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ORIGINAL ARTICLE **OPEN ACCESS**

Biosorption of Heavy Metal by *Ocimum Sanctum* in Waste Water

Nileema Tiwari[1] and Rakesh Kumar Yadav[1*]
[1&1*]Department of Chemistry, Dr. C.V. Raman University Kargi Road Kota Bilaspur (C.G.) 495113 India
E-Mail: nileema1987.np@gmail.com

ABSTRACT
Contamination of heavy metals like Pb, As, Zn, Cr and Fe into ground water or surface water could be cause of toxicity in environment, fauna and flora. Treatment of waste water process use to remove heavy metal contamination in water including treatment of chemical precipitation via ion exchange, membrane filtration, adsorption using activated carbon methods. But those methods were highly expensive and also had various disadvantages like high consumption of energy and inefficiencies of trace amount heavy metals contamination. Biosorption was an alternative method to eliminate trace amount of heavy metals through Ocimum Sanctum. Here, we investigate the ability of Ocimum Sanctum in adsorbing heavy metals of Pb, As, Zn, Cr and Fe from water sample. Where, plant of Ocimum Sanctum(Tulsi) easily obtained from nature. After being fully washed with deionised water, dried it in furnace. Ocimum Sanctumwere characterized through UV-visible spectroscopy and FT IR to determine their functional groups. Effect of adsorption time, adsorbent concentration, and pH also analysed with it. Resultant, Ocimum Sanctum had high ability to adsorb heavy metals. In conclusion, various functional groups, hydroxyl, carboxylic and amine groups, present in leaves of Ocimum Sanctum, which enable to adsorb heavy metals like Pb, As, Zn, Cr and Fe in ground water. Because of fast speed bio adsorption properties of it, and their potential method to treat surface water or post-treatment of wastewater.
Keywords: *Ocimum Sanctum, heavy metals, surface water, biosorption, ground water, waste water.*

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INTRODUCTION

Growth of industries, an essential part of development of any country but it also cause of pollution in environment. Because of industrial wastes, the contamination of heavy metals to ground water had become a major global issue. Discharge of effluents from mining, mineral processing and metallurgical activities were main cause of contamination of heavy metals to natural ground water resources [1]. Heavy metals were toxic in nature and cause of pollution in ground water as well as potential public health problems and risks[2]. Thus, it was highly important to remove heavy metals like lead (Pb), zinc (Zn), copper (Cu) and cadmium (Cd) which were common which found in waste water discharge. Lead, was naturally occurring metal which found in earth. Lead and Copper sulphate could in filtrate water supply through its use in mining industries. However, copper sulphate was extremely soluble in water, Thus, it could be easily seep into water bodies and supplies[3]. Whereas, cadmium was naturally occurring metal and found with metal ores as natural deposits in earth. Their compounds used in various industries like pigments in plastics, an anticorrosive layer electroplated onto steel, electrical batteries and components. It also used to in filtrate the water supply through zinc pipes and solder contained cadmium which used to supply water. Zinc pipes used in pipe water. Low pH in tap water could increase the amount of zinc leaching into the water [4].

Once heavy metals enter into water resources, it persists there for long duration because of its non-degradable nature, as well as also easily enters into human body via human food chain by aquatic environment. Once it entered into living cells, it could be bio accumulated and cause of various health issues like gastro intestinal and kidney dysfunction, vascular damage, immune system dysfunction, nervous system disorders, skin lesions, birth defects and cancer are some of the complications arises due to heavy metals toxic effects [5]. Thus, removal of heavy metals from water has essential for human health. Now, there were common methods which used for removal of heavy metals from water like adsorption [6, 7], ion exchange [8, 9],chemical precipitation [10, 11], membrane filtration [12, 13], reverse osmosis [14, 15], solvent extraction[16]and electrochemical treatment [17, 18]. But some methods necessary for special techniques and some of them highly expensive which limits their use for removal of metals from water.

Adsorption was one of the effective methods due to low cost and high efficiency [19]. The adsorbents like carbon foam [20], activated carbon [21], zeolite [22], clay minerals [23, 24],organic polymers[25], and bio char [26], fly ash[27], reused sanding wastes [28], biomass [29], and water treatment residuals (WTRs) [30, 31],used for removal of heavy metals by adsorption. Out of those adsorbents, bio-adsorbents were highly important for removal of heavy metals from water and waste water. Both terrestrial and aquatic plants could be used for this purpose. Previously, researchers report heavy metal removal from aquatic environment via medicinal plants like *Acacia nilotica* (Babool), *Bacopamonnieri* (Brahmi), *Commiphorawightii* (Guggul), *Ficusreligiosa* (Peepal), *Glycyrrhizaglabra* (Mulethi), *Hemidesmusindicus* (Anantmul), *Salvadoraoleoides* (Jaal, Pilu), *Terminaliabellirica* (Bahera), *Terminaliachebula* (Harītak,Harad) and *Withaniasomnifera* (Ashwagandha) [32-34]. Utilization of adsorption capabilities of a medicinal plant for water purification was one of the cheapest and safe methods. Here, we report heavy metal removal through Tulsi (*OcimumSanctum*) from water.

Ocimum Sanctum commonly known as Tulsi and belongs to member of Lamiaceae family which was highly useful for its therapeutic potentials. In nature, generally two varieties of Tulsi occurs: black (Krishna Tulsi) and green (RamaTulsi); their chemical constituents were almost similar [1].Different parts of the plant (leaves, stem, flower, seeds) used as medicines for treatment of various diseases like bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites etc. It also possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardio protective, analgesic, antispasmodic and adaptogenic actions. Their active constituent was 1-hydroxy-2-methoxy-4-allylbenzene, which commonly known as Eugenol present in the plant is mainly responsible for its therapeutic actions (Figure 1)[35].

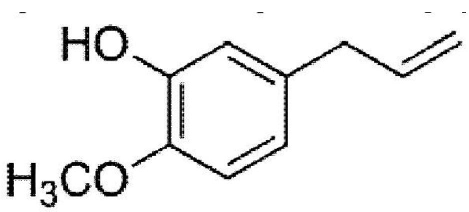

Figure 1. Structure of Eugenol (1-hydroxy-2-methoxy-4-allylbenzene).

Here, we explore the capability of *Ocimum Sanctum* in adsorption of heavy metals like lead (Pb), arsenic (As), zinc (Zn), chromium (Cr) and Iron (Fe) in water medium using deionised water and collected water samples. Characterisations of it conducted via UV-spectroscopy and identify the presence of many functional groups via FTIR analysis. The concentrations of all those metals were measured through latest technology of ICP-OES analytical instrument.

MATERIAL AND METHODS

Chemical and Reagents

Starting reagents were used from Merck 99.8%.All metal standard solutions were purchased from Sigma Aldrich.

Instrumentation

Ocimum Sanctum characterized through UV-spectroscopy, as well as FTIR used for determination of functional groups of samples of it. There was no pre-treatment required for the samples.In the absorbance mode, UV-Visible spectra were acquired using a UV-1900i Double beam spectrophotometer. The vibration spectra were recorded using an Avtar 370, Thermo Nicolet, Fourier transform infrared (FT-IR) spectrophotometer equipped with a DTGS detector with a set resolution of 4 cm^{-1}, and the samples were prepared as KBr discs for this study Solution YC-80 rotary mixing, Sartorius Quintix 224 1S Analytical Balance, plastic spinning tubes.

Methodology

The Tulsi leaves were collected from Korba, Chhattisgarh. The collected leaves were washed with distilled water, then the washed samples were drenched in 3M NaOH solution for 24 hrs. After 24 hours the drenched samples were washed with distilled water and the washed samples were kept in hot air oven for overnight at 60° C. Then the samples were crushed [36].

Figure 2: Plant of *Ocimum Sanctum*(Tulsi).

Batch adsorption Experiment with Synthetic heavy metal solution

10 mg of powder form of *Ocimum Sanctum* was measured through analytical balances which were accurate up to four decimal places and placed directly into the plastic spinning tube. Required amount of deionized water added into it. After that, those tubes were placed on to rotary mixing solution in order to determine the percentage of metal adsorption at various equilibrium times. Before, the appropriate plastic spinning tubes were placed on the rotator, Pb solution was added in tubes to make 2 ppm metal concentration. That whole process also repeated with other metals of As, Zn, Cr, and Fe.

The tubes were rotated at 300 RPM at 45 degrees to mix the whole solution. Once spinning was completed, plastic spinning tubes were set to rest for 5 min to allow particles of *Ocimum Sanctum*to settle bottom of tube. Once particles get settled, solute was extracted and placed into two 15 ml plastic centrifuge tubes and centrifuged for 3 minute at 6000 RPM to force the plant particle to side of the plastic centrifuge tube to separate particles of *Ocimum Sanctum* from the solution. Solution was extracted from the centrifuge tubes via droppers and placed into clean plastic spinning tubes. The remaining concentration of Pb, As, Zn, Cr, and Fe ions in the solution were measured via UV-visible spectrophotometer (Shimadzu 1900 i) at 220 nm, 228 nm, 324 nm, 213 nm and 212 nm respectively. The whole process repeated twice for each metal ion.

For observation of effect of adsorbent concentration via adding 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg sample into 30 ml plastic tubes. 2 ppm of metal standard solution with amount of deionized water was added into it. Rotation in the rotary mixer and centrifugation procedures were repeated, rotation time was fixed up to 60 mins and fully ensure the metal adsorption process. In a subsequent batch experiment, the effect of pH was conducted by altering the pH of spiked solution with NaOH and HCl, ranging from pH 2 to 12 and that process repeated, except a fixed rotation set up at 30 mins.

RESULT AND DISCUSSION

Characterisation

UV-Absorption

UV-vis spectroscopy used for optical properties of *Ocimum Sanctum* (Figure 3). According to UV spectrum, the corresponding measuring range was 400 -800 nm. There were four absorption bands occurs at 417 nm, 437 nm, 468 nm and 665 nm with one hump at 617 nm, respectively.

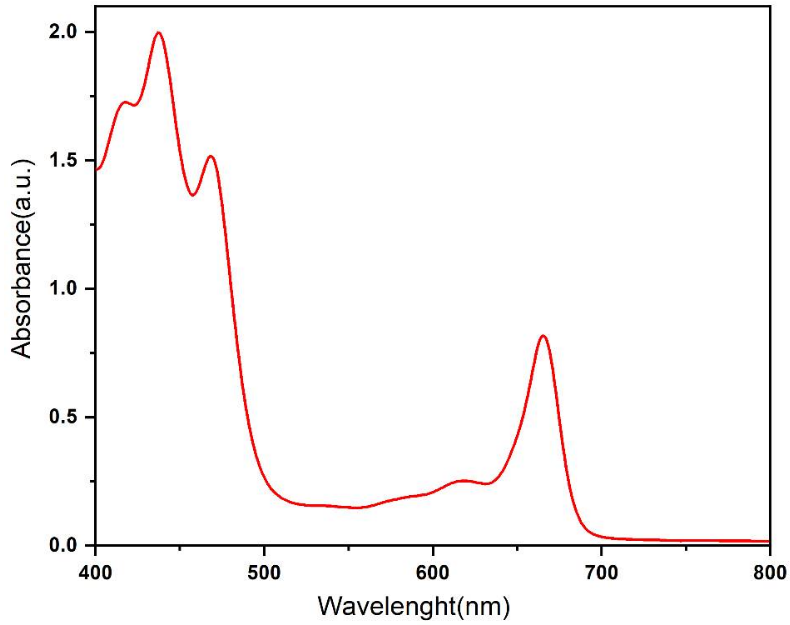

Figure 3. UV-vis absorption spectrum of *Ocimum Sanctum*.

Fourier transformed infrared spectra (FTIR spectra)

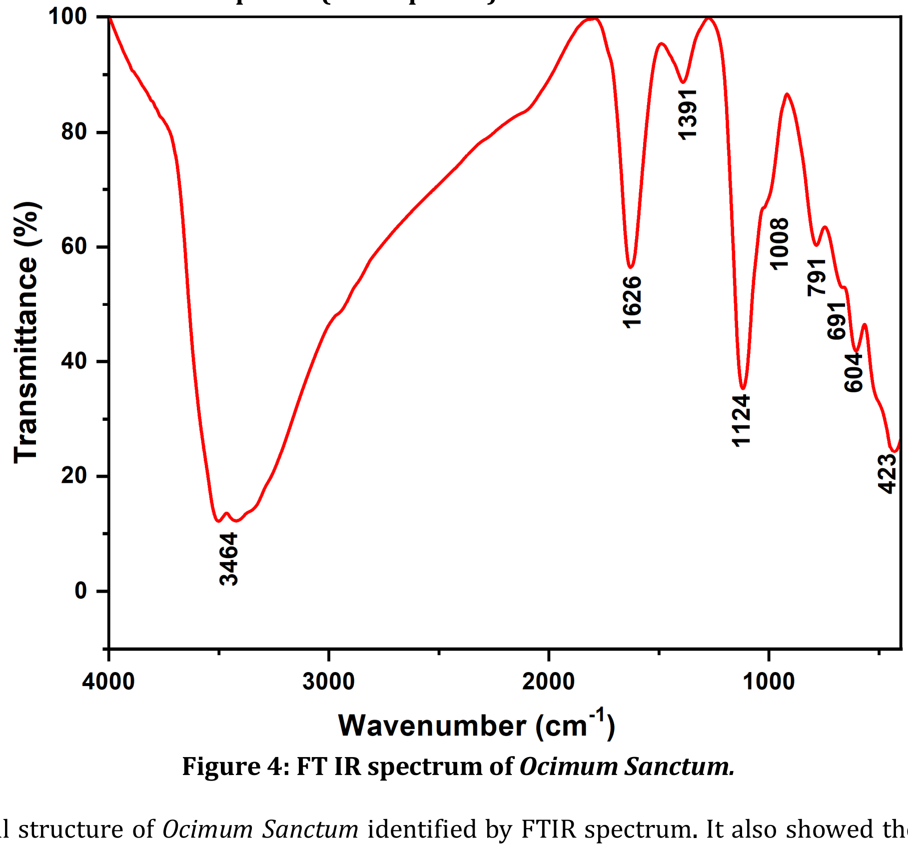

Figure 4: FT IR spectrum of *Ocimum Sanctum.*

The chemical structure of *Ocimum Sanctum* identified by FTIR spectrum. It also showed the presence of various functional groups in it. According to Figure 4, it corresponding the range of 400 – 4000 cm^{-1}. There were several absorption bands observed like; the very strong and broad infrared bands at 3464 cm^{-1} corresponds the broad natured band originated from overlapping of O-H and N-H stretching modes of vibration of water or alcohol present in carotenoid and amide present in all plant and leaves.

Sample showed a weak absorption infrared band at 2944 cm^{-1}region because of asymmetric C-H stretching of CH_3 of alkane. Another very weak and extended band at 2110 cm^{-1} because of asymmetrical

stretching of c ≡ c of alkynes. A small weak band at 1733 cm-1 assigned because of C=O stretching of aromatic ester[37].
A strong band at 1626 cm^{-1} assigned because of C=O stretching vibration of derivative amide. The band at 1391 cm-1 assigned because of C=N stretching vibration . The band at 1124 cm^{-1} correspond as C-O stretching mode in aromatic acetate group [38]. The strong band at 1008 cm-1 assigned as C-O stretching vibration of primary and secondary alcohols in cellulose [39]. A specific absorption band at 791 cm^{-1} assigned because of bending vibration mode of O-N-O of NO_2 group which confirmed the presence of nitro group in sample. The absorption band at 691 cm^{-1} and 604 cm^{-1} which correspond due to ring deformation. At 423 cm band exhibited aromatic H and -CH vibration.
It elucidate the chemical structure and understand the significance of functional groups as bio active constituents for treatment of various diseases. An active constituent which present in *Ocimum sanctum* was Eugenol. The chemical name was 1-hydroxy-2-methoxy-4-allyl benzene. 0.27% of it present in leaves of *Ocimum sanctum* plant. The presence of carboxylic acid in plants depicted as main pharmaceutical agent in treatment of diseases like ulcers, jaundice, headache, stomatitis, fever, edema and rheumatic joint pains. Where, amine, amides and amino acids are the main group of protein synthesis.

Effect of Adsorption time

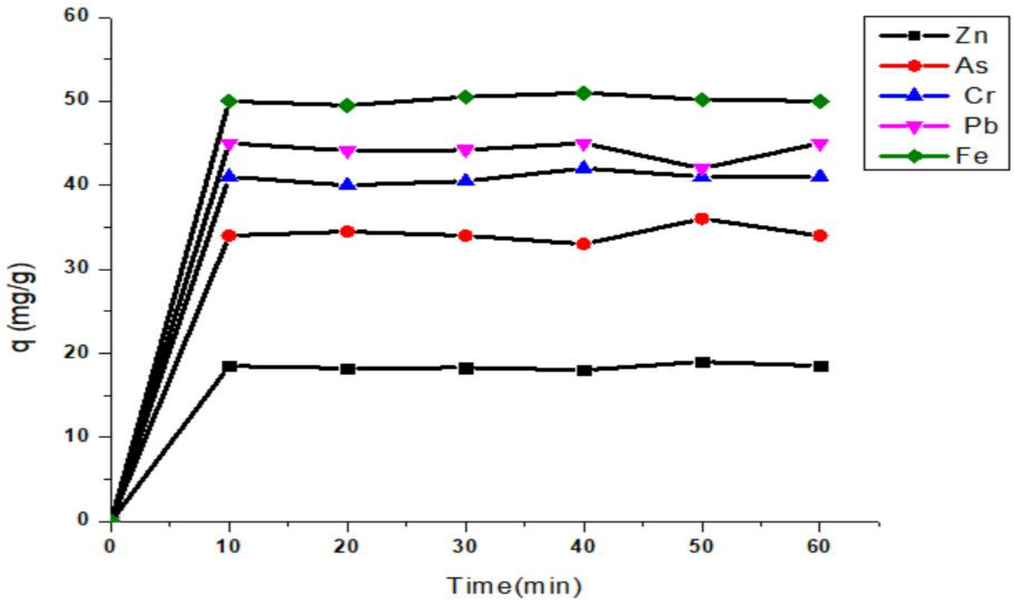

Figure 5. Adsorption Kinetics of Pb, As, Zn, Cr, Fe onto *Ocimumsanctum*. Where, metal salt= 2.0 ppm, *Ocimumsanctum*= 10.0 mg at 30°C, pH=6.5.

Ocimum sanctum was able to absorb much more Fe than Cr, Zn, Pb, and As when tested with each metal in its own solution (Figure 5). Within minutes, the adsorption process had begun, and after 10 minutes, the rate of adsorption had stabilised. Therefore, the subsequent investigation into the impact of pH and adsorbent concentration would employ a minimum fixed time of 60 minutes. The amount of Fe absorbed by *Ocimum sanctum* was 51 mg/g within 10 minutes of reaction. Results showed that after 10 minutes, *Ocimum sanctum* has absorbed 42 mg/g of Pb, 25 mg/g of Zn, 34 mg/g of As and 45 mg/g of Cr. It could also be observed that the percentage of heavy metal adsorbed remained constant throughout the entire 60 min.

Effect of Ocimum Sanctum (adsorbent) concentration on heavy metal adsorption

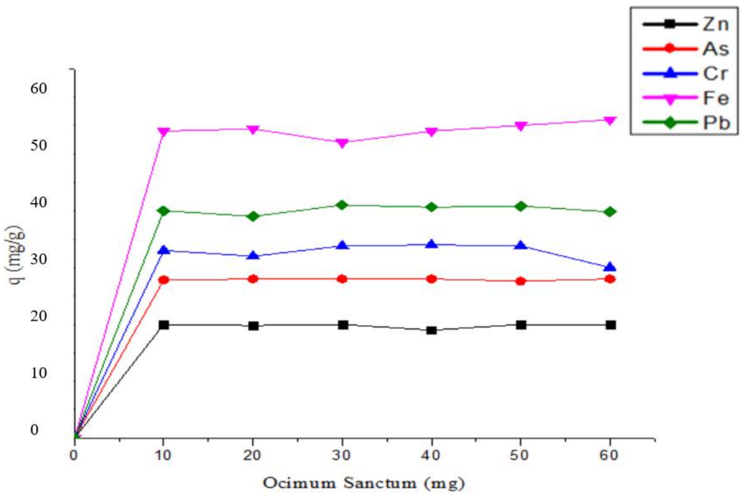

Figure 6. Adsorption Kinetics of Pb, As, Zn, Cr, Fe onto different concentration of *Ocimumsanctum*. Where, metal salt Where, metal salt= 2.0 ppm at 30°C, pH=6.5.

The removal of lead, zinc, arsenic, chromium, and iron are displayed in Figure 6. When the concentration of metals in the water stabilises, a greater fraction of the *Ocimum sanctum* plant may be able to absorb the metals, or reach balance. Figure 6 displays the removal of each metal adsorbed by *Ocimum sanctum* at different concentrations, i.e., blank and 10 mg, 20 mg, 30mg, 40 mg, 50 mg, and 60 mg. The results demonstrated that Ocimum sanctum's ability to absorb metal in water increased with the mass percentage of Ocimum sanctum used. *Ocimum sanctum* was reached a constant plateue around 10 mg. If we compare the absorption efficient with different concentration of Ocimum sanctum, we observed a common pattern for heavy metal removal and lead shows highest removal with least removal of Zn.

Effect of temperature on heavy metal removal by Ocimum Sanctum

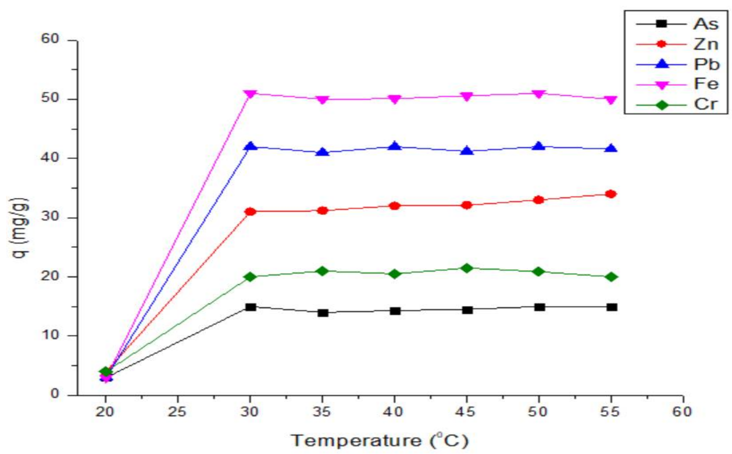

Figure 7. Adsorption Kinetics of Pb, As, Zn, Cr, Fe onto Ocimumsanctum at different temperature. Where, metal salt= 2.0 ppm, Time =60 minutes, pH=6.5.

Ocimum sanctum was able to absorb much more Fe and Pb than Cr, Zn, and As when tested with different temperature (Figure 7). Within 20 minutes, the adsorption process had begun, and after 10 minutes, the rate of adsorption had stabilised. Results showed that between 30-40 °C, *Ocimum sanctum* has absorbed 45-52 mg/g Fe and Pb, 30 mg/g Zn, 19 mg/g Cr and 14 mg/g As shows least removal. There is no much effect of temperature seen for heavy metal removal.

Effect of pH on heavy metal adsorption onto *Ocimum sanctum*

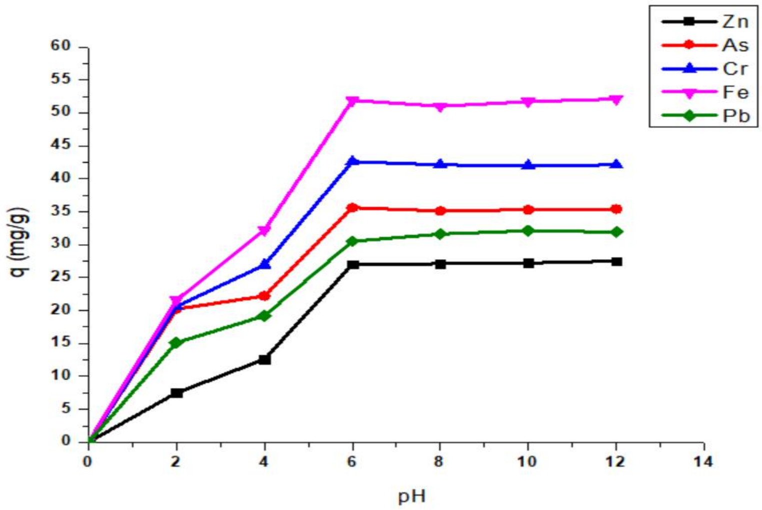

Figure 8. Effect of pH on adsorption of Pb, Zn, Cr, Fe onto *Ocimum sanctum*. [Metal salt]= 2 ppm, [Ocimum sanctum]= 10 mg, at 30°*C* pH=6.5.

With a pH range of 2-12, as shown in Figure 8, Ocimum sanctum has a high capacity for adsorbing heavy metals. Fe showed 6-8 pH at highest concentration of 52 mg/g, similarly, other metals showed pH 6-8 at concentration of 30 mg/g, 34 mg/g, 26 mg/g and 42 mg/g for Pb, As, Zn and Cr, respectively. The results show that Fe had the highest adsorption rate while Zn had the lowest. Maximum adsorption was likewise measured at pH 6.5.

CONCLUSION

At *Ocimum sanctum* (Tulsi) had various functional groups like hydroxyl, carboxyl and amine groups occurs. It was able to adsorb heavy metals like Pb, As, Zn, Cr and Fe from ground water. Because of their absorptive properties and fast adsorption capability, *Ocimum sanctum* had potential to surface water or post-treatment of wastewater. *Ocimum sanctum* had capability to adsorb heavy metals at intermediate pH, ranging from pH 5 to 8. Over all conclusions was *Ocimum sanctum* use as scavenger of heavy metal.

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