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Original Article

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The Efficiency of Bio-adsorption of Heavy Metals from Pharmaceutical Effluent by Rumex crispus L. Seed

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Abstract

The programs of managing waste materials in developing countries are often unsatisfactory and the unreasonable disposal of waste is a major issue in the world. The main aim of the current study is to assess the applicability of Rumex crispus L. in removing heavy metals from the contaminated wastewater effluent from Pharmaceutical laboratories by the bio-adsorption method. The dried R. crispus L. seeds were purchased from recognized herbal plant markets randomly in Tehran in May 2016 in order to investigate the influence of R. crispus seeds as an amendment to remove or decrease chemical forms of Co, Pb, Cu, Zn, and Ni. The experimental parameters including pH, temperature, a dose of bio-adsorbent R. crispus L. seeds, contact time, particle mesh size were studied. Results revealed that bio-adsorption capacity of Lead, Zinc, Copper, Cobalt, and Nickel increases with increasing initial adsorbent concentration and reaches a maximum level after a 2% initial concentration of S. incisa seeds concentration value. Heavy metals adsorption ranged from 83.5 -91 % after agitation for 1 week (equilibration time) and there was no further significant increase in sorption of them after the equilibration time ($P \geq 0.05$). This research area of using models for resolving nature of heavy metals complexation and sequestrations mechanism at the heavy metals-bio-adsorption interface has been less explored. The results represent a critically important mechanism in the scientific ability, which should be investigated in future research to unravel complex surface heavy metal sorption mechanism on the bio- sorbent's surface using various chemical modeling approaches. Current research is suggested for the characterization of novel bio-adsorbents from other waste of herbal plants, agriculture/food-industry with maximum heavy metals sorption capacities to promote the large-scale use of bio-adsorbents.

Keywords: Rumex crispus L.; Seed; Bio-adsorption; Pharmaceutical Effluent; Removal Heavy Metals.

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Introduction

Rumex crispus L. is a tap rooted stationary perennial forb, belonging to the Polygonaceae family. In the categorization of plant life forms made by Raunkiaer (1934), Rumex spp. belongs to the hemicryptophytes. Representing for this class, which consists of many kinds of grass and rosette plants, is that bloom is based at the soil surface, Characterizing for this group, that includes many grasses and rosette plants, is that buds are located at the soil surface, protected by leaf and stem bases (Mauseth , 2008). R. crispus is considered one of the most widely allocated non-cultivated plants in the flora of the world (Hughes, 1938). It has spread to every continent and is known as a serious weed of agriculture in many countries (Cavers & Harper, 1964). The exact original distribution specific primary distribution cannot be recognized but was undoubtedly much more restricted. The species is probably native to Europe and Africa. It does not occur in higher abundance in native plant communities but is clearly stimulated and distributed by human activities (Zaller, 2004a; Zaller, 2004b). R. crispus, also known as curled dock, is native to Europe, northern Africa (i.e., Algeria, Egypt, Libya, Morocco, and Tunisia), and western Asia (i.e., Afghanistan, Iran, Iraq, Lebanon) (USDA, 2010). This species grows in a wide variety of habitats, including disturbed soil, waste areas, roadsides, fields/meadows, shorelines, and forest edges and prefers rich, moist, and heavy soil in general. This species can be used as a wild leaf vegetable because its leaves are an excellent source of vitamin A, protein, iron, and potassium. R. crispus is propagated through the contamination of crop seeds and by sticking to clothing. It is classified as an "injurious weed" under the United Kingdom Weed Act of 1959 (Crofts & Jefferson, 1999). As a widespread naturalized species throughout the temperate world, R. crispus is now present in continental Asia, Japan, North and South America, North and South Africa, Australia, and New Zealand (USDA, 2010). Weed is a serious pest that damages most of the crops and is an everlasting problem for our agriculture (Samad et al., 2008). Weeds compete for light, nutrients, moisture, and space with the crop and thus cause severe loss to yield (Hassan and Marwat, 2001). More use of herbicides may be cause increasing tolerance of weeds to some herbicides. Studying on allelopathy can solve these problems (Macias et al., 1998) and allelopathic compounds can be used as a model for herbicide production (Schabes and Sigstad, 2007).

Several studies show that Rumex species can cause reduced grass yields (Courtney, 1985; Oswald & Haggar, 1983). They can constitute a considerable part of the biomass (up to 70 percent in the mentioned studies) and the yield loss is proportional to the area of ground covered by dock plants. The reduction in grass growth is caused by competition from the dock plants, mainly by shading but also by below ground competition for water and nutrients (Oswald & Haggar, 1983). Courtney (1972) found that grass yields increased with up to 50 percent in fields where the dock population had been controlled.

Many of the water systems that keep ecosystems thriving and feed a growing human population have become stressed. Water pollution, especially heavy metal contamination, is a major global problem and it leads to death and diseases (OEHHA, 2011; Ziarati et al., 2016). The environmental sustainability of the human society predominantly lay on the organization of the natural ecosystems and the environment, which empowers the stage upon which our civilization is based (Linnaeus, 2012). The programs of managing waste materials in developing countries are often unsatisfactory and the unreasonable disposal of waste is a major issue worldwide. Furthermore, conventional methods for water

and soil remediation are mostly expensive and energy consuming and the elevated costs involved in the removal of toxic substances from contaminated water and soils prevent repudiation from being carried out; especially in areas of little money-making value (Pourzare et al, 2017; Ziarati et al., 2017). Wastewater generated from the educational and research pharmaceutical laboratories varies drastically with the pH ranging from acidic to alkaline For example, the pH of an alkaline waste stream from a synthetic organic pharmaceutical plant ranges from 9 to 10, whereas a pH of = 0.8 has been reported for acidic waste streams7, 8 and from Nutrition and Food Sciences Research Center Food Industries Research center 2-4. Nevertheless, almost all types of waste streams produced from the pharmaceutical research laboratories are either alkaline or acidic and require neutralization before biological treatment. Therefore, neutralization/pH adjustment of the waste prior to the biological system biological treatment is a very vital treatment task for the biological treatment of pharmaceutical wastewater. The pH of the wastewater in this unit is adjusted by adding base or acid, depending upon the requirement of the raw wastewater effluent. Utilizing waste of food industries and agricultural waste material and even waste of herbal teas from our previous studies had very successful results (Jafari et al., 2016; Ziarati et al., 2015a; Ziarati et al., 2015b; Mehrarad et al., 2016; Alimardan et al., 2016; Ziarati et al., 2013). In the current study, Rumex crispus L. of the Polygonaceae family is considered one of the most widely allocated non-cultivated plants in the flora of the world. The main aim of the current study was to assess the applicability of Rumex crispus L. to the removal of heavy metals from the contaminated wastewater effluent from pharmaceutical laboratories by the bio-adsorption method.

Materials & Method

Waste water Effluent

Effluents from 5 research laboratories in pharmaceutical sciences branch, Azad university in Tehran, including, Nutrition and Food Sciences Research Center (Effluent 1&2), Toxicology (Effluent 3&4), Analytical chemistry (Effluent 5) were used in current study. Effluent 1 and 2 were from the same laboratory but were collected on separate occasions with a 2 week time interval. Although Effluent 1 and Effluent 2 come from the same wastewater treatment plant, they were examined and considered as 2 different effluents due to the variability of their characteristics. The characteristic is attributed to the significant experiments which occurred following the first sampling event. After collection, the wastewater effluent was instantly transported to the main research laboratory for analysis. Physico-chemical parameters such as total solids, total hardness, pH, electrical conductivity, total dissolved solids, Chloride, Sulphate, dissolved oxygen, , Calcium, Sodium, Cadmium, Lead, Zinc, Copper, Chrome, Manganese, Iron and Potassium were analyzed as per the standard methods (Manshadi et al., 2013; Shokri et al., 2016).

The initial concentration of heavy metals/ metalloid such as Nickel, Zinc, Copper, Cobalt, and Lead in the untreated effluents and treated by R. crispus were analyzed. After specific times: 48, 72 hours and 1 and 2 weeks (with/ without stirring), the final concentration of heavy metals in effluent samples 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 required precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines (APHA,

2012; Ziarati et al., 2012; Ziarati, 2012). The efficiency of R. crispus seed in accumulating heavy metals was investigated.

Preparation of S. incisa Seeds

The dried R. crispus L. seeds were purchased from recognized herbal plant markets randomly in Tehran in May 2015 in order to investigate the influence of R. crispus seeds as an amendment to remove or decrease chemical forms of Co, Pb, Cu, Zn, and Ni.

The R. crispus seeds were grounded with a grinding mill and sieved to a particle size of 0.25 to 0.4 mm. This was to allow for shorter diffusion path, thus allowing the non-living Bio-adsorbent (R. crispus L. seeds) to puncture immersed and immediately into the contaminated wastewater resulting in a higher rate of bio-adsorption (Adeyinka et al., 2007). The plant seeds were dried at 80º C in an oven for 24 hours, then ground and weighed. Approximately 1.0 g of these plant samples were soaked in 1% citric acid, for 1 hour and then digested with concentrated HNO3 and H2O2 (Aziz et al., 2008; Ziarati et al., 2016). First, the digested solution was filtered and then analyzed by Flame Emission Spectrophotometer. Experimental parameter were as follows: Effect of various solution pH adjusted with a 0.1M HCl /0.1M NaOH solution); temperature; dose 0.1%, 0.2%, 0.3%, 0.4%, 0.6%, 1% and 2% mg dried R. crispus L. seeds /100 mL of pharmaceutical effluent contaminated solution; contact time 24, 48, 72 hours , one and two weeks; particle size mesh >30, mesh <30, mesh >20; and agitation speeds 100, 300, 400, 800 rpm were studied. Time of each experiment was kept at specific times. The factor of time as a variance was equivalent in a factor model for each test. These flasks were shaken on the shaker with different speed, though the optimum was considered to be 400 rpm.

Total Dissolved Solids (TDS)

The total solid concentration in wastewater pharmaceutical effluent represents the colloidal form and dissolved species. The presumed reason for the inconstancy of the value of total solid and subsequently, the value of dissolved solids due to the content collision of these colloidal particles. The rate of collision of aggregated process is also influenced by pH of these effluents (Ziarati & Tosifi, 2014; ORA LABORATORY MANUAL FDA, 2013).

Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is an indirect measurement of the amount of organic matter in a sample. With this test, you can measure virtually all organic compounds that can be digested by a digestion reagent. The chemical oxygen demand test (COD) regulates the oxygen required for chemical oxidation of organic matter with the help of strong chemical oxidant. The COD is an important test which commonly measures pollution of domestic and industrial waste. The waste is measured 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 biologically resistant substances. For COD determination, samples were preserved using H2SO4 and processed for COD determination after the entire sampling operation was complete (Pouzare et al., 2017; Ziarati et al., 2107; ORA LABORATORY MANUAL FDA, 2013).

Biochemical oxygen demand (BOD)

For BOD, 10 samples were processed immediately after collection, for the determination of initial oxygen and incubated at 20 °C for 5 days for the determination of BOD5 (Pouzare et al., 2017; Ziarati et al., 2107; ORA LABORATORY MANUAL FDA, 2013; Shokri et al., 2016; APHA, 2012).

Heavy metal in Effluent

The heavy metals: Zinc, Copper, Lead, Cadmium, and Nickel in the wastewater effluent samples were analyzed by AAS in different studied samples after treatment by different dose of bio-adsorbents in different contact times according to AOAC method (Pouzare et al., 2017; Ziarati et al., 2107; ORA LABORATORY MANUAL FDA, 2013; Shokri et al., 2016; Mehrarad wt al., 2016; Jafari et al., 2016). The R. crispus L. seed samples were washed in deionized water dried (72 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 was thoroughly mixed and a 4 to 6-g aliquot withdrawn for analyses and storage((Pouzare et al., 2017; Ziarati et al., 2107; ORA LABORATORY MANUAL FDA, 2013; Shokri et al., 2016; Mehrarad wt al., 2016; Jafari et al., 2016). The powdered samples then subjected to the acid digestion using Nitric acid (65% Merck, Germany), Sulfuric acid (96.5% Merck, Germany) and perchloric acid(70% Sigma-Aldrich).One gram of air-dried of each homogeneously R. crispus seed samples accurately weighed and 15.0 mL of the digestion mixture(3 parts by weight of concentrated Nitric acid:1 parts of concentrated Sulfuric acid & 1 parts by weight concentrated perchloric acid) and slowly heated by an oven and then the temperature was raised. The remaining dry inorganic residues were dissolved in 10.0 mL of Nitric acid and the solution used for the determination of heavy metals. Simultaneously, blanks and samples were also processed and analyzed. All the chemicals used were of analytical grade (AR). Standardized international protocols (AOAC) were followed for the preparation of material and analysis of heavy metals contents (Ziarati, 2012; AOAC, 1998; AOAC 2000; Ziarati, Tosifi, 2014).The samples were analyzed by Flame Emission Spectrophotometer, using six standard solutions for each metal and determination of potassium content was followed by FDA Elemental analysis(ORA LABORATORY MANUAL,2013; AOAC 2000). In addition, periodic testing of standard solutions was performed in order to verify the reliability of the measuring apparatus.

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 contaminations in wastewater and the dose of bio-adsorbent and contact time parameters before and after being treated by R. crispus seeds. 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 & Discussion

The main content of heavy metals: Zinc, Cobalt, Cadmium, Copper, Lead, and Nickel in presence of R. crispus seeds samples collected from recognized herbal markets in Tehran, Iran are shown in Table 2 and Figures 1 and 2. The Pharmaceutical wastewater effluent samples were analyzed by wet digestion method and standardized international protocols were followed for the preparation of material and analyses of heavy metal contents and analyzed by Atomic Absorption Spectrophotometer in Nutrition and Food Sciences Research Center in Pharmaceutical Sciences Branch, Islamic Azad University. The data obtained from chemical analyses, mean values were calculated and are given in table 2, with their standard errors.

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

Parameters	Concentration Range	Average
pH	0.18 – 6.8	2.5
BOD5 at 208C (mg/L)	1962–3165	2560
COD (mg/L): chemical oxygen demand	1200 – 7000	2370
TSS (mg/L): total suspended solids	30 – 58	41
Total alkalinity as CaCO3(mg/L)	70 – 1100	560
TVA (mg/L)	70 – 2100	735
Lead (mg/L)	0.54 – 11.03	4.46
Tin (mg/L)	0.5 – 6.1	2.8
Cadmium (mg/L)	1.8 – 3.7	2.9
Mercury (mg/L)	0.15 – 0.42	0.21
Zinc (mg/L)	5.18 – 24.32	13.92
Cobalt (mg/L)	1.78 – 8.75	3.22
Chromium (mg/L)	2.67 – 7.65	3.72
Chloride (mg/L)	500 – 1310	870
Sulfide (mg/L)	2-8	5
Nitrate (mg/L)	350-1300	910

As compared to BOD, COD was very high, which is normal for effluent of such pharmaceutical laboratories. The minimum and maximum values ranged between 1962–3165 and mean of 2564 mg/L and the average values ranged between 1200 – 7000 and mean of 2370 mg/L for the studied effluent.

Results in Figure 1 showed a significant difference in Zinc up-taking after 7 days (1 week), but the potential of taking up Zinc was not as much as Cu and Co. Moreover, time factor of putting adsorbent in contaminated effluent wastewater by different heavy metals in the study showed significant ($p < 0.05$) and positive correlation with contents of Pb ($r = +87$ to $r = +91$), Zn ($r = +88$ to $r = +90$), Co ($r = +913$ to $r = +91$), Ni ($r = +67$ to $r = +73$) in the contaminated wastewater and R. crispus in one week contact, respectively. The amounts of Lead and Nickel adsorption increased significantly with increasing contact time ($p<0.005$).

For 1 and 2 weeks (2W) stirred exposure stirred samples within the 0.1 to 2% Bio-Adsorbent range, there is a steady decrease in solution lead from the Pb contents in studied solutions around 52.1% in 1 week stirred to 42.3% in 2 weeks stirred samples. The effect of stirring the Pharmaceutical effluent and bio-adsorbent concentration is evaluated in the sorption isotherm, which provides information on estimating maximum sorption capacity of a bio-adsorbent at a constant pH= 4 and temperature 25 C0 (Figure 1). Our survey revealed that Lead can transfer from the aqueous contaminated heavy metal solution to the bio-sorbent surface as a result of a driving force produced by the initial content of an

Table 2-Removal of heavy metals: Cobalt and Nickel contents from contaminated water by pharmaceutical effluent using different contents of S. incisa seeds.

Co Content (mg/L ±SE) bio-adsorbent	**48h**	**72h**	**1week**	**1week (stirred solution)**	**2week**	**2week (stirred solution)**
0.1% bio-adsorbent	[a]104.26±1.34	[a]100.01±1.67	[b]86.56±3.44	[c]73.44±5.09	[b]80.55±2.19	[d]54.21±1.65
0.2% bio-adsorbent	[a]103.78±1.04	[a]98.65±1.22	[b]84.87±0.78	[c]70.78±0.78	[b]80.13±0.56	[d]53.22±1.04
0.3% bio-adsorbent	[a]103.76±1.45	[a]97.34±0.46	[b]84.32±0.84	[c]71.28±0.76	[c]75.62±0.78	[d]50.08±0.89
0.4% bio-adsorbent	[a]102.92±0.78	[a]97.65±0.56	[b]85.28±0.67	[c]70.15±0.87	[c]76.09±0.56	[d]50.01±0.45
0.5% bio-adsorbent	[a]101.89±0.46	[a]96.98±0.94	[b]84.10±0.06	[c]68.92±0.05	[c]74.32±0.04	[d]50.17±0.34
0.6% bio-adsorbent	[a]103.25±0.54	[a]95.49±0.76	[b]83.67±1.11	[c]68.43±1.03	[c]74.10±0.08	[d]48.76±0.21
1% bio-adsorbent	[a]98.77±1.03	[a]94.72±0.44	[b]80.22±0.45	[c]68.71±0.04	[c]73.29±0.03	[d]45.39±0.18
2% bio-adsorbent	[a]95.44±0.67	[a]95.38±0.54	[b]78.92±0.67	[c]65.29±1.04	[c]72.17±0.06	[d]40.17±0.45
Untreated bio-adsorbent	[a]107.48±1.03	[a]105.44±1.06	[a]104.39±0.12	[a]107.02±0.14	[a]106.55±1.04	[a]103.29±1.09
Cu Content (mg/L ±SE) bio-adsorbent	**48h**	**72h**	**1week**	**1week (stirred solution)**	**2week**	**2week (stirred solution)**
0.1% bio-adsorbent	[a]102.28±0.45	[a]98.71±0.89	[a]95.42±0.45	[c]55.63±0.32	[b] 83.21±0.18	[d]30.33±0.06
0.2% bio-adsorbent	[a]91.06±0.37	[a]89.41±0.28	b87.66±0.25	[c]54.21±0.43	[b]80.44±1.03	[d]30.18±0.06
0.3% bio-adsorbent	[a]89.27±0.56	[a]86.04±0.05	[a]84.21±0.18	[c]55.01±0.65	b78.28±0.09	[d]30.06±0.04
0.4% bio-adsorbent	[a]83.21±0.76	[a]82.33±0.06	[a]80.09±0.07	[c]52.39±0.52	[a]76.54±0.06	[d]31.06±0.03
0.5% bio-adsorbent	[a]79.17±0.34	[a]78.61±0.16	b76.48±0.04	[c]50.67±0.04	[a]75.35±0.03	[d]30.05±0.56
0.6% bio-adsorbent	[a]76.57±0.06	[a]76.02±0.17	b75.34±0.06	[c]50.17±0.05	b71.09±0.06	[d]29.89±0.42
1% bio-adsorbent	[a]73.87±0.03	[a]72.19±0.16	[a]70.01±0.04	[c]45.29±0.06	b67.43±0.04	[d]27.67±0.06
2% bio-adsorbent	[a]74.32±0.76	[a]70.41±0.16	b65.44±0.03	[c]43.21±0.03	b68.76±0.16	[d]28.46±0.04
Untreated bio-adsorbent	[a]108.56±0.98	[a]109.34±0.76	[a]108.72±0.93	[a]108.25±1.17	[a]110.01±1.03	[a]106.54±1.07
Cu Content (mg/L ±SE) bio-adsorbent	**48h**	**72h**	**1week**	**1week (stirred solution)**	**2week**	**2week (stirred solution)**
0.1% bio-adsorbent	[a]86.55±0.52	[a]80.34±1.63	65.44±1.19	33.67±1.14	62.93±1.54	30.16±0.07
0.2% bio-adsorbent	[a]84.33±1.34	[a]80.11±1.53	64.56±0.06	33.19±2.26	60.87±0.65	30.02±0.07
0.3% bio-adsorbent	[a]80.28±1.05	[a]80.09±2.16	b63.18±1.14	[c]32.45±0.89	b61.76±0.04	[d]30.16±1.09
0.4% bio-adsorbent	[a]76.54±1.18	[a]74.44±1.67	b62.87±1.37	[c]34.78±1.09	b62.33±1.07	[d]31.21±1.17
0.5% bio-adsorbent	[a]76.52±0.94	[a]76.56±1.85	b63.09±1.05	[c]34.89±0.06	b61.14±0.75	[d]29.86±1.54
0.6% bio-adsorbent	[a]75.66±0.96	[a]75.33±0.54	b60.42±0.06	[c]31.29±0.43	b60.56±0.45	[c]30.16±1.33
1% bio-adsorbent	[a]70.34±0.67	[a]70.19±0.62	b58.54±0.54	[c]28.44±1.15	b60.16±0.98	[d]25.44±0.95
2% bio-adsorbent	[a]70.29±0.66	[a]68.31±0.72	b50.89±0.45	[e]29.76±1.11	b60.05±0.07	[e]23.49±0.06
Untreated bio-adsorbent	[a]98.77±1.14	[a]98.84±0.67	[a]99.44±0.87	[a]96.78±1.09	[a]98.16±1.23	[a]96.45±1.89

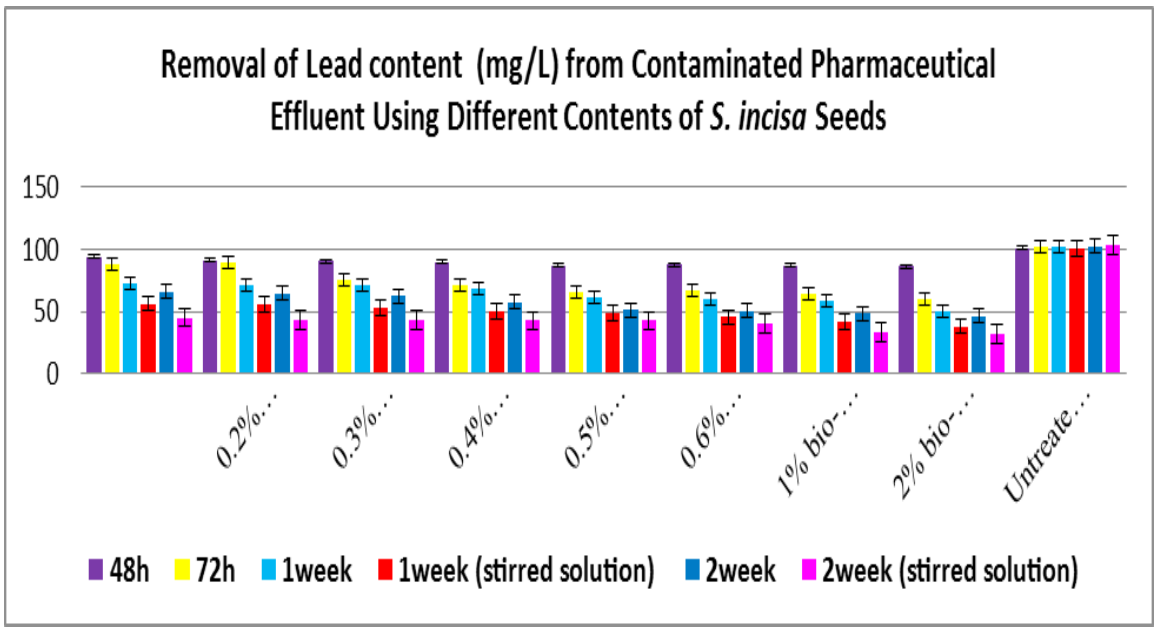

Figure 1- Effect of contact time on the removal of Lead (initial Lead concentration=100 mg/l, bio-adsorbent dose=0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 1 and 2 mg/100 ml of S. incisa seeds, temperature=25 ± 1 ºC, agitation speed= 400 rpm), pH = 4.0.

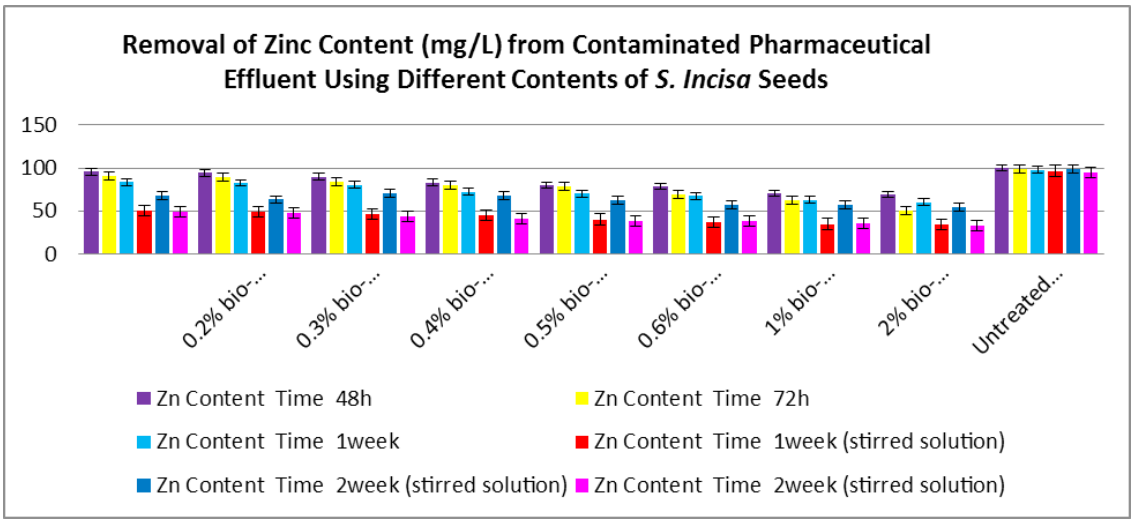

Figure 2- Effect of contact time on the removal of Zinc (initial Zinc concentration=100 mg/l, bio-adsorbent dose=0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 1 and 2 mg/100 ml of S. incise seeds, temperature=25 ± 1 ºC, agitation speed= 400 rpm), pH = 4.0.

analyte in aqueous solution. Due to increases in the initial absorbent concentration, there are more metal oxyanions available for bio-sorption reaction in the solution and our data was the same as previous studies.

For 1 and 2 weeks (2W) stirred exposure samples within the 0.1 to 2% Bio-Adsorbent range, there is a steady decree decrease in solution zinc from around 49% in 1 week stirred to 33% in 2 weeks stirred samples. That is except for the fact at 0.6% and 1% Bio-Adsorbent (Bio-A) zinc increased in

solution very slightly in 2 weeks stirred (2WS) compared to 1W stirred (1WS).

With two anomalies exceptions being 72h and particularly 2W exposures, 48h, and 1W exposure samples without stirring within the 0.1 to 2% Bio-Adsorbent range, there is a general decline of zinc in solution from 96% in 48h exposure, to a low of 60% with 1W exposures and 2% Bio-A, and anomalously low 51% with 72h, while 2W exposure experienced a decline to 56% zinc in 2% Bio-A. Anomalous behavior is seen with 72h and 2W sample exposures herein termed the 72h sample exposures Zn anomaly and 2W sample exposures Zn anomaly respectively. With 72h sample exposures, with over ≥0.6% Bio-A, near equal to and finally significantly less zinc in solution compared to 1W exposures with 2% Bio-A occurs, whereas from 0.1 to 0.5% Bio-A, the behavior is as expected, systematically with more zinc in solution in 72h exposures. The decline in 72h sample exposure solution zinc is more rapid, from 1% to 2% Bi-a, compared to 1W, which is more systematic in decline.

With the 2W sample exposure anomaly, while there is an overall decline in solution zinc within the 0.1 to 2% Bio-absorption range, it is no systematic, with a rise in solution zinc Zinc content in solution in the mid-Bio-adsorption range of 0.3 to 0.5% Bio-adsorption.

72h Sample exposure Zn anomaly

One reason may be strong initial Bio-adsorption site affinity kinetics at 2% concentration become preoccupied with other motives due to competitive affinities, relative to zinc, releasing more zinc to the solution in time.

2W sample exposure Zn anomaly

It is interesting that comparing this aforementioned anomalous inflection between 72h and 1 W exposure with the 2W sample exposure Zn anomaly. For it appears to begin at 0.6% Bio-A, which is conversely the Bio-A concentration that the other anomalous behaving exposure time, comprising the 2W exposure anomaly corrects itself to more systematic zinc solution decline with increasing Bio-A. Prior to this for 2W sample exposures, between 0.3% and 0.5 % Bio-A, solution zinc anomalously increases with increasing Bio-A concentration to finally level nearer to the 0.2% Bio-A Zn levels not until 0.5% Bio-A is reached.

The reasons may once again relate to those explaining the 72hr anomaly relative to 1 W exposure. For 2W exposures, from a low 0.1% to 0.2% Bio-A, solution Zn decline appears normal, presumably where Zn adsorption sites are satisfied without competitive elements. Yet, with higher Bio-A concentrations, adsorption sites may compete more strongly for other motives comprising either elements or complexes. That is until even stronger Bio-A concentration co-hobbit to regain Zn affinity. It is possible, should the Bio-adsorption levels have exceeded 2%, a time depended on competitive kinetic oscillation in declining Zn would appear. This may have occurred at a leveling of solution Zn at 1% Bio-A, before decline again at 2% Bio-A zinc levels. However, the range of analyses suggests this may not be significant ($P \geq 0.05$).

In this context, it may be significant from the see ligand perspectives, as the competitive motive responsible, given control 2W sample exposure samples appear to promote re-absorption of adsorbed zinc back into solution.

Bio-adsorption capacity of Lead, Zinc, Copper, Cobalt, and Nickel increases with increasing initial adsorbent concentration and reaches at maximum after a 2% initial concentration of S. incisa seeds concentration value (Table 2; Figures 1 and 2). As the sorption capacity reaches to its maximum, further increase in initial heavy metals concentration results in a gradual decrease of heavy metals-sorption on the bio-sorbent surface.

However, this research area of using models for resolving nature of heavy metals complexation and sequestrations mechanism at Pb-bio-adsorbent, Ni- bio-adsorbent,

Cu-bio-adsorbent, Co-bio-sorbent, and Zn-bio-sorbent interface have been less explored.

This represents a critically important mechanism in the scientific ability which should be investigated in future research to unravel complex surface heavy metal sorption mechanism on the bio-sorbent's surface by using various chemical modeling approaches.

Contact Time

Biosorption of heavy metals can increase with increasing contact time (Figures 1 and 2, and Table 1). This could be attributed to the transfer of a higher amount of bio-sorbent from solution phase to the bio-sorbent active sites as contact time increases.

Another reason for increased sorption of heavy metals with protracts stirring time could be due to the reduced bound covering covering bond resistance to the transfer of biomass in solution and enhanced heavy metal kinetic energy.

The results of the current study revealed that heavy metals adsorption ranged from 83.5 -91 % after agitation for 1 week (equilibration time), and there was no further significant increase in % sorption of them after the equilibration time ($P \geq 0.05$).

Bio-adsorbent Dose

The bio-adsorbent dose is one of the most important factors, which significantly affects specific uptake of all the heavy metals studied: Nickel, Cadmium, Cobalt, Copper, and Lead from Pharmaceutical effluent (Table 2). Generally, for low bio-adsorbent dose, there is an enhanced heavy metal sorption, especially for Pb and Sorption capacity of different bio-adsorbents have been observed to reduce with increasing bio-adsorbent dose (Table 2 and figure 1 and 2).

A potential bio-adsorbent for Lead and Copper removal must be selected on the basis of its high sorption capacity so that the bio-adsorbent could be reconstructed and rephrased. Bio-adsorbents from agricultural waste and also herbal plants or food industry are considered to be cost-effective and easily available; therefore, post-bio-adsorption disposal of Pb-loaded biomass at landfill sites could be another option. The current research indicated that this could reduce the volume of total biomass and release heavy metals mainly as a safe form.

Conclusion

Heavy metals contamination of pharmaceutical effluent wastewater, which could affect groundwater, is a major health issue and environmental concern at global align, due to its carcinogenic and toxic nature. Treatment of Lead, Nickel and other studied heavy metals, which leads to water contamination, the process of bio-adsorption can play a vital role by providing a cost effective and eco-friendly solution to heavy metals contamination of water constituent. Researchers have studied the potential of various bio-sorbents for heavy metal removal from contaminated water, however, the data on the use of organic solid waste materials (discussed above) as bio-sorbents for remediation of contaminated water is limited. In this review, the effect of solution chemistry including solution pH, cations, anions and other environmental factors (temperature, contact time, dynamic/ static states) which can interact with the biosorption of heavy metals in aqueous solutions has been analyzed. Although limited research has been carried out to evaluate the effect of competing herbal plants on Lead, Nickel or other heavy metals, removal ability of bio-adsorbents. Although little research has been carried out on the potential of herbal plants on removal of heavy metals such as Nickel, Lead and Cadmium from aqueous solutions. The non-conventional, inexpensive and locally available wild herbal plant effective bio-ad sorbents can be utilized alternately as available

common sorbents.

The current research was suggested for the characterization of novel bio-adsorbents from other waste of herbal plants, agriculture/ food-industry with maximum heavy metals sorption capacities to promote the large-scale use of bio-adsorbents.

Further studies are required to increase the understanding of the adsorption mechanism at the bio-sorbent-water interface. The intention of using bio-sorbents could be helpful in the development of an affordable green environmental technology for purification of heavy metals- contaminated drinking water, which could be presented for low-income communities to savor heavy metals-free drinking water to protect them from health hazards and toxins.

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Conflicts of Interest

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

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