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Using of *Calendula officinalis* L. Plant Extracts in Removing Some Heavy Metals from Polluted Water

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ABSTRACT

In recent years, heavy metals have become major threats to human health. Over the past decades, many traditional methods have been used to remove these pollutants from water, including ultrafiltration, reverse osmosis, ion exchange, solvent extraction, and chemical precipitation. However, these methods have disadvantages such as high cost. In this study, aqueous and alcoholic extracts of *Calendula officinalis* L. were introduced to remove heavy metals from polluted river water as a new environmental sustainability. Five heavy metals (Fe, Ba, As, Mn, and Sr) were monitored, and their concentrations in river water were tested using inductively coupled plasma (ICP) spectrometry. The results showed that the alcoholic and aqueous extracts of *Calendula officinalis* L. were highly effective in removing 100% Fe, 96% Ba, 100% As and Mn, and 92% Sr. The best removal rates were recorded at pH 8, removal time 30min, and extract concentration of 0.3g/ L of contaminated water. *C. officinalis* extract has been shown to be effective in removing heavy elements and is considered a promising future alternative in the field of water treatment.

INTRODUCTION

The high demand for drinking water for various human activities may lead to an increased discharge of sewage into waterways. This increase negatively affects the aquatic environment, particularly in countries that suffer from water shortage problems **(Zaboon *et al.*, 2022)**. Sewage usually enters surface waters due to the pollution of rivers and lakes by organic and inorganic pollutants resulting from industrial facilities discharging their waste directly into rivers **(Salem *et al.*, 2023)**. Pollutants have led to changes in the physical, chemical, and biological properties of water owing to the disposal of various industrial wastes since the Industrial Revolution, especially in the last two centuries **(Alzaidy *et al.*, 2023)**. Recently, industries have produced wastewater that contains a variety of pollutants, including small and large suspended and dissolved solids, organic and inorganic particles, and heavy metals **(Azeez, 2021; Chasib *et al.*, 2021)**. The high toxicity of heavy metals in the soil causes harmful and serious effects on plants and human health. They reach the soil through various means such as excessive use of fertilizers, sewage sludge, and pesticides **(Amjad *et al*., 2023)**. As a result, researchers have turned to environmentally friendly alternatives, such as low-cost methods that do not generate secondary waste or require maintenance and do not require specialized labor, only requiring sunlight as a source of energy **(Al-Nabhan *et al*., 2021)**.

Heavy metal pollution in wastewater is important for many industries, such as metal plating, mining and tanning, chlor-alkali, refrigeration, smelting, alloying, and storage battery industries. On the other hand, toxic heavy metals are pollutants that have a direct and significant impact on humans, animals, and the environment in general. Industrial wastewater containing heavy metals can directly pollute groundwater resources, leading to serious groundwater pollution. Good quality water is essential for human life, and water of acceptable quality is essential for agricultural, industrial, domestic, and commercial use **(Renge *et al*., 2012)**. Recently, considerable attention has been paid to the use of various biomaterials for the treatment of contaminated water and wastewater. The use of original natural adsorbents has become a source of great confidence due to their good performance, high efficiency in absorbing heavy elements, cost-effectiveness, and environmental friendliness **(Madadgar *et al*., 2023)**. Phytoextraction is a widely used phytoremediation technique that uses natural or genetically modified plants to remove heavy metals **(Cao *et al*., 2022)**.

Over the past few years, many researchers have favored the absorption process because of its low cost and high efficiency. Therefore, there is a need to develop absorbent materials with high feasibility, availability, low cost, and high absorption capacity **(Sireesha *et al*., 2023)**. *Calendula officinalis* L., with a long history of cultivation and consumption, is the most prominent perennial root herb of the genus *Calendula* in the family Asteraceae. It is commonly referred to as one of the "Four Masters of Flowers" in China and ranks among the "Four Master Cut Flowers" in the world. It is also considered a natural source of phytochemicals, as it is rich in nutrients such as carbohydrates, dietary fiber, and proteins **(Zhang *et al*., 2024)**.

The present study aimed to use alcoholic and aqueous extracts of the *Calendula officinalis* L. plant to remove heavy elements present in samples taken from the Ashar River section in Basra city, in addition to determining the concentration of some heavy metals before and after treatment with plant extracts.

MATERIALS AND METHODS

1. Materials

Materials used include *Calendula officinalis* powder (local plant), a Soxhlet extractor (China), a pH meter (China), a jar tester (India), river water samples, 36% HCl (India), 99.8% NaOH (India), a water bath (Germany), and a rotary evaporator (China).

2. Method

2.1 Water sampling

The most polluted internal rivers in Basra have been identified. Reports from local and international organizations assessed pollution levels in the Ashar River, one of the oldest tributaries of the Shatt al-Arab Canal, based on studies conducted by **Akesh (2017)**, **Alhello *et al.* (2020)** and **Kahami *et al.* (2023)**. Samples were collected from the Ashar River at three different stations (Fig. 1) along the river in the middle of the city, where pollution has been observed to have increased recently.

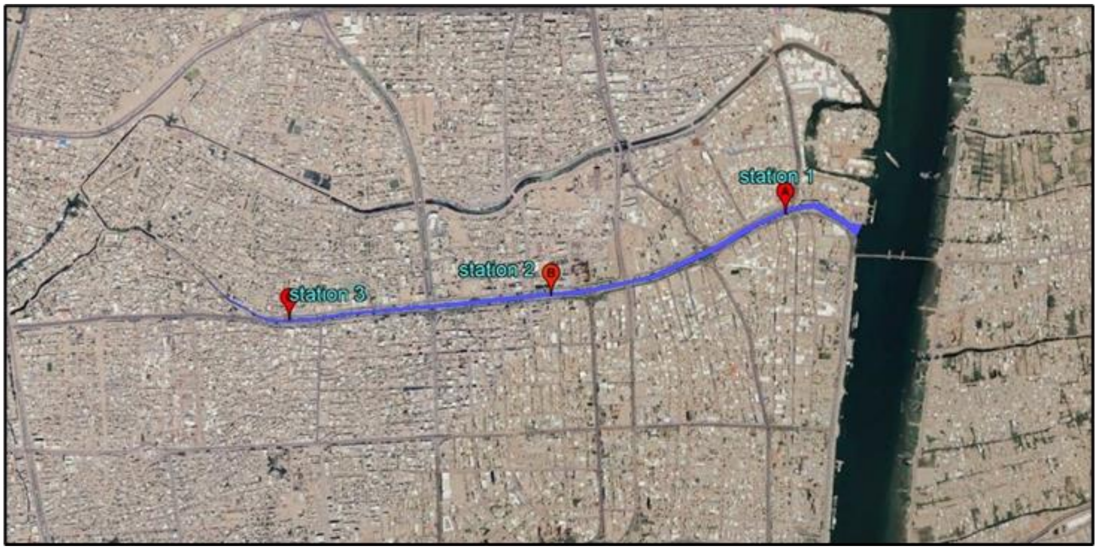


Fig. 1. Location of study area (Ashar River)

2.2 Plant collection and preparation

The *Calendula officinalis* L. plant (Fig. 2) was collected locally from selected nurseries in Basra City to ensure it was free from heavy metal contamination. Five anvils were used to extract the plant from the soil, which was then washed, and the roots and flowers were removed. The remaining green parts, consisting of the stem and leaves, were dried away from sunlight at room temperature, taking 13 days to dry completely. Once dried, the plant was ground in an electric mill and stored in plastic containers for extraction.

Fig. 2. *Calendula officinalis* L. plant

2.3 Extraction method

A Soxhlet extraction device was used to extract active compounds from plants **(Dib *et al.*, 2021)**. It took eight hours for the rosemary plant to be fully extracted using both aqueous and alcoholic extraction methods. Ten grams of rosemary powder were weighed, placed in a paper thimble, and fixed in the main chamber of the device (Extraction Tube). Then, 150mL of distilled water was added for aqueous extraction, and 150 mL of ethanol was added for alcoholic extraction. Both the aqueous and alcoholic solvents were heated to the boiling point to start the extraction process. At the end of the extraction, the volume of the extracted solvent was reduced using a rotary evaporator to obtain a concentrated extract of approximately 20 mL, which was left to dry at room temperature (Fig. 3). The resulting extract was weighed, and the extraction yield was calculated according to Equation 1, as shown in Table (1).

$$Y\% = \left(\frac{We}{Wi}\right) \times 100 \qquad (1)$$

Y%= Extraction yield
Wi= Weight the powder before extraction
We= Weight of the resulting extract

Table 1. *C. officinalis* extraction yield

Extract type	Weight of the resulting extract (g)	Extraction yield (%)
Alcoholic *Calendula officinalis* L.	1.6	16
Water *Calendula officinalis* L.	2.6	26

Fig. 3. *C. officinalis* alcohol extract after drying

2.4 Gas chromatography–mass spectrometry (GC-MS) analysis

The sample analysis was conducted at the Nahran Omar Laboratories of Basra Oil Company, located in the northern part of the city, using a GC-MS system. The Agilent Technologies 7890 B GC was coupled with an Agilent Technologies 5977A MSD featuring an EI signal detector and equipped with an HP-5ms column (5% phenyl, 95% methyl siloxane; dimensions: 30m × 250µm × 0.25µm). The oven temperature was initially set at 40°C for 4 minutes, then increased to 300°C at a rate of 10°C/min for 20 minutes. Helium was used as the carrier gas with a flow rate of 1mL/ min and a purge flow rate of 0.3mL/ min. The injection mode was split, with an injection temperature of 290 °C and a sample volume of 0.5µL. Mass spectrometry was conducted with an ion source temperature of 230 °C and a mass range of 44-650m/ z. Data were recorded using the NIST 2020 and 2014 databases as additional sources for confirming compound identity.

2.5 FT-IR spectrophotometry analysis

Infrared spectroscopy was used to identify the functional groups of the phytochemicals present and compared with GC-MS analysis. Infrared spectra of the plant extracts were obtained in the 4000–400cm^{-1} region using a Shimadzu FTIR–8400 spectrophotometer using the KBr disc technique at the Department of Chemistry, College of Science, University of Basrah.

2.6 Water treatment

A jar test device was used by filling each beaker in the jar test with 100mL of river water sample. Different concentrations of the extract were added to each beaker (0.01 -

0.04g. The stirring speed was set to 200rpm for 4min, followed by 40rpm for 30min **(Lea, 2010)**.

2.7 Optimization of biosorption parameters

The parameters required for the optimum pH value for heavy metal adsorption were determined using 100mL of water. Biosorption experiments were carried out using the jar tester method to optimize the pH, metal ion concentration, contact time, and fixed temperature. Batch studies were carried out using different concentrations of plant extract (0.01 - 0.04g, pH 4 - 9, and contact time 15 min - 60min).

RESULTS AND DISCUSSION

1. Gas chromatography–mass spectrometry (GC-MS) results

The results of the plant extract sample examination revealed the presence of many types of aromatic and aliphatic compounds. Table (2) and Fig. (4) indicate the components of the alcoholic *Calendula officinalis* L. extract, as they showed the presence of compounds with high concentrations belonging to the organic categories of alkanes and aromatic compounds in addition to cyclic esters and amides, with concentrations ranging from 15.63 to 2.10%. Table (3) and Fig. (5) represent the plant components of the aqueous *Calendula officinalis* L. extract, as the presence of active compounds, cyclic and acyclic esters, alcohols, and aromatic and aliphatic compounds was also observed, and their concentrations ranged from 16.62 to 1.52%.

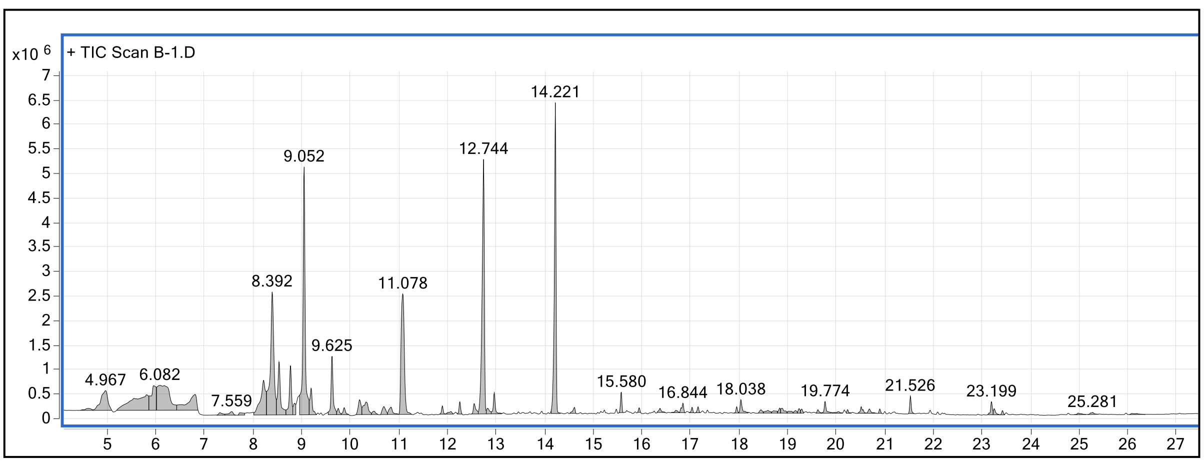


Fig. 4. GC-MS analysis of the alcoholic extract of *C. officinalis*

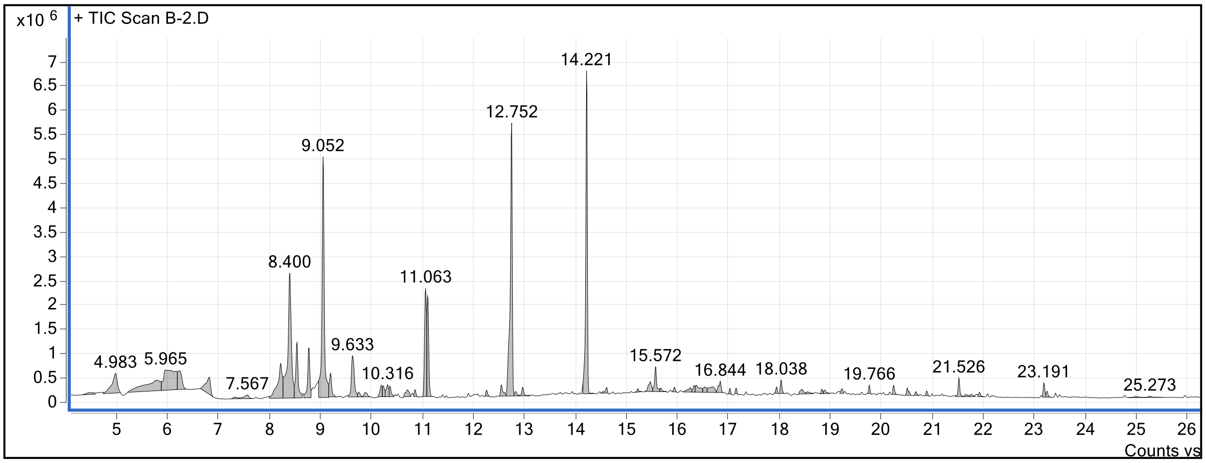


Fig. 5. GC-MS analysis of *C. officinalis.* aqueous extract

2. FT-IR spectrophotometry results

Infrared spectroscopy was used to identify the phytochemicals present in the active groups and compared with GC-MS analysis. The infrared spectroscopy data of the alcoholic extract of *Calendula officinalis* L. showed absorption bands at 1589, 1397, and 1037 cm^{-1}, as shown in Fig. (6). Whereas, the aqueous extract of *Calendula officinalis L.* showed absorption bands at 1593, 1387, and 1076 cm^{-1} (Fig. 7). The first and second bands of the extracts can be interpreted as being due to the aromatic C=C vibration and aliphatic C-H frequency, respectively **(Jasim *et al.*, 2020)**, while the bands at 1037 and 1076 cm^{-1} of the extracts are due to the C-O stretching of the cyclic ester groups **(Hameed *et al.*, 2019)**.

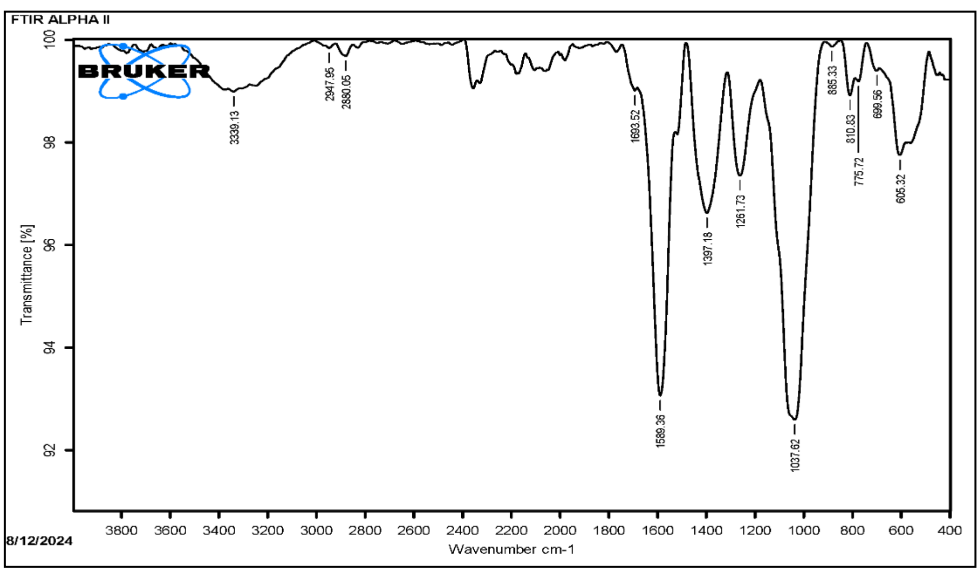


Fig. 6. FT-IR spectrum of alcoholic extract of *C. officinalis*

Table 2. GC-MS data for the alcoholic extract of *C. officinalis*

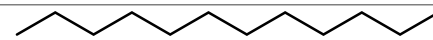
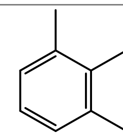
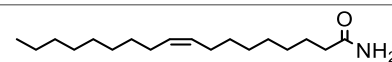
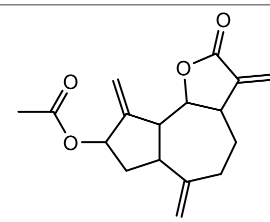
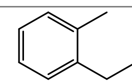
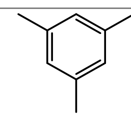
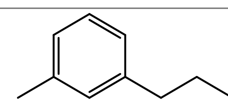
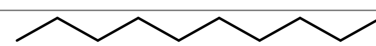
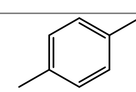
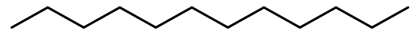
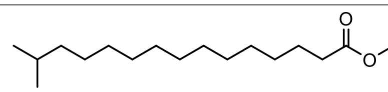
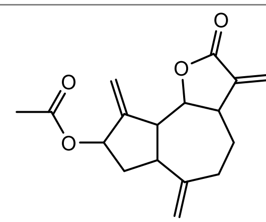
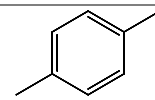
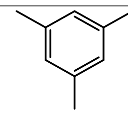
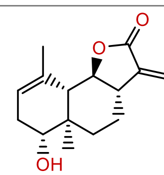
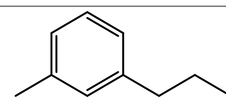
Peak	R.T.	Area Pct	Library/ID	Chemical structure
28	12.744	15.6368	Dodecane	
11	9.052	14.8857	Benzene, 1,2,3-trimethyl-	
31	14.221	14.6037	9-Octadecenamide, (Z)-	
21	11.078	10.9427	(3,6,9-Trimethylidene-2-oxo-3a,4,5,6a,7,8,9a,9b-octahydroazuleno[4,5-b] furan-8-yl) acetate	
7	8.392	7.932	Benzene, 1-ethyl-2-methyl-	
6	8.219	4.1977	Mesitylene	
13	9.625	3.7049	Benzene, 1-methyl-3-propyl-	
8	8.533	2.8668	Decane	
9	8.769	2.1073	p-Xylene	

Table 3. GC-MS of *C. officinalis* aqueous extract

Peak	R.T.	Area Pct	Library/ID	Chemical structure
26	12.752	16.6295	Dodecane	
29	14.221	15.9849	Pentadecanoic acid, 14-methyl-, methyl ester	
8	9.052	13.242	(3,6,9-Trimethylidene-2-oxo-3a,4,5,6a,7,8,9a,9b-octahydroazuleno[4,5-b] furan-8-yl) acetate	
5	8.4	8.3999	p-Xylene	
21	11.063	6.6346	Mesitylene	
4	8.219	5.3266	Santamarine	
9	9.201	1.526	Benzene, 1-methyl-3-propyl-	

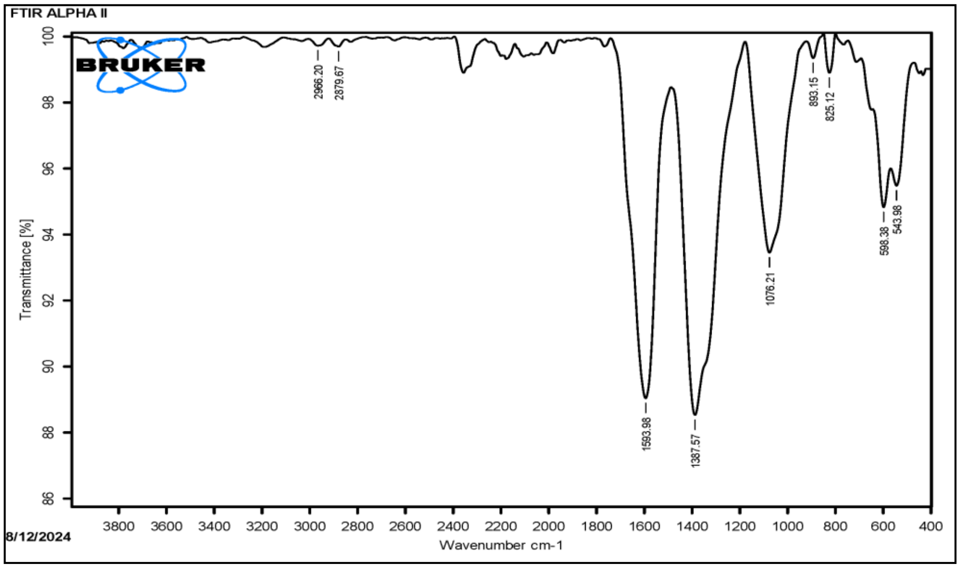


Fig. 7. FT-IR spectrum of C. officinalis aqueous extract

3. Removal of heavy metals by *C. officinalis* aqueous extract

3.1 Effect of contact time

After collecting the selected samples from the Al-Ashar River, a test was conducted on the effect of contact time on the removal of heavy metals from the river water sample for a period of 15–60min. Table (4) presents the results of this study. It can be inferred that the removal of heavy metals (As, Ba, Mn, Fe, and Sr) using both alcoholic and aqueous extracts showed that the highest removal rate at a contact time of 30min was for all elements.

Table 4. Effect of contact time on removal percentage of heavy metals using *C. officinalis* extract

Extract type	Contact time (min)	Removing Efficiency (%)				
		As	Ba	Mn	Fe	Sr
Alcoholic	15	71.06	75.96	57.74	67.40	84.10
	30	100	84.17	100	100	87.58
	45	100	84.79	100	100	87.52
	60	100	84.48	100	100	87.56
Aqueous	15	53.29	87.20	82.94	92.29	89.91
	30	100	96.87	100	100	92.88
	45	100	96.89	100	100	92.86
	60	100	96.97	100	100	92.83

The process of absorbing heavy elements from water using the active compounds present in the plant extract depends on several basic factors, the most important of which is the time factor for interaction, diffusion, distribution, and accumulation of these elements in water. The results are shown in Table (4), where the removal rate was low at 15min, which may be attributed to the uneven distribution of heavy elements in water. With the passage of time, specifically at 30min, the highest removal rate of the elements was noticed, as shown in Figs. (8, 9). This is evidence that the distribution became more homogeneous, allowing for increased opportunities for interaction with the plant extract. These results agree in principle with the results of **Bernard *et al.* (2013)**, and differed in determining the period only due to the difference in the plant used in the experiment.

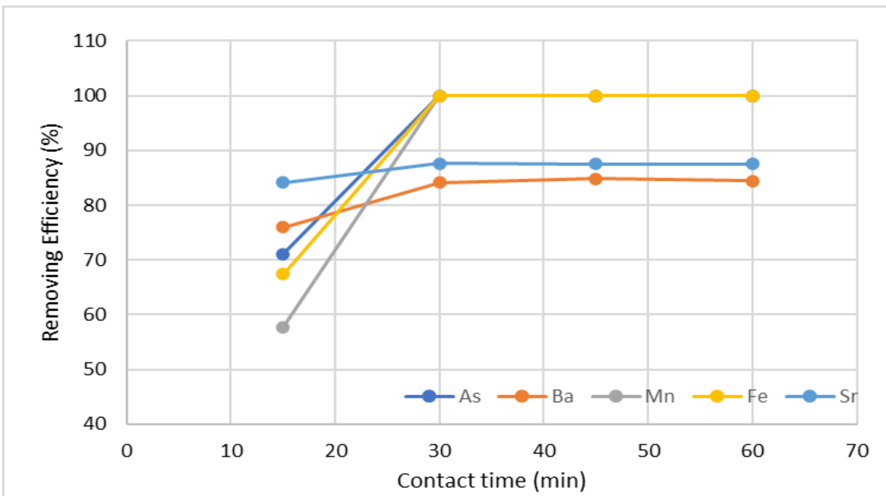


Fig. 8. Effect of contact time on removing efficiency (%) of heavy metals using alcoholic *C. officinalis* extract at pH 8

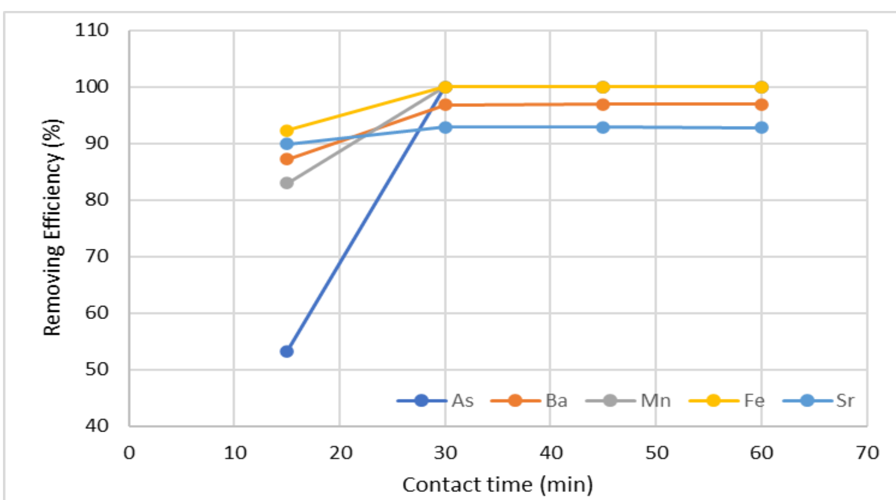


Fig. 9. Effect of contact time on removing efficiency (%) of heavy metals using aqueous *C. officinalis* extract at pH 8

3.2 Effect of pH

The effect of pH levels of 4, 6, 7, 8, and 9 on the removal efficiency of heavy metals was tested using the two types of extracts, as shown in Table (5). The data for the removal of metal ions showed a clear convergence of the removal rate at pH 7 and 8, as shown in Figs. (10, 11). At pH 8, the removal rate increased significantly, which may be attributed to the fact that a basic pH increases the ability of plant extracts to adsorb heavy metals because of the change in electrical charges on the surface of the adsorbents, which enhances the interaction with heavy metals **(Huang *et al.*, 2017)**. In addition, many heavy metals are less soluble in basic than in acidic environments. That is, at pH 8, an accumulation of insoluble compounds or hydroxides may form, which can be easily removed from water **(Zhangabay & Berillo, 2023)**.

Table 5. Effect of pH level on removal percentage of heavy metals using *C. officinalis* extracts

Extract type	pH	Removing Efficiency (%)				
		As	Ba	Mn	Fe	Sr
Alcoholic	4	59.23	71.16	65.09	70.31	84.12
	6	80.86	75.10	73.48	79.94	85.10
	7	82.05	78.15	86.09	87.90	87.30
	8	100	84.78	100	100	87.55
	9	62.18	80.24	76.65	89.71	86.50
Aqueous	4	70.96	78.43	69.22	86.47	89.01
	6	71.07	83.57	74.47	92.20	90.65
	7	82.21	92.36	79.81	95.24	92.39
	8	100	96.86	100	100	92.98
	9	80.45	94.47	87.17	89.40	91.99

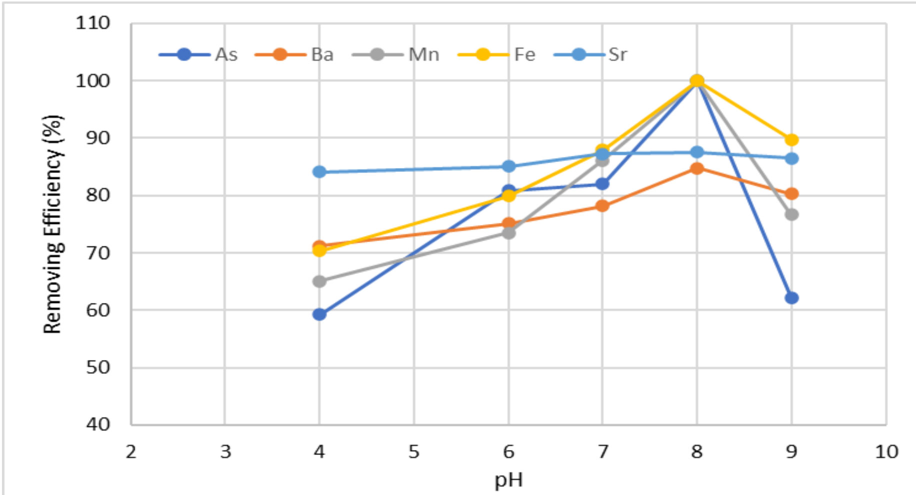


Fig. 10. Effect of contact time on removing efficiency (%) of heavy metals using alcoholic *C. officinalis* extract at contact time of 30min

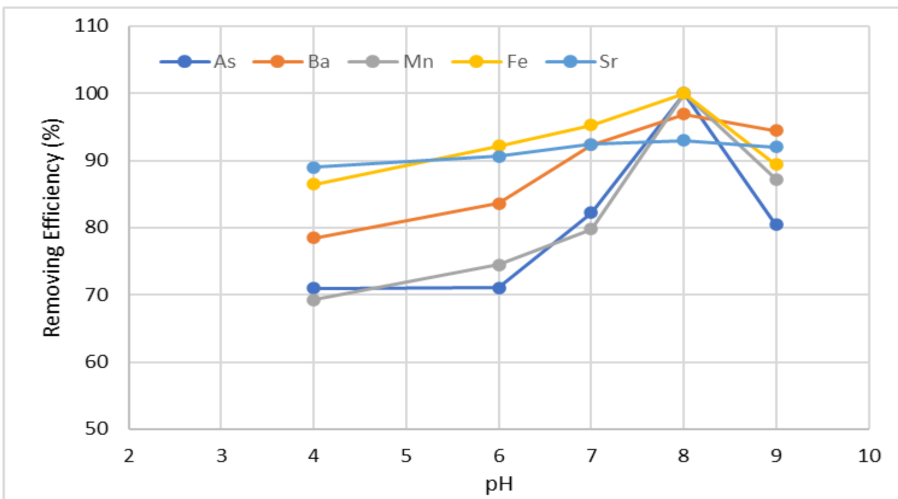


Fig. 11. Effect of contact time on removing efficiency (%) of heavy metals using aqueous *C. officinalis* extract at contact time of 30min

3.3 Effect of extract concentration

The effect of concentration selection was carried out to obtain the best efficiency for removing heavy elements using the two types of extract, and the quantities of 0.1 - 0.4g/ L were tested as shown in Tables (6, 7) using a jar test device. The results showed that the appropriate extract quantity for the alcoholic extract of the *Calendula officinalis L.* plant was 0.3g/ L, while the appropriate extract quantity to achieve the best removal rate for the aqueous extract was 0.2g/ L, owing to the difference in the extracted compounds of the two extracts (Fig. 12). Gas chromatography and infrared spectroscopy showed that the alcoholic extract contained the active compounds alkanes and aromatic compounds in addition to cyclic esters and amides. The aqueous extract contained high concentrations of active compounds, cyclic and acyclic esters, and alcohols, in addition to aromatic and aliphatic compounds. This may be the reason why a lower concentration of aqueous extract became more efficient, whereas alcoholic extract required a higher dosage to achieve optimal removal efficiency of heavy metals from water **(Ak *et al.*, 2021)**.

Table 6. Effect of aqueous *C. officinalis* extract concentration on removal of heavy metals

Concentration (g/L)	**Removing Efficiency (%)**				
	As	**Ba**	**Mn**	**Fe**	**Sr**
0.1	69.12	65.94	63.70	81.91	71.01
0.2	100.00	96.87	100.00	100.00	93.88
0.3	100.00	96.59	100.00	100.00	92.92
0.4	100.00	100.00	100.00	100.00	92.89

Table 7. Effect of alcoholic *C. officinalis* extract concentration on removal of heavy metals

Concentration (g/L)	**Removing Efficiency (%)**				
	As	**Ba**	**Mn**	**Fe**	**Sr**
0.1	60.60	23.01	68.96	51.28	55.88
0.2	100.00	82.75	100.00	100.00	85.22
0.3	100.00	84.78	100.00	100.00	87.61
0.4	100.00	84.45	100.00	100.00	87.19

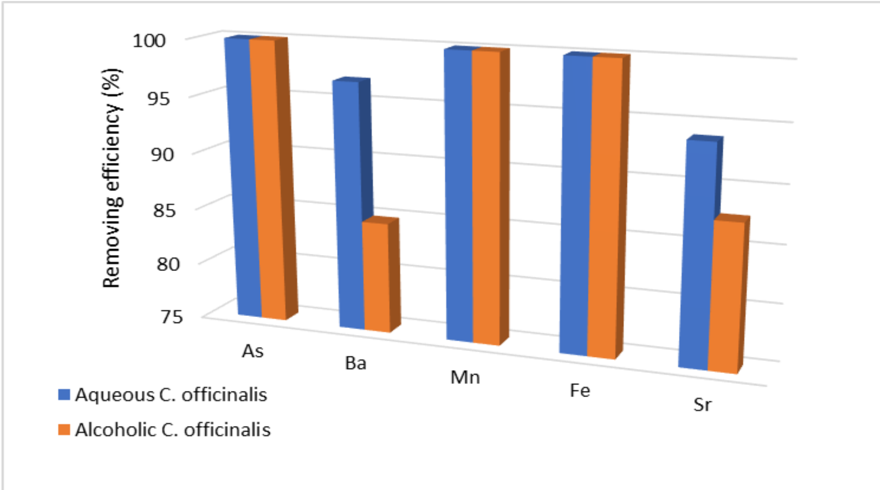


Fig. 12. Removing efficiency of aqueous and alcoholic *C. officinalis* extracts on heavy metals

From the results of the adsorption capacity of alcoholic and aqueous extracts of the *Calendula officinalis* L. plant for toxic heavy metals (Fe, Ba, As, Sr, and Mn), we noticed some important points, as the properties of the extract shown by the results of FTIR and GC-MS indicate that the *Calendula officinalis* extract has good adsorption properties and is effective in removing heavy metals from aqueous solutions. The results also showed that the iron removal rate reached 100%, and this result is completely consistent with what was reached by **Nwagbara *et al.* (2022)**. On the other hand, the percentage of barium removal reached 96%, which was reached by **Azhar (2012)**. The percentage of manganese removal was 100%, and this result is relatively close to what was reached by **Melaku (2023)** upon using the moringa plant to remove manganese from wastewater. The results showed an arsenic removal rate of 100%, which is almost consistent with the results obtained by **Karimi *et al.* (2019)**, whereas strontium showed a removal rate of 92%, which corroborates with some studies **(Hassan *et al.*, 2020)**. The effects of different parameters known to affect adsorption were investigated, and the results revealed that under the specified experimental conditions, all elements showed the highest removal rate at pH 8, contact time at 30min, and concentrations of 0.3g/ L for the alcoholic extract and 0.2 g/L for the aqueous extract.

CONCLUSION

It has been proven that *Calendula officinalis* is a good means for removing heavy metals from heavily polluted river water. Through two parts, alcoholic and aqueous extracts, this plant achieved excellent rates of heavy metal removal, and the rate of heavy metal removal increased until the optimum concentration was used. Moreover, the removal of elements was within the optimum pH of the river water, the pH value was not affected, and there was no need to adjust the pH. Therefore, the *Calendula officinalis* extract can be recommended as a good coagulant and heavy metal removal agent from water.

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