated heating and 30% H_2O_2 additions until digest is clear. Add additional HNO_3 as needed to maintain a wet digest. After sample digest is clear, removed watch glass and lowered temperature to 80°C. Continued heating until near dryness. Added dilute HNO_3 (10%), and deionized water to dissolve digest residue and bring sample to final volume.

Total dissolved solids (TDS)

The total solid concentration in waste effluent represents the colloidal form and dissolved species. The probable reason for the fluctuation of value of total solid and subsequent the value of dissolved solids due to content collision of these colloidal particles. The rate of collision of aggregated process is also influenced by pH of these effluents.

Chemical oxygen demand (COD)

The chemical oxygen demand test (COD) determines, the oxygen required for chemical oxidation of organic matter with the help of strong chemical oxidant. The COD is a test which is used to measure pollution of domestic and industrial waste. The waste is measure in terms of equality of oxygen required for oxidation of organic matter to produce CO2 and water. It is a fact that all organic compounds with a few exceptions can be oxidizing agents under the acidic condition. COD test is useful in pinpointing toxic condition and presence of biological resistant substances. For COD determination samples were preserved using H2SO4 and processed for COD determination after the entire sampling operation was complete.

Biochemical oxygen demand (BOD)

For BOD, 5 samples were immediately processed after Collection for the determination of initial oxygen and incubated at 20 °C for 5 days for the determination of BOD5.

Chlorides

Chlorides are generally present in natural water. The presence of chloride in the natural water can be attributed to dissolution of salts deposits discharged of effluent from chemical industries, oil well operations, sewage discharge of effluent from chemical industries, etc.

Sulphates

Sulphate in one of the major ions occurring in natural water. Sulphate being a stable, highly oxidized, soluble form of sulphur and which is generally present in natural surface and ground waters. Sulphate itself has never been a limiting factor in aquatic systems. The normal levels of sulphate are more than adequate to meet plants need.

Bio-concentration Factor

The bio concentration factor is a measure of bioaccumulation of heavy metals. It can be calculated by dividing the trace element concentration in plant tissues (ppm) at harvest by initial concentration of the element in the external nutrient solution (ppm)[34-36].

Statistical Analysis

For testing statistical significance, student's t-test (SPSS-20) was used. Independent sample t-test was used for finding the mean difference of each parameter control with plants.

RESULTS AND DISCUSSION

Chemical extraction of the soil profile before and after planting *A.Vera* samples during 60 days were studied in pH= 5.6-7.1 , as plant availability of certain heavy metals depends on soil properties such as soil and contains exchange capacity and on the distribution of metals among several soil fractions . All the soil data are expressed on a dry basis.

Results in figures 1,2 and 3 showed significant differences in cadmium, Cr (III) , Cr(VI) and lead up taking by roots and leaves of plant. The best results for uptake of Nickel and copper was in the soil with pH=6.2 among different samples while for lead up taking was in 6.4 and for copper up taking was in pH=5.9. This range of pH had no affecting in zinc up taking. Chemical extractions of the soil profile before treatment of heavy metals by *Aloe Vera* L. is shown in the table 1. Data is averages of the profiles.

Results showed that *A. Vera* transition factors for all heavy metals in treated soil were higher than one and *A. Vera* can up-take lead and Cadmium after 20 days ($p<0.01$) more than other studied metals. In figures 1 and 4, the phytoremediation of lead and Cadmium trend by this plant indicates that *A.vera* cultivated in the soil can be consider as a suitable hyper-accumulator by its relatively large ratio of biomass concentration of the contaminant to soil concentration. Although A. Vera can uptake Cr (III) and (VI) after 10 days significantly ($p < 0.05$), but Cr (III) can be absorbed in a slightly manner up to 60 days (figures 2 and 3).

The heavy metals uptake rate by this plant is significantly affected by number of plant cultivated as for lead uptake ($p<0.005$) while for Cd and chrome the *p*-value was less than 0.01. The percentage of copper uptake by leaves and roots of this plant has been demonstrated in figure 5, it has been indicated that plants had more potential to uptake Nickel and Copper after 10 days by root and the absorbing trend were decreased after 20 days. A highly significant, although low, positive correlation ($r= 0.52$, $p=0.01$, $n=200$) was found

Table 1: Heavy Metal concentration in studied Pharmaceutical Laboratories ' effluent before treating

Heavy Metal Content	Concentration (mg/kg DW ± SE*)
Lead	30.731 ± 0.106
Cadmium	9.879 ± 0.011
Nickel	30.98 ± 0.318
Chrome	19.043 ± 1.204
Copper	101.650 ± 10.006
Iron	42.089± 1.819

SE* = Standard Error

between Lead and Cadmium and chrome (III) of the greenhouse –grown *A. Vera* , compared to a non-significant and much lower correlation between the three other heavy metals: Nickel, Cr(VI) and Copper .

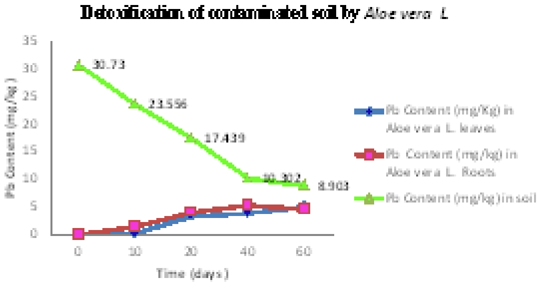

Fig. 1. The mean content of Pb (mg/kg) in *Aloe Vera L.* leaves and root parts and studied soil samples after treatment during 60 days

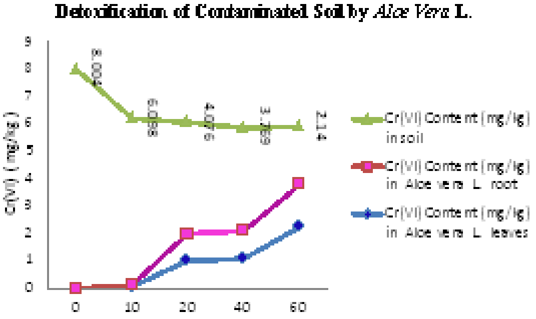

Fig. 2. The mean content of Cr(VI) (mg/kg) in *Aloe Vera L.* leaves and root parts and studied soil samples after treatment during 60 days

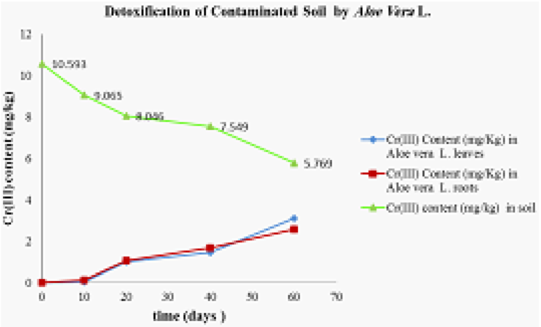

Fig. 3 . The mean content of Cr (III) (mg/kg) in *Aloe Vera L.* leaves and root parts and studied soil samples after treatment during 60 days.

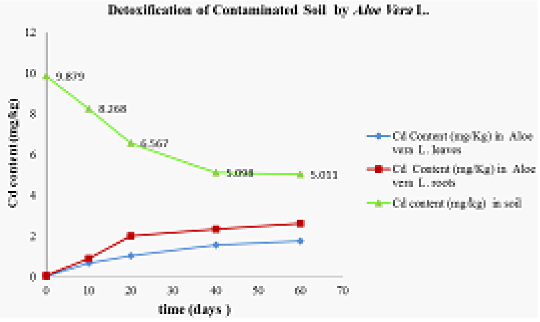

Fig. 4 . The mean content of Cd (mg/kg) in *Aloe Vera L.* leaves and root parts and studied soil samples after treatment during 60 days.

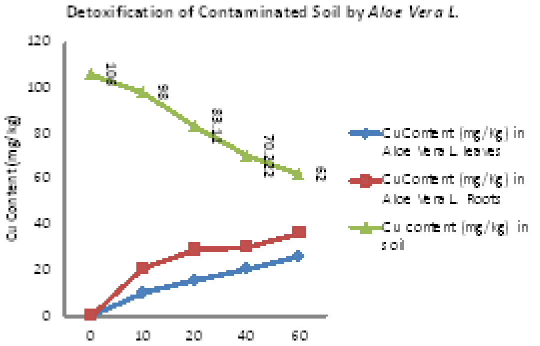

Fig. 5 : The mean content of Cu (mg/kg) in *Aloe Vera L.* leaves and root parts and studied soil samples after treatment during 60 days.

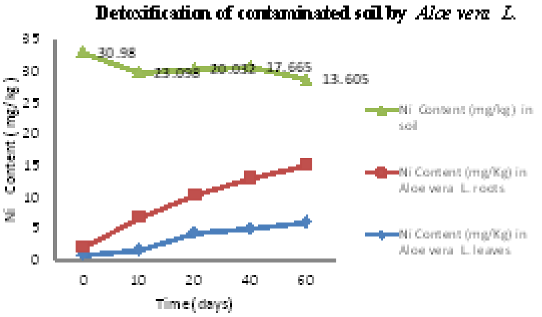

Fig. 6: The mean content of Ni (mg/kg) in *Aloe Vera L.* leaves and root parts and studied soil samples after treatment during 60 days

CONCLUSION

Phytoremediation has been advanced in the last few years by increased understanding of the mechanisms of plant uptake and the various types of enzymatic metabolism that occur. Sorption and uptake constants such as the RCF and TSCF may help model plant uptake rates of various chemicals, allowing more accurate prediction of treatment times required for phytoremediation technology. Research into enzymatic transformation pathways will help determine the ultimate fate of chemicals in a plant remediation system. Our results for chrome translocation indicate that *A. Vera* has high potential for taking –up this toxic heavy metal and Translocation factor in all conditions were higher than one which proves that metal concentrations in shoots were higher than roots and the plant is suitable for phyto-remediation and the heavy metals uptake rate by this plant is significantly affected by time of growing plant as for lead uptake ($p<0.01$) while for Cd and Cr(III) the *p*-value was less than 0.03. The present investigation shows that the *A. Vera* plant is effective and inexpensive adsorbent for the removal of Pb, Cd, Ni, Cu, Cr (III) and Cr (VI) from contaminated soil by heavy metals. Aloe vera, is considering as a medicinal plant with diverse therapeutic applications, also used in many different countries. Through human trade and migration, this plant came to be known and was widely used to cure burns and wounds throughout ancient civilizations. Successful cultivation of this plant is economically attractive provided marketing is not a problem. The cultivation of Aloe vera has acquired great commercial importance for medicinal products and cosmetics processing. Aloe vera cultivation demands skill and it is also very labor intensive. Better management can results in much higher income and net profit .The nutrient make up of Aloe Vera is one of a kind and has amazing natural healing properties. Its uses are multiple and undoubtedly, the nature's gift to humanity and it remains for us to introduce it to ourselves and thank the nature for its never-ending gift. This plant also could be used for treating contaminated soil and rescue the environment. This research conduct adsorption of heavy metals by its leaves and root and proved that this friendly method should gain more attention and research interest for the removal of heavy metals from contaminated soil due to its surface area, adsorption capacity and plenty abundant in nature must be followed seriously. Also, this research suggests more investigations by other genera and families of cost-effective plants.

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Conflicts of Interest

None of the authors have any conflicts of interest associated with this study.

REFERENCES

1. Adams, C., Wang, Y., Loftin, K., Meyer, M. Removal of antibiotics from surface and distilled water in conventional water treatment processes. *Journal of Environmental Engineering – Asce*., 2002; **128** (3): 253–60.
2. Daughton, C.G. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rational for and avenues toward a green pharmacy. *Environ Health Perspect.* 2003;**111**:757–74.
3. Deegan, A. M., Shaik, B., Nolan, K., Urell, K., Oelgemöller, M., Tobin, J., Morrissey, A. Treatment options for wastewater effluents from pharmaceutical companies *Int. J. Environ. Sci. Tech*., 2011;**8**(3): 649-66.
4. Khetan, S., Collins, T. Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry. *Chem. Rev*., 2007;**107** (6): 2319-64.
5. Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Lõhmus, R., Ivask, M., Kuu, A., Kutti, S., Litvin ,S.V., Nei, L. Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting *Int. J. Environ. Sci. Tech*., 2010; **7** (2):307-12 .

6. Heberer, T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.,* 2002;**131** (1-2): 5-17 .
7. , F., Ziarati, P., Mousavi, Z. Removing Heavy Metals from Pharmaceutical Effluent by *Pelargonium grandiflorum* . *Biomedical & Pharmacology Journal*, 2016; **9**(1): 151-161.
8. Watkinson, A., Murby, E., Costanzo, S. Removal of antibiotics in conventional and advanced wastewater Treatment *Water Res.,* 2007; **41**(18): 4164-76.
9. Webb, S. Indirect human exposure to pharmaceuticals via drinking water. *Toxicology Letters*., 2003; **142**:157–67.
10. Suarez, S., Lema, J. M., Omil, F. Pretreatment of hospital wastewater by coagulation-flooculation and flotation. *Bioresource Technology*., 2009; **100** : 2138–46.
11. Barakat, M.A. New trends in removing heavy metals from industrial wastewater. *Arabian Journal of Chemistry*, 2011; **4**: 361–77.
12. Mirbagheri, S.A., Hosseini, S.N. Pilot plant investigation on petrochemical wastewater treatment for the removal of copper and chromium with the objective of reuse. *Desalination.,* 2004; **171**: 85–93.
13. Aziz, H.A., Adlan, M.N., Ariffin, K.S. Heavy metals (Cd, Pb, Zn, Ni, Cu and Cr (III)) removal from water in Malaysia: post treatment by high quality limestone. *Bioresour. Technol*., 2008; **99**:1578 – 83.
14. Jalilian ,Z., Ziarati, P. High Potential of Ferulago angulate (Schlecht) Boiss in Adsorption of Heavy Metals*Biomedical and Pharmacology Journal*., 2016; **9**(1): 201-8.
15. Ziarati, P., Alimardan, M. Study on Increasing Efficiency of Phytoremediation in Cadmium and Nickel Contaminated Soil. *Chemistry Journal* .2015; **5**(6): 86-92.
16. Ziarati, P., Asgarpanah , J ., MirMohammad Makki, Phytoremediation of Heavy Metal Contaminated Water Using Potential Caspian Sea Wetland Plant: Nymphaeaceae. *Biosciences Biotechnology Research Asia*, 2015; **12**(3): 2467-2473.
17. Ziarati, P., Nazif, M. H., Khandehrouy, M. . Decreasing Bio-toxicity of Fume Particles Produced in Welding Process by Aloe vera L. *Oriental Journal of Chemistry*. 2015; **31**(4): 113-20.
18. Ziarati, P., Alaedini, S. The Phytoremediation Technique for Cleaning up Contaminated Soil By sp*. J Environ Anal Toxicol* ,2014; **4**: 208. doi: 10.4172/2161-0525.1000208.
19. Ziarati, P., Iranzad-Asl, S., Asgarpanah, J. companion pelargonium roseum and rosmarinus officinalis in cleaning up contaminated soil by phytoextraction technique the role of companion plants in boosting phytoremediation potential*. IJPAES* . 2014 ; **4**(3): 424-430.
20. Ziarati, P., Zolfaghari, M., Azadi, B . the effect of tea residue in promoting phytoremediation of lavandula angustifoli mill. *IJPAES* . 2014; **4**(2): 479-486.
21. S Babel ; TA Kurniawan .*J Hazard Matter*., 2003, **97**(1-3): 219-43.
22. GH Dixie; MJ Hossain; SA Imran. A publication by Intercooperation and South Asia Enterprise Development Facility. 2003 , 8-22.
23. F Nejatzadeh-Barandozi; MR Naghavi; S Tahmasebi-Enferadi; A Mousavi; Y Mostofid; M Esmaiel-Hassani. *Industrial Crops and Products*. 2012, **37** : 347–51.
24. S Rai; D Kr Sharma; SS Arora; M Sharma; AK Chopra. *Annals of Biological Research*. 2011*,* **2**(6): 575-79.
25. Alimardan, M., , Ziarati, P., Jafari Moghadam, R.Adsorption of Heavy Metal Ions from Contaminated Soil by integerrima Barberry. *Biomed Pharmacol J*., 2016; **9**(1): 169-75.
26. Manshadi, M., Ziarati, P., Ahmadi, M., Fekri, K. Greenhouse Study of Cadmium and Lead phytoextraction by five Pelargonium spices. *International Journal of Farming and Allied Sciences* . 2013; **18**: 665-9.
27. South African National Biodiversity Institute's plant information website: http://www.plantzafrica.com.
28. APHA. Standard methods for the examination of water and waste water.22th Edn., APHA,AWWA,WPCF, Washington .D.C.USA. 2012.
29. Ziarati, P., Khoshhal, Z. , Asgarpanah , J., Qomi, M. Contaminations of heavy metals in tea leaves, finished tea products and liquor in Gilan province, Iran. Int. J. Farm Allied Sci.,

2013; **2**: 383-7.

30. Ziarati, P. Determination of contaminants in some Iranian popular herbal medicines. *Environ. Anal. Toxicol*., 2012; **2**: 1. DOI: 10.4172/2161- 0525.1000120.

31. Ågerstrand, M., Berg, C., Bjorlenius, B., Breitholtz, M., Brunstrom, B., Fick, J., Gunnarsson, L., Joakim Larsson, D.G., Sumpter, J.P., Tysklind, M., Rudeìn, C. Improving Environmental Risk Assessment of Human Pharmaceuticals. *Environmental Science & Technology.* 2015. DOI: 10.1021/acs.est.5b00302.

32. Kümmerer, K. The presence of pharmaceuticals in the environment due to human use present knowledge and future challenges. *J. Environ. Manage*., 2009; **90**: 2354-66.

33. W Fu; A Franco; S Trapp. Methods for estimating the bioconcentration factor of ionizable organic chemicals. *Environ Toxicol Chem*., 2009; **28**: 1372-9.

34. Zayed, A., Gowthaman, S., Terry, N. Phytoaccumulation of trace elements by wetland plants: I. Duck weed. J. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*., 1998; **537**: 29 -41.

35. Kavitha, G.V., Beebi, S.K. A review on characterization and bioremediation of pharmaceutical industries' wastewater: an Indian perspective. *Asian J Microbiol, Biotechnol Environ Sci*., 2003; 5(2):157 –9.

36. Ahmadi,M., Ziarati ,P., Manshadi,M., Asgarpanah, J., Mousavi, Z . The Phytoremidiation Technique for Cleaning Up Contaminated Soil by Geranium (pelargonium roseum) . *Intl J Farm & Alli Sci*. 2013; **2(**15): 477-81.