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Research Article

Removing Cadmium and Nickel Contents in Basil Cultivated in Pharmaceutical Effluent by chamomile (Matricaria chamomilla L.) Tea Residue

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Background: Pharmaceutical waste effluent could be dumped into a sink and if these chemical compounds are not biodegraded or eliminated during sewage treatment, they eventually reach drinking water.

Objectives: Chamomile as a one of the most common herbal teas people drink was chosen for adsorbing the heavy metals from pharmaceutical effluent.

Materials and Methods: Pharmaceutical research laboratories effluent samples in 2016 were collected and added to the soil. Meanwhile the residue of Chamomile tea in different concentrations added as an adsorbent in contaminated soil and Basil as an edible vegetable has been grown in these soil samples. The soil leaves and roots of basil samples under different experimental conditions have been studied after every 10 days in 60 days and digested by wet method according the standard protocol. The data were analyzed using Analysis of Variance, (ANOVA).

Results: Results revealed that Chamomile residue have more potential to adsorb Nickel and Cadmium first days of study ($p<0.001$) and adsorption capacity varied by considering the effects of various parameters like contact time, initial concentrations, pH, temperature, absorbent dose. Chamomile can accumulate high level of Cadmium in a short time and the uptake rate by vegetable edible plant is significantly affected by their concentrations in the contaminated soil ($p<0.05$).

Conclusion: A contact time of 30 days by 5% Chamomile was found to be optimum and 41% of Ni was taken by 15% adsorbent from contaminated soil while a few amounts of these heavy metals being uptake by leaves of edible vegetable basil.

Keywords: Pharmaceutical effluent; Adsorbent; Chamomile Tea, Nickel, Cadmium.

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Introduction

The environmental sustainability of the human society principally relays on our administration of the natural environment and the ecosystems that establish the platform upon which our civilization is based (1). Waste management programs in developing countries are often unsatisfactory and the disproportionate disposal of waste is a major issue worldwide. One the other hand, traditional methods for soil remediation are mostly expensive and energy consuming and the elevated costs involved in removal of toxic substances from contaminated soils prevent remediation from being carried out; especially in areas of little economic value. Historical evidence suggests that compost tea (CT) were used in agriculture by Romans and ancient Egyptians (2).

Hazardous chemical materials and Pharmaceutical waste from educational laboratory systems usually is thrown into the trash or dumped into a sink and thereby placed in the sewer waste stream. If these chemical compounds are not biodegraded or eliminated during sewage treatment, they eventually reach drinking water (3-6). In fact, most sewage

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and water treatment facilities do not take pharmaceutical contaminants into consideration, so these wastes are left untreated to enter our surface, ground, and drinking water. The majority of the effluent from laboratories consists primarily of a mixture of water and acid and as its toxicity to the staff; they were confined to fume hoods. Information regarding the extent and concentration of all the chemicals expected to be used in the laboratory were mainly obtained from the end user. These chemicals have founded their way into the drain pipe (3-5). Due to vast consuming of herbal tea which essentially prepared of herbal mixture made from leaves, seeds and/ or roots of various plants the residue and waste of them could be the best choose for cleaning –up the soil.

Dramatically wastewater breeding from the pharmaceutical laboratories varies in pH, ranging from acidic to alkaline. For example, the pH of an alkaline waste stream from a synthetic organic pharmaceutical plant research center ranges from 9 to 10, while a pH = 0.8 for acidic waste streams has been addressed (7, 8). Nevertheless, essentially all types of waste streams produced from the pharmaceutical industry and research laboratories are either alkaline or acidic, and require neutralization before detoxification and treatment.

Significant criterions should be contemplated in construction of a treatment for pharmaceutical wastewater. Determination of Biochemical oxygen demand (BOD) in the waste should be considered with dilution, in order to indicate if toxic or inhibitory substances and materials in some pharmaceutical effluents are presented or not (9, 10). Economic restraints may also proscribe the treatment of adsorbing pharmaceutical wastewater by activated carbon. It has also been mentioned that activated carbon adsorption may not always be successful in removing some recalcitrant organics such as toluene, phenols, nitrophenols, nitroaniline, TCMP: trichloromethyl propanol (9-11).

Nowadays the environmental pollution with toxic and heavy metals has become a multinational crisis by affecting agriculture and subsidizing to bioaccumulation and bio exaggeration in the food chain(12-16).

Adsorption is considered to be entirely alluring in terms of its performance of removal from dilute waste solutions. Despite the fact that the use of common materials such as activated carbon (17), chitosan (18), zeolite, clay (19, 20) is still completely recognized in order to the high adsorption capacity, they lead to overpriced process, too. Along these longs, there is a growing demand to find relatively efficient, low-cost and easily ecofriendly and available adsorbents for the adsorbing process of heavy metals, particularly if the adsorbents are the wastes from agricultural or food industries (21-24). The new orientation is occurred towards no expensive adsorbents by researches. However, there is a lack of literature dealing with the possible application of commercial herbal tea wastes as adsorbents. Under the title of "Herbal tea wastes" are generally called the solid wastes discarded from the infusion of tea, and the final residues could be utilized as adsorbent.

In current study *Matricaria recutita chamomilla* of the Asteraceae family is an annual plant indigenous to Europe and Asia (25) possessing branched, erect, and smooth stems (26,27) by the common name of Chamomile is selected due to being one of the most important herbal teas in Iranian traditional medicine selected. It has a mild sedative effect, so be the perfect for posing as a sleep aid. It also soothes stomach pains and acts as a gentle laxative (28). It helps alleviate menstrual cramps and some researches revealed that chamomile raises levels of glycine, a substance that calms muscle spasms (28).

In general, the main aim of the current study is to assess the applicability of chamomile in removing heavy metals from the contaminated soil irrigated by pharmaceutical waste effluent.

Materials and Methods

Samples

Pharmaceutical research laboratories effluent samples, mostly from Food Science & Technology research laboratory were collected between 1 April - 1 December 2016. Sample collection containers (1 L, amber glass) were washed in hot water (75 degree) , rinsed three times with deionized water and then rinsed 3 times with acetone. Then they baked in a heated oven at 250 ℃ for 6 hours. A 24-hour composite sample (500 mL of effluent) was collected by wastewater treatment plants (WWTP) operators from each WWTP, using their own equipment. Due to the large number of sampling sites and laboratories and chemical analytes, it was rationally too difficult and overpriced to collect and analyze field blanks as well as duplicates from each lab. Field blanks were collected from 20% of the sampling sites, with the field blanks being prepared from laboratory deionized 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 10 educational and research laboratories in pharmaceutical sciences branch, Azad university in Tehran, including, Food Science and Technology research (Effluent 1-4), General Chemistry (Effluent 5-8), Analytical chemistry (Effluent 9, 10) were studied in current investigation. Effluent 1, 2, 3 and Effluent 4 were from the same laboratory but were collected on separate occasions with a 3 week time interval. Although these effluents come from the same wastewater treated, they were conducted as 4 different effluents in order to the variance of their characteristics. This difference is ascribed to the significant experiments which occurred following the first sampling event. After collection, the effluent was immediately transported to the Nutrition and Food Sciences research laboratory for analysis. Physico-chemical parameters such as pH, Electrical Conductivity, Total Solids, Total Dissolved Solids, Total hardness, Chloride, Sulphate, Dissolved oxygen, Nitrate , Calcium, Sodium, Cadmium, Lead, Zinc, Copper, Chrome, Iron and Potassium were analyzed as per the standard methods and results are shown in table 1(45).

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 every 10 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 [46-47].

Estimation of Heavy Metals in Effluents

The heavy metals/metalloid in the effluent samples such as Chrome, Zinc, Copper, lead, cadmium, Manganese and Iron were analyzed before treatment by herbal tea waste by Atomic Absorption Spectrophotometer Model AA-6200 (Shimadzu, Japan) using an air-acetylene flame for heavy metals: Nickel and Cadmium, using at least five standard solutions for each metal by AAS after digestion of plant materials by AOAC method (31-32). The samples were analyzed by an. All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (18-20).

The plant samples were washed in deionizer water dried (16 hrs at 80 ℃) 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 (5, 33). Weighed 1.0 g of dried (80 ℃) 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 HNO3 (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℃ for 1 hour. Removed the digestion tube and allowed cooling. Added 3 to 5 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 HNO3 as needed to maintain a wet digest. After sample digest is clear, removed watch glass and lowered temperature to 80℃. Continued heating until near dryness. Added dilute HNO3 (10%), and deionized water to dissolve digest residue and bring sample to final volume (48-49).

Vegetable Sampling Method

Aerial parts of Basil in every 10 days in companion of Chamomile Tea residue were separated in 60 days and washed and digested by wet method according the standard protocol for measuring Cadmium and Nickel. Bioaccumulation factors (BAF-s) were calculated for heavy metal content of plant parts (mg/kg) / heavy metal content of soil (mg/kg), for each metal (17).

All Basil samples were watered each day by tap water (Tehran tap water). The studied samples were managed by the same light situation and some circumstances in order to be compared with each other due to determine the ability of Chamomile in adsorbing Cadmium and Nickel from soil and its potential to avoid transferring heavy metals to coriander and keep safe the eating vegetable .

Physical and chemical properties and concentrations of heavy metals in soils, before and after adding herbal tea

Chamomile residue in the growth period of cultivated Basil were measured every 10 days. In order to assess amount of heavy metals in the soil samples, heavy metal concentrations in soils of studied vases were determined by atomic absorption spectrophotometer (21-26).

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 (48-53).

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 H_2SO_4 and processed for COD determination after the entire sampling operation was complete (49-58).

Biochemical oxygen demand (BOD)

For BOD, 5 samples were immediately processed after collection for the determination of initial oxygen and incubated at 20 ℃ for 5 days for the determination of BOD5 (49-58).

Heavy metal in Effluent

The heavy metals/metalloid in the effluent samples such as Chromium, Zinc, Copper, lead, cadmium, Tin and Cobalt were analyzed before and after treatment by AAS after digestion of plant materials by AOAC method [48, 50]. The plant samples were washed in deionized water dried (24 hrs at 80 ℃) 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 storage33. Weighed 0.5 to 1.0 g of dried (80 ℃) 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 HNO3 (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 ℃ for 1 hour. Removed the digestion tube and allowed cooling. Added 1 to 2 ml 30% H2 O2 and digest at the same temperature. Repeated heating and 30% H2 O2 additions until digest is clear. Add additional HNO3 as needed to maintain a wet digest. After sample digest is clear, removed watch glass and lowered temperature to 80 ℃. Continued heating until near dryness. Added dilute HNO3 (10%), and deionized water to dissolve digest residue and bring sample to final volume (9, 53-58).

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 (50).

Sulphates

Sulphate is one of the major cations 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 (50-53).

Statistical Analysis

The values reported here are means of five values. Data were tested at different significant levels using student t-test to measure the variations between the concentration of herbal tea residue of Chamomile and Basil leaves parameters before and after treated soil by Chamomile residue during the time factor. One way analysis of variance (One-ANOVA) was used for data analysis to measure the variations of metal concentrations using SPSS 22.0 software (SPSS Inc, IBM, Chicago, IL).

Results and Discussion

Chemical composition of the wastewater effluent of studied research laboratories profile in pharmacy faculty before treatment by Chamomile is shown in the table 1. Data is the average of the effluent profiles.

Table 1. Characteristics of Wastewater from Research pharmaceutical laboratories in Oct 2016, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran.

Parameters	Concentration Range	Average
pH	0.79 – 7.2	3.1
BOD5 at 208C (mg/L)	1915–3130	2525
COD (mg/L): chemical oxygen demand	1200 – 7000	2430
TSS (mg/L): total suspended solids	30 – 55	42
Total alkalinity as CaCO3(mg/L)	70 – 1100	510
TVA (mg/L)	70 – 2,000	750
Lead (mg/L)	0.22 – 6.9	2.44
Tin (mg/L)	0.3 – 5.5	1.2
Cadmium (mg/L)	1.6 – 2.45	1.70
Mercury (mg/L)	0.15 – 0.50	0.22
Zinc (mg/L)	5.18 – 20.11	10.47
Cobalt (mg/L)	1.34 – 7.83	2.98
Chromium (mg/L)	1.1 – 3.43	2.14
Chloride (mg/L)	500 – 1250	950
Sulfide (mg/L)	2-8	5
Nitrate (mg/L)	340-1200	870

As compared to BOD, COD was very high which is normal for effluent of such pharmaceutical laboratories. The minimum and maximum values ranged between 1915–3130 and the averaged values ranged between 1200 – 7000 mg/L for the studied effluent.

The physical properties of the soil profile before planting holy basil (*Ocimum tenuiflorum* is shown in table 2. The mean content of some mineral elements such as Fe, Mn , Cr(VI) , Zn, Cu, Se, Pb and Nickel (mg/kg DW ± SD) in vases soil after 3 replicate of 5 subsamples is shown in table 3. Vegetables samples during 60 days were studied in pH= 5.78- 7.29, 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.

Table 2. Selected chemical and physical properties of studied soil

Soil texture	Sand (%)	Silt (%)	Clay (%)	Field Capacity (%)	$CaCO_3$ (%W)	OC (%W)	OM %	CEC cmol/kg)	pH	EC ds/m
Clay	38	18	44	52.0	6.73	1.01	1.74	44.5	7.23	6.09

Table 3. The mean content of Mineral elements (mg/kg DW ± SD) in the studied soil

Mineral elements	mg/kg DW ± SD
Fe	298.76 ± 1.17
Mn	152.34 ± 1.10
Zn	287.93 ± 20.11
Cu	67.56 ± 6.72
Ni	47.29 ± 1.76
Cd	2.67 ± 0.09
Se	2.33 ±0.28
Pb	3.87 ± 1.09
Cr(VI)	25.67 ± 1.08

Wastes from food science and Technology research laboratory had acidic state contributed 50% of the total waste flow at 3600 mm^3/day and had a pH of 0.8. The combined toxicology and food science laboratories waste had a pH of 1.9 (including acidic waste stream), whereas the pH of the waste without acidic waste stream was 7.2.

The combined wastewater had average COD, and BOD values of 2430 mg/L and 2525 mg/L. Some heavy metal contents such as copper and zinc of the wastewater was found to be well below the limits according to IS-3306 (1974). Most of the solids present were in a dissolved form, with practically no suspended solids.

This investigation was carried out to determine the accumulation of heavy metals in *Chamomile* in contaminated soils by heavy metals result in food science and technology, chemical and toxicology laboratories' pharmaceutical effluent and waste water irrigated soil in the vicinity of sewage treatment plant (STP), Pharmacy Faculty, Tehran.

The fractionation of nickel in edible leafy vegetable cultivated in control soil (untreated by *Chamomile*) and in soils treated by herbal tea residue in different percentage of is completely determined due to find out the adsorption ability of heavy metals by agricultural waste in contaminated soil samples. In figures 1 and 2 and table 4, the treating contaminated soil trend by *Chamomile* residue indicates that dried plant parts in the soil which is enriched soil by mineral elements and vitamins, it can be consider as a suitable method for rescuing soil by its relatively large ratio of biomass concentration of the contaminant to the soil concentration.

Table 4. Removal of Nickel contents of edible vegetable Basil using different contents of herbal tea Chamomile residue.

Nickel Content (mg/kg DW ±SE) in Basil and Soil	**Day**						
	1	**10**	**20**	**30**	**40**	**50**	**60**
Soil sample : %5 Chamomile	53.706[a]	51.176[a]	50.167[a]	47.666[a]	43.093[a]	41.208[b]	40.220[b]
Root Sample in soil %5 chamomile	8.323[a]	6.453[b]	5.908[b]	4.263[c]	3.333[c]	2.652[d]	2.042[d]
leaves Sample in %5 chamomile	2.033[a]	1.187[b]	1.267[c]	1.070[c]	1.0811[c]	1.001[d]	1.005[d]
Soil Sample : %10 chamomile	54.002[a]	53.000[a]	50.002[a]	45.821[b]	42.967[b]	42.078[b]	41.333[b]
Root Sample in %10 chamomile	7.888[a]	6.167[b]	5.567[b]	4.092[c]	3.444[d]	2.892[d]	1.030[e]
leaves Sample in %10 chamomile	2.000[a]	1.1563[b]	1.106[b]	1.064[b]	1.006[c]	1.000[c]	1.000[c]
Soil Sample : %15 chamomile	54.111[a]	52.067[a]	51.021[a]	46.242[b]	43.610[b]	42.190[c]	41.333[c]
Root Sample in %15 chamomile	7.788[a]	6.903[b]	5.422[c]	4.302[d]	2.443[e]	1.000[f]	0.621[f]
leaves Sample in %15 chamomile	2.111[a]	1.108[a]	1.098[a]	1.054[a]	1.003[b]	1.001[b]	0.847[c]
Untreated Soil samples	54.902[a]	53.190[a]	53.3092[a]	51.908[a]	50.117[a]	48.908[a]	47.028[a]
Untreated Root samples	10.178[a]	13.180[b]	16.724[c]	17.005[c]	17.899[c]	18.032[c]	18.555[c]
Untreated leaves samples	2.555[d]	5.109[c]	7.877[b]	8.180[a]	9.106[a]	8.654[a]	8.334[a]

In *Basil* leaves under herbal tea Chamomile residue treatment of the heavy metal contaminated soil by pharmaceutical effluent indicates that Cd concentration in the basil leaves is appreciably depleting as the time passes, even up to 40 days of observation. In initial 30 days the Cd concentration depletion is very fast compared with the remaining 60 day period, observed and analyzed on a 20 day interval. The treatment of Chamomile residue nurtures the rehabilitation of soil by balancing (15 g/100g has a faster rate of reaction compared with that of the 5g/100g and 10 g/100 g mixture). The observed values establish the band of Cd stabilizing across increased time period. The rate of reaction is continuous in basil leaves; the Cd concentration drops significantly as the mixture concentration of herbal tea Chamomile residue treatment increases and the days prolong.

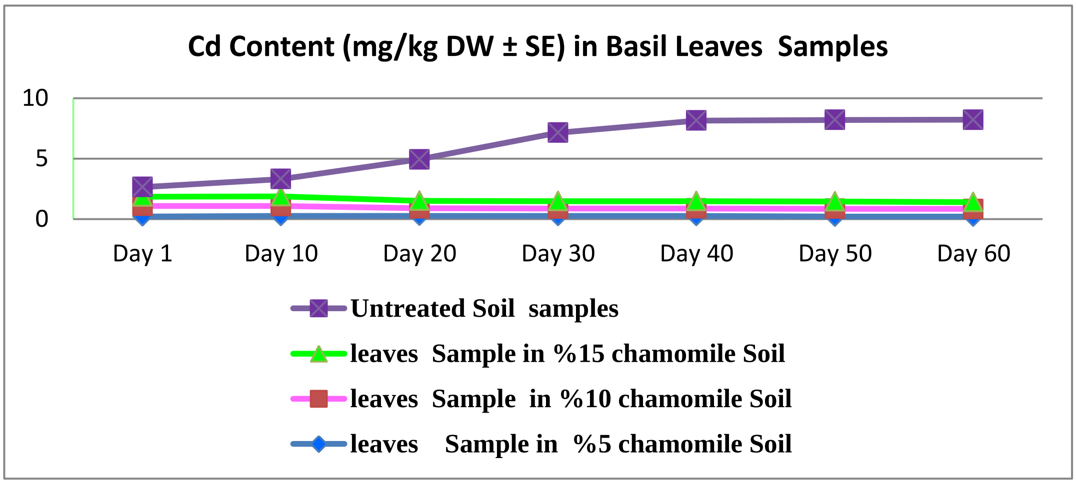

Figure 1- Removal of Cadmium contents from basil (*Ocimum tenuiflorum*) leaves using Chamomile residue (adsorbent dose=5 g/100 g , 10 g/100 g & 15g/100 g), temperature=25 ℃, during 60 days studying.

Results in figures 1 and 2 showed significant differences in Cd up -taking by plant. The best results for uptake of Nickel and cadmium was in the soil with pH=4.1.

Plant availability of certain heavy metals depends on soil properties such as soil pH and contain exchange capacity and on the distribution of metals among several soil fractions. The fractionation of Nickel and Cadmium in Basil cultivated in control soil and in soils treated by Chamomile is completely determined due to find out the adsorption ability of heavy metals by *Chamomile* in contaminated soil samples. Results showed *Chamomile* adsorption for both heavy metals in treated soil were affected significantly by *Chamomile* dried content and the adsorbent not only affected contaminated soil and can adsorb cadmium after 20 days ($p<0.001$) more than other studied but also adding *Chamomile* have reduction and rescue effect in taking up heavy metals especially in bio-adsorbing Cadmium and Nickel more than other heavy metals studied in short time and it keeps edible vegetable safe for eating . In figures 1 and 2, the treating contaminated soil trend by *Chamomile* indicates that dried plant parts in the soil which is enriched soil by mineral elements and vitamins, it can be consider as a suitable method for rescuing soil by its relatively large ratio of biomass concentration of the contaminant to the soil concentration.

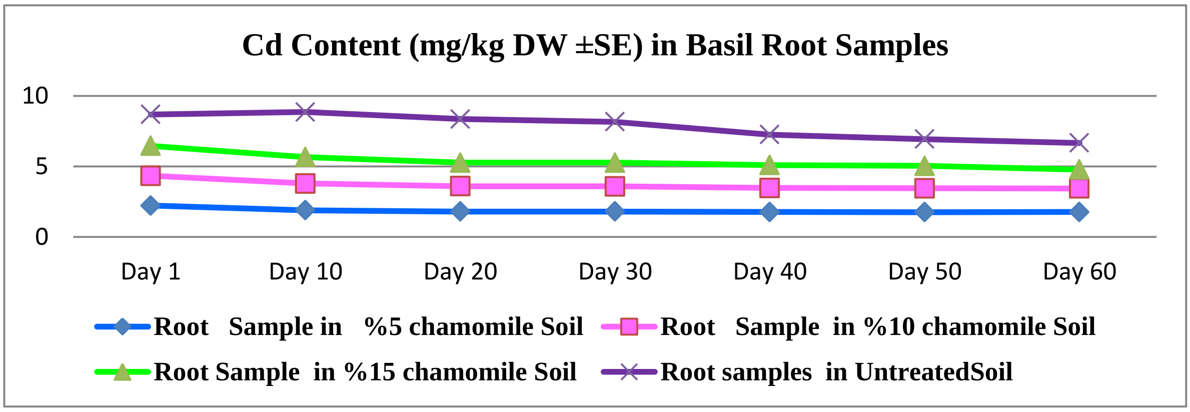

Figure 2. Cadmium contents in basil (*Ocimum tenuiflorum*) Root samples using Chamomile residue (adsorbent dose=5 g/100 g , 10 g/100 g & 15g/100 g), temperature=25 ℃, during 60 days studying.

Results in table 4 showed significant difference in Nickel up -taking by after 10 days by 5%, 10% and 15 %, but the potential of taking up Nickel by 15% was not as much as different from lower percent's ($p> 0.05$). Moreover, time factor of putting adsorbent in contaminated soil by different heavy metals in the study showed significant ($p <0.05$) and positive correlation with contents of Ni (r = +87 to r = +91), Cd (r = +79 to r = +83) in the contaminated soil and *Chamomile* respectively. The amounts of Nickel adsorbed increased significantly with increase contact time (p<0.005).

Conclusion

The present study focused on adsorption capacity of Cd and Ni by *Chamomile* was investigated in a batch system by considering the effects of various parameters like contact time, initial concentrations, pH , temperature, absorbent dose. The results of this study revealed that *Chamomile* fruit can accumulate high level of Cadmium in a short time and their uptake rate by vegetable and edible plant is significantly affected by their concentrations in the contaminated soil (p<0.05). A contact time of 20 days by *Chamomile* was found to be optimum and 91.2% Cadmium and 89.2% Ni in presence of 15% herbal tea residue , was taken by adsorbent from contaminated soil while a few amounts of these heavy metals being uptake by edible vegetable – basil. To quantify the occurrence and the distribution of heavy metals, to evaluate their effects, and to prevent them from passing through waste water collection and treatment systems into soil and ground water bodies represents an urgent task for applied environmental sciences in the coming years. Public acceptance of green technologies is generally higher than that of industrial processes. The responsible organizations should stimulate research to upgrade existing waste water treatment by implementing phytoremediation modules and demonstrating their reliability to the public.

Competing interests

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

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