



Original Articles

Characterization of Ethanolic Seeds Extract of *Silybum marianum* and Evaluation of Its Ability to Removal Efficiency of Three Heavy Metals from Wastewater

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Abstract:

As an alternative to the traditional methods, which are difficult to manage and need a significant amount of expensive equipment, the purpose of this study is to make use of inexpensive materials such as *Silybum marianum* in order to remove heavy metal ions from waste water. It is a subject of grave worry that heavy metals have the potential to cause harmful impacts on both the environment and the health of the general populace. Recently, biosorption has emerged as a promising and environmentally friendly technology. Among the most significant environmental issues that nations face, heavy metals in their water resources are among the most problematic. There has been a considerable increase in the amount of heavy metal pollution in water resources, which poses a hazard to both terrestrial and aquatic life. The expansion of industrial activity and the strain on the environment are two key factors that have led to this dilemma. Due to the non-biodegradability of metal ions, the toxicity of metal pollution is both slow and endless. It was determined how well *Silybum marianum* adsorbed metal ions including Zn²⁺, Cd²⁺, and Fe³⁺. Various process factors, including sorption duration and pH, were tested in batch studies to determine the adsorption capacity. At a pH of 4, with an initial concentration of 10 mg/L of metal ions, the experimental results demonstrated that the removal percentages of these ions increased in the following order: Zn²⁺ < Cd²⁺ < Fe³⁺ after 90 minutes of shaking.

Keywords: *Silybum marianum*, Heavy metals, Removal of metals, Absorption.

Introduction:

Water is an essential component in all operations carried out by humans, including agricultural production. The amount of freshwater resources available on the planet is extremely restricted, and only 0.6% of the total water resources on the

planet are freshwater [1]. They may not be able to meet the requirements of the many human demands in the future [2]. Freshwater resources have been decreasing at an alarming rate due to increased population development and demand for

fresh water. This is a concern because freshwater resources are essential to human survival. There has been an increase in the amount of wastewater that has been produced as a consequence of the increased demand for freshwater. Instead of being considered a waste that needs to be disposed of, treated wastewater is increasingly being seen as a significant additional water supply for the agricultural, industrial, and municipal sectors. Water and wastewater collection systems are provided in the majority of the cities in Palestine [3]. The past twenty years have seen the construction of a limited number of secondary-level wastewater treatment plants serving a number of cities, while untreated wastewater continues to flow through valleys. Untreated wastewater is still being discharged into the Jordan Valley in a number of other cities, including Nablus east [4, 5]. Because they are located within the boundaries of the municipality, a great number of small industries discharge their effluent into the municipal infrastructure. There are significant levels of heavy metals present in these wastewaters, in addition to other types of contaminants [6]. The usage of milk thistle, also known as *Silybum marianum* (L.) Gaertn, which belongs to the family Asteraceae, for medicinal purposes has been documented as far back as the ancient Greeks. In modern times, a particular concentrated extract from milk thistle fruits, which is also referred to as silymarin, is utilized in the field of medicine. Its primary purpose is to alleviate digestive issues and to provide supportive therapy for liver ailments, as stated by the Committee on Indigenous Medicinal Products. Typically, between 70 and 80 percent of silymarin is composed of a combination of diastereomeric and regioisomeric flavonolignans. This is the predominant component of silymarin. They are generated from flavanonol-taxifolin, which is also included in the combination. Most of them are generated from flavanonol-taxifolin; this includes silybins A/B, isosilybins A/B, silychristins, and silydianin. Multiple in vitro and in vivo pharmacological effects have been shown for flavonolignans isolated from milk thistle. Some of

these actions include protecting the liver, fighting viruses, easing diabetes, protecting the heart, and fighting cancer [7-9]. Still, milk thistle flavonolignans have a very low oral bioavailability. This is due to their high susceptibility to phase II metabolism and their poor water solubility. Due to their abundance and limited absorption in the upper GI tract, milk thistle flavonolignans may interact with the gut flora. They are mainly removed through faeces. The quantities of heavy metals that are found in sewage effluents are often quite low; however, the application of heavy metals over an extended period of time frequently leads to the accumulation of higher metal concentrations in soils [10-12]. On top of harming plants, the application of high amounts of heavy metals can also harm soil and groundwater. Several methods, such as ion exchange, membrane filtration, and adsorption, have been developed for the purpose of removing heavy metals from wastewater [13, 14]. When it comes to purifying polluted water, adsorption is among the most successful processes. Among the several advantages of the adsorption process are its accessibility, affordability, and positive impact on the environment. Using low-cost substances, such as *silybum marianum*, to remove heavy metal ions from effluent is the objective of this research.

Materials and Methods:

Preparation of Adsorbent

In the laboratory, the size of *Silybum marianum* has been reduced to very small sizes, less than one millimeter, after a continuous crushing and fine grinding process. After that, the resulting sieved powder was directly applied as a ready-made natural laboratory sorbent.

Gas Chromatography-Mass Spectrometry

For this study, a GC-MS analysis was performed on the ethanolic seed extract of *Silybum marianum* L. utilizing a Thermo Scientific Trace Ultra GC in conjunction with a Thermo Fisher Scientific SpA. Strada Rivoltana in Rodano-Milan, Italy. A TG-5 column with a film thickness of 0.25 micrometers and an internal diameter of

30 meters by 0.25 millimeters was installed, as per reference [15]. An injection volume of 0.1 microliters was used, and the injector was heated to 230 degrees Celsius. The injection was performed in n-hexane with a split ratio of 1:50. The mass spectroscopy procedure made use of a 40-450 amu range with a power setting of 70 eV. Those are the figures. The retention index (RI), which was calculated by comparing the homologous series of n-alkanes C8-C25 under the same experimental conditions, was used to identify the components. In addition, we compared the results with data from Microsoft publications and searched the Microsoft library (including NIST and WILEY). Using the GC peak area (FID response) as a starting point, a calculation was run to determine components' relative quantities. The adjustment factor, however, was completely disregarded.

Fourier transform infrared spectroscopy (FTIR) analysis of *Silybum marianum*

An FTIR instrument (model/make: IFS 25, Bruker, Germany) has already been used to collect FTIR spectra of native and defatted GLVs in a controlled laboratory setting. A computer-based programme was used for instrument operation and electronic data processing [16]. In preparation for FTIR analysis, in the meantime a small amount of crushed leaf samples was converted into small pellets by KBr, and at the same time a thin layer of them was created by constant pressure. Over a wavenumber range extending from the 4000 cm wave to the 500 cm wave, all data on infrared light transmittance were collected. Meanwhile, three separate chemical analyzes were performed on each sample studied, with simple KBr pellets used as a control. In order to identify the functional groups present in the sample, the spectral data were compared to the reference.

Laboratory preparation of artificial wastewater

A qualitative laboratory chemical analysis approach was used to prepare metal ion solutions in distilled water. Simultaneously, the solutions described before were diluted to meet the specific laboratory concentration standards.

A laboratory calibration curve was prepared for each of the ions (Zn^{2+} , Cd^{2+} , Fe^{3+}) studied in this experiment. The curve was prepared according to their concentration versus the volume of EDTA in order to reach the end point. This curve was used to analyse the results of the current practical experiment [17].

Experimentally achieving single batch adsorption

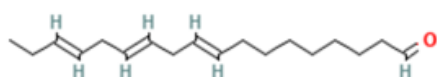
Here is how the targeted metal ion was experimentally generated in the lab by dissolving salt: Each solution contains exactly one gramme of the ion needed by the lab in just one litre of distilled water. Following this, the same solution was supplemented with the concentration of the relevant adsorbent as determined experimentally and vigorously mixed for a duration of one hour. The samples were collected, filtered, and tested for the elimination of the target metal ion immediately afterward. In the meantime, the following phase of the laboratory experiment required this balanced mixture to be held for an additional hour. Lastly, the percentage of elimination was determined by dividing the difference between the original and experimental concentrations of metal ions.

Results and Discussion:

Adsorption is a very effective approach for removing heavy metals from wastewater. In most cases, heavy metals are major harmful contaminants. Because of its high adsorption capacity, activated carbon is a popular adsorbent in the adsorption process. But activated carbons are pricey, so businesses are searching for more economical adsorbents. Many scientists have looked at agricultural byproducts and garbage and found promising adsorbents. Conventional methods of heavy metal removal, such as precipitation, have their limitations; adsorption is considered a sufficient alternative [18–21]. The major constituents of ethanolic seeds extract of *Silybum marianum* were octadeca-9,12,15-trienal, 6-Nonenal, (6Z)-, Santolina alcohol, Dodecane, beta-Bisabolol, 2,6,11-trimethyl, Diethyl dodecanedioate, dien-4-ol, menthyl

acetate, Linalool. By using FT-IR technique the major constituents were: 669.30 (Strong), 684.73 (Strong), 827.46 (Strong), 873.75 (Strong), 927.76 (Strong), 1010.70 (Strong), 1236.37 (Bending), 1313.52 (Unknown), 1417.68 and 2358.94 (Unknown) (Wave number cm^{-1}) with Functional group assignment alkyl halides, Alkenes, Alkenes, alkyl halides, alkyl halides, alkyl halides, Amide, and Amide respectively. A effective removal of the heavy metals was achieved by *Silybum marianum*. A measure of the percent of Zn^{+2} , Cd^{+2} , Fe^{+3} were 38%, 48%, and 70% respectively. While recorded (42%, 25%) for Cd^{+2} and Zn^{+2} , (37%, 63%) for Cd^{+2} and Fe^{+3} , (21%, 57%) for Zn^{+2} and Fe^{+3} , (31%, 18%, 49%) for Cd^{+2} , Zn^{+2} , Fe^{+3} respectively Table 1. With a 25 g/L adsorbent loading ratio, the clearance percentage

was reached within one hour of contact. Competition and contact among the ions led to a low clearance % of mixed ions. The *Silybum marianum* is a cheap, abundant, and effective adsorbent for metal ions. Section 2. The proportion of Zn^{+2} , Cd^{+2} , and Fe^{+3} ions removed from single ions was 43.5, 27, and 65.5, respectively, as shown in the table. Tertiary ions saw their values drop to 34.5, 23, and 60.5, whereas binary ions saw theirs rise to 40-41.5, 24.5-25, and 61-63.5, respectively. The efficiency of removing metal ions decreases as the concentration of mixed ions rises. Mixed ions in the solution compete with one another and interact with one another, leading to a drop in performance.



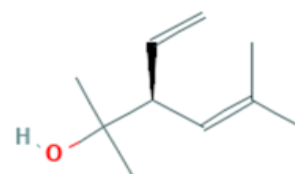
(9E,12E,15E)-octadeca-9,12,15-trienal

Molecular Weight: 262.4 g/mol



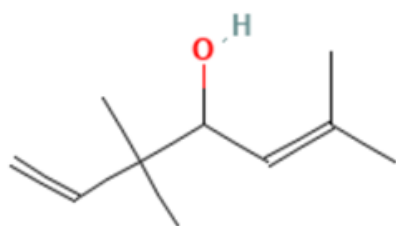
6-Nonenal, (6Z)-

Molecular Weight: 140.22 g/mol



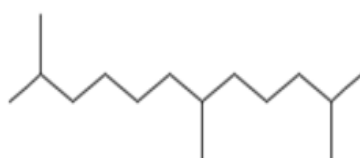
Santolina alcohol

Molecular Weight: 154.25 g/mol



3,3,6-Trimethylhepta-1,5-dien-4-ol

Molecular Weight: 154.25 g/mol



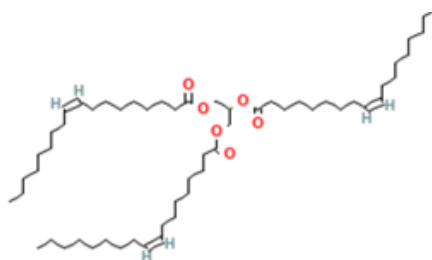
Dodecane, 2,6,11-trimethyl

Molecular Weight: 212.41 g/mol



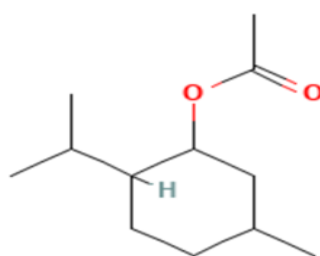
Diethyl dodecanedioate

Molecular Weight: 286.41 g/mol



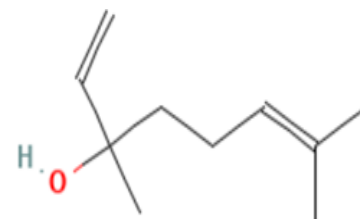
Glycerol trioleate

Molecular Weight: 885.4 g/mol



menthyl acetate

Molecular Weight: 198.30 g/mol



Linalool

Molecular Weight: 154.25 g/mol

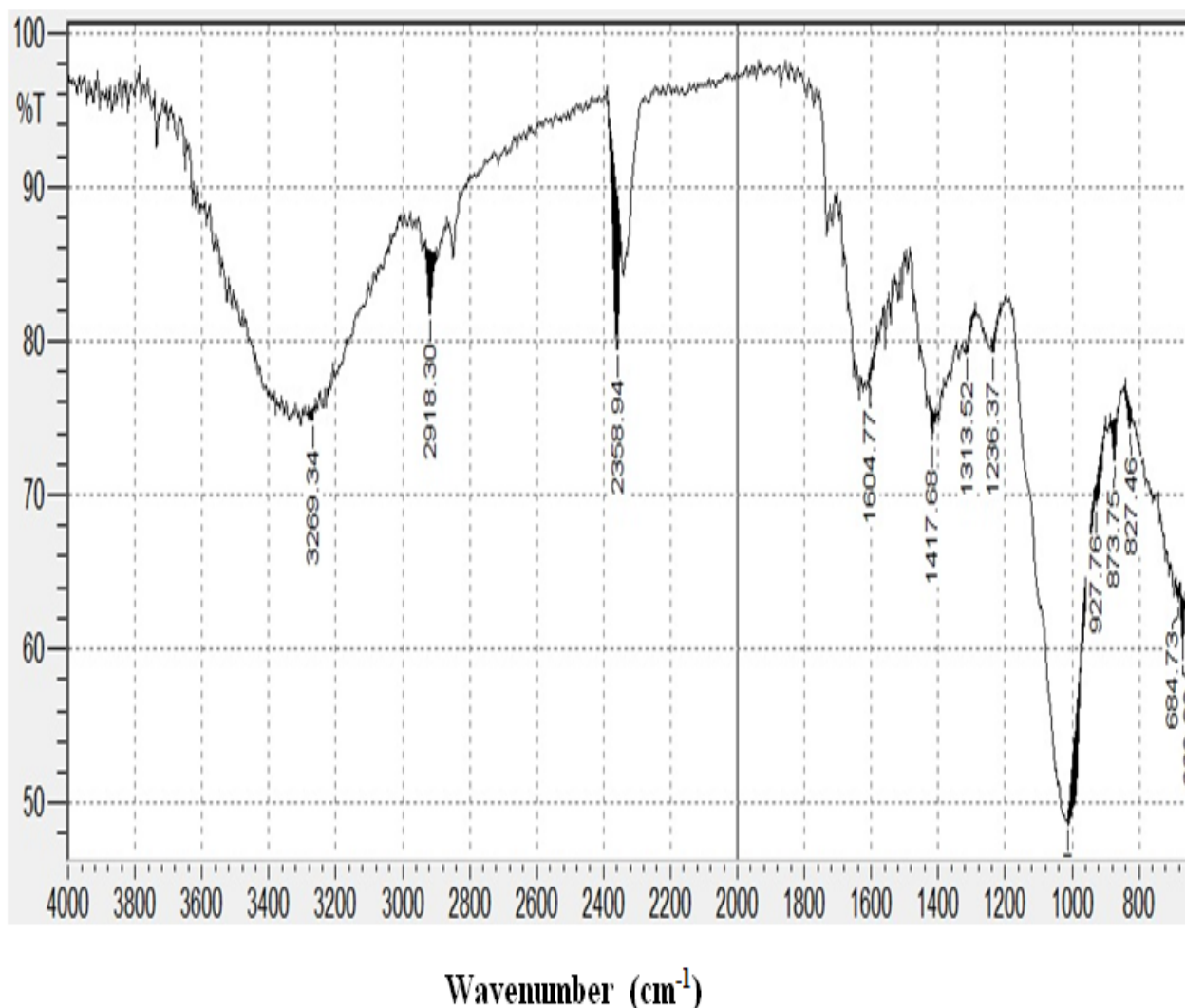
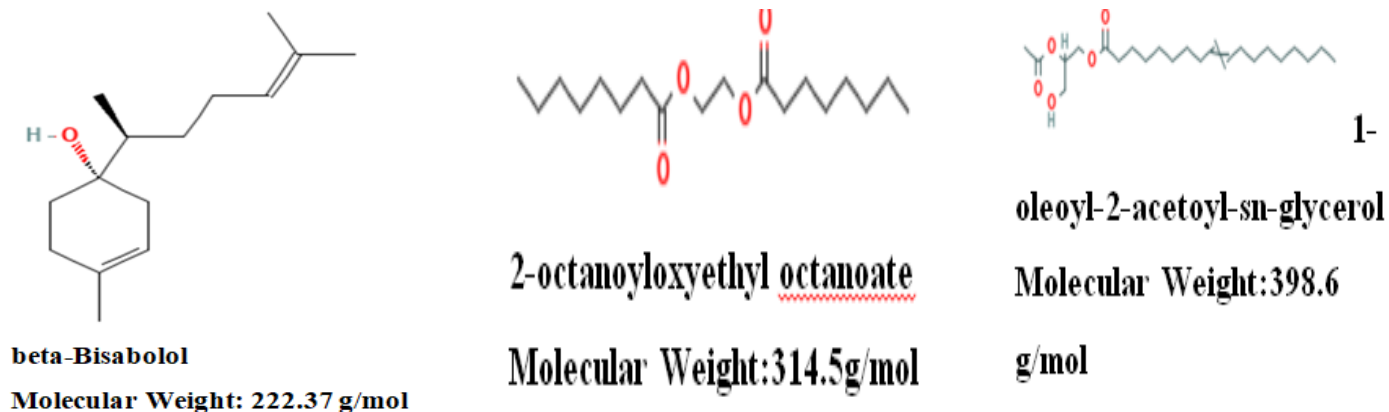


Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Silybum marianum*.

Table 1. FT-IR peak values of solid analysis of *Silybum marianum*.

No.	Peak (Wave number cm ⁻¹)	Intensity	Corr. Intensity	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	669.30	59.416	3.865	2.859	0.176	Strong	C-Cl	Stretch	alkyl halides	600-800
2.	684.73	63.115	0.860	2.285	0.038	Strong	C-Cl	Stretch	alkyl halides	600-800
3.	827.46	74.505	1.152	2.387	0.081	Strong	=C-H	Bending	Alkenes	650-1000
4.	873.75	72.300	2.593	2.555	0.131	Strong	=C-H	Bending	Alkenes	650-1000
5.	927.76	69.360	0.661	4.511	0.180	Strong	=C-H	Bending	Alkenes	650-1000
6.	1010.70	48.730	0.709	18.575	0.677	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1236.37	79.328	0.352	2.182	0.052	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1313.52	79.285	1.798	2.296	0.008	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1417.68	73.681	0.430	2.204	0.073	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1604.77	77.448	9.752	2.817	0.060	Bending	N-H	Stretch	Amide	1550-1640
11.	2358.94	79.466	3.998	2.488	0.880	Unknown	-	-	-	-
12.	2918.30	81.850	1.636	2.468	0.286	Strong	C-H	Stretch	Alkane	2850-3000
13.	3269.34	74.844	0.747	2.381	0.034	Bending	N-H	Stretch	Amide	3100-3500

Table 2. Mixed ion solution percentage removal adsorbent.

Mixed ion conc. (1000 ppm)	Conc. ppm after 1 hour	Removal %
Cd ⁺²	460	48%
Zn ⁺²	590	38%
Fe ⁺³	277	70%
Cd ⁺²	390	42%
Zn ⁺²	543	25%
Cd ⁺²	387	37%
Fe ⁺³	182	63%
Zn ⁺²	538	21%
Fe ⁺³	171	57%
Cd ⁺²	342	31%
Zn ⁺²	521	18%
Fe ⁺³	135	49%

Table 3. Percentage of removal for single, Binary and tertiary ions at same conditions.

Metal ions %	Single	Binary ions	Tertiary
Cd ⁺²	43.5	40-41.5	34.5
Zn ⁺²	27	24.5-25	23
Fe ⁺³	65.5	61-63.5	60.5

Conclusions:

The cheap adsorbent *silybum marianum* is effective in removing heavy metal ions from synthetic wastewater, with the following order of concentration: $Zn^{2+} < Cd^{2+} < Fe^{3+}$. Competition and contact among the ions led to a low clearance % of mixed ions.

References:

1. Sharma J.K., Kumar N., Singh N.P., Santal A.R. Phytoremediation Technologies and Their Mechanism for Removal of Heavy Metal from Contaminated Soil: An Approach for a Sustainable Environment. *Front. Plant Sci.* 2023;14:78.
2. Křen, V., Valentov K. Silybin and its congeners: from traditional medicine to molecular effects. *Nat. Prod. Rep.* 2022; 39: 1264–1281.
3. Hosseinabadi, T., Lorigooini, Z., Tabar zad, M., Salehi, B., Rodrigues, C.F., Martins, N., Sharifi-Rad, J. Silymarin antiproliferative and apoptotic effects: insights into its clinical impact in various types of cancer. *Phytother Res.* 2019;33: 2849–2861.
4. Tvrđý, V., Pourova, J., Jirkovský, E., Křen, V., Valentova, K., Mladěnka, P. Systematic review of pharmacokinetics and potential pharmacokinetic interactions of flavonolignans from silymarin. *Med. Res. Rev.* 2021; 41: 2195–2246.
5. Eid E.M., Galal T.M., Sewelam N.A., Talha N.I., Abdallah S.M. Phytoremediation of Heavy Metals by Four Aquatic Macrophytes and Their Potential Use as Contamination Indicators: A Comparative Assessment. *Environ. Sci. Pollut. Res.* 2020;27:12138–12151.
6. Latif A., Abbas A., Iqbal J., Azeem M., Asghar W., Ullah R., Bilal M., Arsalan M., Khan M., Latif R., et al. Remediation of Environmental Contaminants Through Phytotechnology. *Water. Air Soil Pollut.* 2023;234:139.
7. Nedjimi B. Phytoremediation: A Sustainable Environmental Technology for Heavy Metals Decontamination. *SN Appl. Sci.* 2021;3:286.
8. Kafle A., Timilsina A., Gautam A., Adhikari K., Bhattarai A., Aryal N. Phytoremediation: Mechanisms, Plant Selection and Enhancement by Natural and Synthetic Agents. *Environ. Adv.* 2022;8:100203.
9. Shen X., Dai M., Yang J., Sun L., Tan X., Peng C., Ali I., Naz I. A Critical Review on the Phytoremediation of Heavy Metals from Environment: Performance and Challenges. *Chemosphere.* 2022;291:1329–79.
10. Bortoloti G.A., Baron D. Phytoremediation of Toxic Heavy Metals by Brassica Plants: A Biochemical and Physiological Approach. *Environ. Adv.* 2022;8:100204.
11. Kurniawan S.B., Ramli N.N., Said N.S.M., Alias J., Imron M.F., Abdullah S.R.S., Othman A.R., Purwanti I.F., Hasan H.A. Practical Limitations of Bioaugmentation in Treating Heavy Metal Contaminated Soil and Role of Plant Growth Promoting Bacteria in Phytoremediation as a Promising Alternative Approach. *Heliyon.* 2022;8:e08995.
12. Landaburu-Aguirre Junkal, Garcia Veronica, et al, Applicability of Membrane Technologies for the removal of heavy Metals 2006 *Desalination* 200 pp. 272-273.
13. X. Yang, Y. Wan, Y. Zheng, F. He, Z. Yu, and J. Huang, Surface functional groups of carbon-based adsorbents and their roles in the removal of heavy metals from aqueous solutions: A critical review 2019 *Chem. Eng. J.* 366 pp. 608–621.
14. V. Bahadur, R. Gadi, and S. Kalra, Clay based nanocomposites for removal of heavy metals from water: A review 2019 *J. Environ. Manage.* 232 pp. 803–817.
15. M. Wołowiec, M. Komorowska-kaufman, A. Pruss, G. Rzepa, and T. Bajda, Removal of Heavy Metals and Metalloids from Water Using Drinking Water Treatment

- Residuals as Adsorbents: A Review 2019 Minerals 487 pp. 1–17.
16. Adeyiga Adeyinka A, Hu Liang, Removal of Metal ions from Wastewater with natural wastes. School of engineering and technology, Hampton University, Hampton, VA23668, 2000.
 17. Ibrahim Naimah, et al, Kinetics Studies of heavy metal ion sorption by modified oil palm waste material ICOSM 2007 school of environmental Eng. University of Malaysia.
 18. Wan M.W., Kan C. C., Rogel B. D., Dalida M.L.P., Adsorption of copper (ii) and lead (ii) ions from aqueous solution on chitosan-coated sand 2010 Carbohydr. Polym. 80 pp. 891- 899.
 19. Kong Z., Li X., Tian J., Yang J., Sun S., Comparative study on the adsorption capacity of raw and modified litchi pericarp for removing cu (ii) from solutions 2014 J. Environ. Manag. 134 pp. 109-116.
 20. E. Leita Teresa, Zakharova Tatiana, "Competitive adsorption of heavy metals" simulation in laboratory experiments, Seminar on SOBRE AGUAS SUBTERRANEAS (APRHCEAS), Lisboa, 27-28 de Fevereiro de 2003.
 21. Ben-Ali S., Jaouali I., Souissi-Najar S., & Ouederni A., Characterization and adsorption capacity of raw pomegranate peel biosorbent for copper removal 2017 Journal of cleaner production 142 pp. 3809-3821.