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Fluoride removal efficiency of *Tulsi (Ocimum Sanctum)* from water

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ABSTRACT

Fluoride concentration in drinking water higher than the recommended value imposes different health problems and there are advanced and chemical based defluoridation techniques, even if they are not feasible for developing countries and have limitations. Due to this, defluoridation by using locally available plants is one of the most efficient and sustainable options. Therefore, the current study was intended to investigate fluoride removal efficiency of *Tulsi (Ocimum Sanctum)* from water, which can be an alternative means to reduce the problem related to its high concentration. A laboratory-based experimental study was implemented by using potentiometric determination in Haramaya University. The leaves of *Tulsi* were collected, washed with tap water, rinsed with distilled water, and then dried at room temperature, crushed and sieved through a 500-μm stainless steel sieve. The experiments were conducted on water artificially fluoridated by anhydrous fluoride and natural water samples collected from deep well water sources from Adama and Harar town. Data was analyzed using Design of Expert (DOE) and Microsoft Excel. Twenty-nine runs for aqueous solution were conducted at different factor combinations and the optimum combinations were applied for natural water samples. The study depicts that the plant has an efficiency of removing 68.4% of fluoride from water. The best factor combinations to achieve this efficiency was 0.2 g/100 ml, 22.6 min, 5.7 and 6.6 mg/l, adsorbent dose, contact time, pH and initial concentration respectively. pH and initial concentration have a negative effect and adsorbent dose and contact time have a positive effect on removing fluoride from water. Hence, people living in fluorosis endemic areas can use the processed plant as a de-fluoridating agent to minimize adverse health effects.

Key words: fluoride concentration, fluoride removal, *Ocimum Sanctum*, removal efficiency, *Tulsi*

HIGHLIGHT

- This research article paves the way to further study to remove contaminants from water, wastewater, which can pose a substantial effect on public health. The study was conducted to investigate the removal mechanism of fluoride from drinking water with a low cost, locally available plant and software technologies were applied to find the optimum conditions at which the adsorbent works best.

BACKGROUND

The presence of fluoride in drinking water in minute quantities is essential for normal mineralization of bones and formation of dental enamel (reducing dental cavities, preventing tooth decay). However, it causes various effects if it is found in excessive concentration in drinking water (Dissanayake 1991; Wiem *et al.* 2017; Yadav *et al.* 2019).

Globally, more than 260 million people drink water from sources with high concentrations of fluoride. Moreover, its concentration is very high in countries within the East African rift valley like Ethiopia, Kenya and Tanzania (Malago *et al.* 2017).

In Ethiopia, especially in rift valley areas, over 80% of the children have developed varying degrees of fluorosis (Kebede *et al.* 2016). A recent study in the country shows that the mean level of fluoride in ground water is 6.03 mg/l and the pooled prevalence of dental fluorosis among residents in the rift valley area is 32% (Demelash *et al.* 2019). Because of its health impacts, the World Health Organization (WHO) cut the maximum value of fluoride concentration in water to be 1–1.5 mg/l and any water having higher than this level of fluoride needs to be treated with appropriate defluoridation techniques (WHO 2011).

Different methods have been developed to remove excess fluoride from water; of which ion exchange, coagulation-precipitation, membrane processes (Reverse Osmosis), electrolytic defluoridation and electro-dialysis (Maheshwari 2006) are the common ones. However, the above technologies have their own limitations, mainly incomplete removal and undesirable taste of water after treating (Apparao & Karthikeyan 1986), use of chemicals and generation of toxic sludge (Nayak 2002), reduction of efficiency if other ions exist in the water (Maheshwari 2006), removal of all the ions present in water and increased water acidity, which needs pH correction, and lot of water wasted as brine, which makes disposal of brine a problem (Maheshwari 2006). Most importantly, the technologies are not affordable by the rural community (Berhanu 2006; Dubey & Shiwani 2012; Getachew *et al.* 2014).

Thus, a viable option is to look for low-cost processes that can be built using local expertise and based on locally available and cost-effective, eco-friendly adsorbents for the removal of fluoride from water. Adsorption processes using natural adsorbents or agricultural products become the new alternatives for the removal of fluoride as they are cheap, simple, sludge-free, re-generable, environment friendly, and involve small initial cost and minimal chemical use (Saka *et al.* 2012).

In recent years, various studies have been conducted on the removal of fluoride from water by using different plant species, of which study on *Moringa oleifera Lam. (Drumstick tree)* (Lea 2010; Agnihotri *et al.* 2013; Ravikumar & Sheeja 2014), banana peel, groundnut shell and sweet lemon peel (Ravikumar & Sheeja 2014; Mohammad & Majumder 2014; Ravikumar & Sheeja 2014; Aravind *et al.* 2015), (*Musa paradisiacal)* peel and coffee *(Coffea arabica)* husk (Getachew *et al.* 2014) are a few.

Leaves of *Tulsi* had been used from ages past as a water cleanser, as indicated in different studies (Yevate & Mane 2017; Tamrakar *et al.* 2019). Botanically, the plant is called *Ocimum sanctum*, which belongs to the plant family *Lamiaceae.* In Ethiopia, it is revered for its aroma and flavor but in India, it is venerated for its holiness.

Leaves of *Tulsi* plant yield an essential oil containing eugenol, carvacrol, methyl eugenol and caryophyllene, which possesses antibacterial and insecticidal properties. According to a study conducted in India in 2014, the plant has no toxic effect and peoples can use it for different purposes (Gautam & Goel 2014).

The efficiency of *Tulsi* plant in removing fluoride from ground water has been reported in prior studies (Amgaokar & Kamble 2012; Sudheer & Ahmed 2016). The advantage of using leaves of *Tulsi* (*O. sanctum* herb) for defluoridation of water is to overcome limitations encountering from available technologies such as cost, effectiveness, acceptability, adaptability, affordability, health problems and so on (Sudheer & Ahmed 2016). Moreover, its application is easy and plants are available in most regions in the world; especially, it is a common plant in Ethiopia and India (Pattanayak *et al.* 2010; Kayastha 2014).

However, prior studies on the plant focus on a batch study type that is fixing one factor and varying another factor, with no consideration of the effect of interaction of factors on removal efficiency of *Tulsi* plant. In addition, the studies did not asses its efficacy after adjusting pH and anions like calcium sulphate and nitrates that interfere with the removal efficiency of the plant. Moreover, to the researchers' knowledge, this is the first study to determine the plant's efficacy in removing fluoride from drinking water in the Ethiopian context. Therefore, this study is intended to determine the fluoride removal efficiency of *Tulsi* (*O. sanctum*), a locally available natural adsorbent plant, by using design of expert software in order to support the community residing in rural areas and at the risk of fluorosis health problems.

MATERIALS AND METHODS

A laboratory based experimental study design was conducted to determine fluoride removal efficiency of *Tulsi* (*Ocimum Sanctum)* from water samples in the Department of Environmental Health Science and Department of Chemistry, Haramaya University. The factor range of variables investigated in this study were 0.2–1 g of adsorbent dose, 10–50 minutes of contact time, 1–10 mg/l of initial concentration and 4–12 pH range. In this case, a Box-Behnken for four independent variables, each at high and low levels was employed to fit the model, in which 29 experiments were conducted.

Experimental design

Design of experiments is a very useful tool as it provides statistical models, which help in understanding the interactions among the parameters that have been optimized. Response surface methodology (RSM) is one of the experimental designing methods which can surmount the limitations of conventional methods collectively. RSM is a combination of mathematical and statistical techniques used to determine the optimum operational conditions of the process or to determine a region that satisfies the operating specifications. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions (Ramakrishna & Susmita 2012).

RSM is Response surface methodology, an experimental strategy that is a widely used technique for modelling and optimization, initially developed and described by Box and Wilson, and has been employed with considerable success in a wide variety of situations (Baş & Boyacı 2007). In this study, Box–Behnken design (BBD) was used among Response surface methodology (RSM) to estimate the effect of four process variables on the removal of fluoride.

Preparation of adsorbent (*Tulsi*) powder

First, the fresh leaves of the *Tulsi (O. Sanctum*) were collected and washed with tap water, rinsed with distilled water, and then dried at room temperature (air-dried) before using them as an adsorbent. Leaves were crushed by using a grinder until the sizes of adsorbent become sieve out through 500- μm and sieved through 500-μm stainless steel sieve.

Sample size determination

The number of samples required for testing was determined by using DOE software. Based on this, the software displays to test 29 runs including 5 replicates at center point to increase precision, 100 ml of sample was used for each run at different predetermined factors. Therefore, 300 ml distilled water was taken and aqueous solution of 1, 5.5, and 10 mg/l was prepared. The controls for this study were done by using distilled water and treated in the same manner as for fluoridated aqueous solution at every experiment per a day for 7 days. In addition to this as calibration and control sample, Initial Calibration Verification standard (ICV) was analyzed which is prepared with known fluoride concentration.

Preparation of fluoride solution

Preparation of (total ionic strength acetate buffer) TISAB solution

A stock solution of TISAB was prepared in the laboratory by placing a one-liter plastic beaker containing 700 ml of dH_2O, and slowly adding 57 ml of glacial acetic acid, 58 g of sodium chloride, 4 g sodium acetate, and 0.30 g of sodium citrate to the water. The solution was warm and strongly acidic. Then the solution was titrated to pH 5.2 by slowly adding 0.1N NaOH. Finally, by adding more dH_2O to a final volume of 1.00 liter and become ready to use.

Preparation of stock standards

Stock solution was prepared from stock standard containing a fluoride concentration of 100.0 mg/l by adding 221.1 mg (0.2210 g) of sodium fluoride to a final volume of 1.000 liter. Different standard solutions were prepared beginning with the 100.0 mg/l standard and using the serial dilution method, made a series of stock standards with fluoride concentrations of 50.00, 10.00, 5.000, 2.500, 1.000, 0.500, and 0.250 mg/l. Fluoride standard solution (100 mg/l F^-): Dilute 10.0 ml of 1,000 mg/l fluoride calibration stock solution to 100 ml with reagent water in a polyethylene volumetric flask. Fluoride calibration standards: Prepare a series of calibration standards by diluting the 100 mg/l fluoride standard.

Preparation of aqueous solution

Three liters of distilled water was taken to prepare aqueous fluoridated solution in different concentration by using anhydrous sodium fluoride. In this case, the solution was prepared in three concentration levels (1 mg/l, 5.5 mg/l, and 10 mg/l). These levels were determined by using DOE to try defluoridation efficiency of *Tulsi (O. sanctum)* at different concentration levels. In these trials, 29 Runs were conducted at different levels of the predetermined factors like pH, adsorbent dose, and contact time.

Procedures for testing

Instruments like pH meter and fluoride ion selective electrode were calibrated with buffer solutions. Analytic measuring balance also calibrated to the standard reading. All the materials to be used like beakers, measuring cylinders, flask, stirring glass rod, funnels were washed thoroughly and rinsed by distilled water followed by sampled water to be tested. 100 ml sample water measured and transferred in to each beaker and 5 drops of prepared TISAB solution were added to the samples. Then after stirred completely, the initial fluoride concentrations were measured and recorded. After that each sample was adjusted to the predetermined pH value by using 0.1N HCl acid and 0.1N NaOH in addition to TISAB solution. Then the required amount of adsorbent was added, then mixed by stirring slowly until it distributes completely in the water sample. After that, it was waited for the predetermined contact time before filtration. After the sample water complete the contact time, by using what man filter paper each sample was filtered and the filtrates was collected and analyzed for the final fluoride concentration by potentiometric method of fluoride ion determination using fluoride Ion-selective Electrode (ISE) and the results were recorded in mill volt reading, then changed to mg/l from standard curve equation (Riley 2014).

Percent removal by sorption to the sorbent was computed using the equation:

$$\text{Removal Efficiency } (\%) = \frac{Co - Ce}{Co} * 100 \quad (1)$$

where *Co* is the initial fluoride ion concentration in the water sample before treatment and *Ce* is the final fluoride ion concentration in the water after treatment.

Data analysis

Data were analyzed using latest versions of DOE Version12, and MS Excel. The Box-Behnken design (BBD) from Response surface methodology (RSM) of data analysis was applied to this study.

The factors considered were adsorbent dose, contact time, initial fluoride concentration, and pH whereas the experimental result or the response to treat was the removal efficiency (percentage).

Construction of standard curve

Beginning with the 100.0 mg/l standard and using the serial dilution method, a series of stock standards with fluoride concentrations of 50.00, 10.00, 5.00, 2.50, 1.00, 0.50, and 0.25 mg/l concentrations were prepared. From potentiometric readings of each standard solution, the following equation was developed.

$$Y = 66.981 * x - 286.15 \quad (2)$$

$$R^2 = 0.970$$

where; y = potentiometric reading (mv), X = needed concentration of fluoride (mg/l) to be calculated, 66.981- slop of graph, 286.15-y-intercept, R^2 = correlation coefficient (Figure 1).

RESULTS AND DISCUSSION

Effect of factors and response surface estimation

Perturbation, contour, and 3D surface plots were drawn by using RSM to investigate the effect of all the factors on the responses. In this study, a quadratic model was suggested with 85.97% and 18.10% of maximum and minimum responses respectively whose ratio lets no transformation.

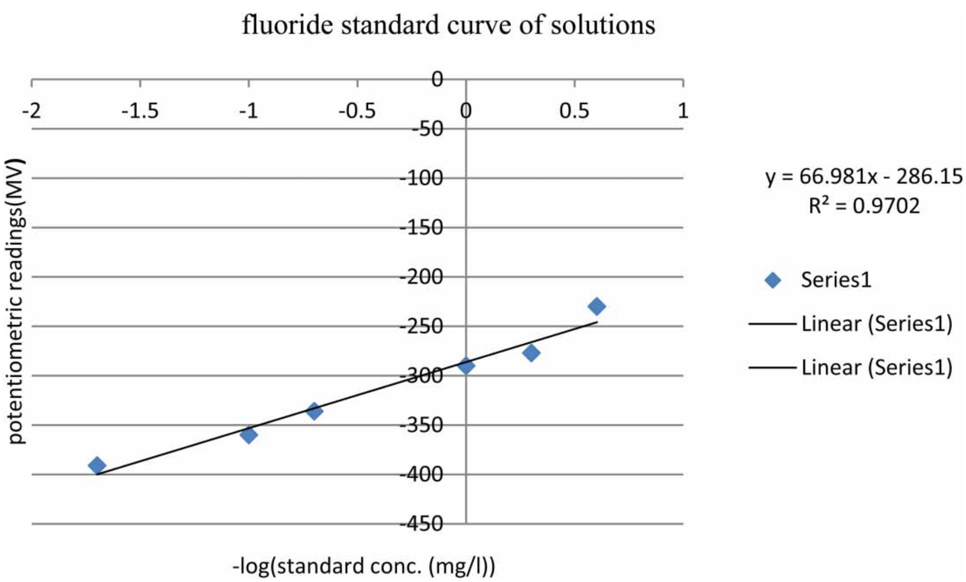

Figure 1 | Fluoride standard curve of solutions prepared.

Fitting the model

Based on the model summary statistics and sequential model sum squares, the quadratic model best fits the experiment and the statistical significance of the model was evaluated by the analysis of variance (ANOVA) as presented in Table 1. The results showed that the regression was statistically significant at *F* value of 54.91. The predicted R^2 of 0.9210 is in reasonable agreement with the adjusted R^2 of 0.9642; that is, the difference is less than 0.2.

Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this experiment ratio of 24.865 indicates an adequate signal. Therefore, this model can be used to navigate the design space. The Model F-value of 54.91 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

The factors and their interactions with *P*-values less than 0.05 indicate model terms are significant. In this case adsorbent dose (A), contact time (B), initial concentration (C), pH (D), the interaction of adsorbent dose and initial concentration (AC), the interaction of adsorbent dose and pH (AD), the interaction of contact time and initial concentration (BC), the interaction of initial concentration and pH (CD), the interaction of contact time (B^2), the interaction of initial concentration (C^2), interaction of pH (D^2) are significant model terms as their p-values are less than 0.0500 as shown table (Table 1). The Lack of Fit F-value of 0.85 implies the Lack of Fit is not significant relative to the pure error. There is a 62.17% chance that a Lack of Fit F-value this large could occur due to noise (Table 1).

The value adjusted determination coefficient (adjusted $R^2 = 0.9642$) is also high, showing a high significance of the model. This also revealed that predicted R^2 of 0.9210 is in reasonable agreement with the adjusted R^2 of 0.9642. The regression equation after the analysis of variances (ANOVA) gave the level of fluoride ion removal as a function of the variables in coded units obtained by the application of RSM is given by: -

$$\begin{aligned}\text{Removal Efficiency (\%)} = {} & +79.64 + 7.03A + 7.54B - 3.89C - 18.22D - 3.39AB \\ & + 8.64AC + 4.52AD - 5.60BC + 2.26BD + 6.26CD + 0.4698A^2 \\ & - 19.18B^2 - 16.23C^2 - 18.73D^2\end{aligned} \tag{3}$$

Table 1 | ANOVA for quadratic model of fluoride removal efficiency from water using *Tulsi (O. sanctum)* as an adsorbent

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value	
Model	11,248.25	14	803.45	54.91	<0.0001	Significant
A-Adsorbent Dose	593.84	1	593.84	40.59	<0.0001	
B-Contact Time	682.47	1	682.47	46.64	<0.0001	
C-Initial Concentration	181.75	1	181.75	12.42	0.0034	
D-pH	3,983.41	1	3,983.41	272.24	<0.0001	
AB	46.07	1	46.07	3.15	0.0977	
AC	298.61	1	298.61	20.41	0.0005	
AD	81.90	1	81.90	5.60	0.0330	
BC	125.23	1	125.23	8.56	0.0111	
BD	20.47	1	20.47	1.40	0.2565	
CD	156.97	1	156.97	10.73	0.0055	
A^2	1.43	1	1.43	0.0978	0.7590	
B^2	2,386.71	1	2,386.71	163.12	<0.0001	
C^2	1,707.95	1	1,707.95	116.73	<0.0001	
D^2	2,275.84	1	2,275.84	155.54	<0.0001	
Residual	204.85	14	14.63			
Lack of Fit	139.33	10	13.93	0.8506	0.6217	Not significant
Pure Error	65.52	4	16.38			
Cor Total	1,1453.10	28				

Model graphs

Perturbation plot as shown in Figure 2 helps to compare the effect of all the factors at a particular point in the design space. A steep slope or curvature in a factor shows that the response is more sensitive to that factor. A relatively flat line shows insensitivity to change in that particular factor (Anderson & Whitcomb 2004). Accordingly, from a plot of perturbation it was observed that, factor 'D (pH)' is more steeply slop curved followed by factor 'B (contact time)' and 'A (adsorbent dose).' Therefore, response variable is more sensitive for these factors.

Effect of independent variables

The linear effects of all four independent variables (adsorbent dose, contact time, initial concentration, and pH) are significant ($P < 0.05$). Thus, each variable in turn can affect removal of fluoride by leaves of *Tulsi* (*O. Sanctum*). In the experimental region, fluoride removal increases with increasing adsorbent dose (with correlation coefficient of 0.23) and contact time (with correlation strength of 0.24). While removal efficiency decreases with increase in initial fluoride concentration (with correlation strength of −0.13) and increase in pH range (with correlation coefficient of −0.59).

Effect of adsorbent dose

Adsorbent dosage is also one of the factors that affect the removal efficiency. As the previous study revealed, it was seen that the removal of fluoride increases with an increase in the amount of adsorbent (Haneef & Kurup 2016). Similarly, in this experiment, the efficiency of removing fluoride ion from water increased from 73.07% by adsorbent dose of 0.2 g/100 ml to 87.14% at adsorbent dose of 1 g/100 ml when other factors kept constant. This increment of efficiency with adsorbent dose is because adding a higher amount of dosage increases the surface area for fluoride adsorption.

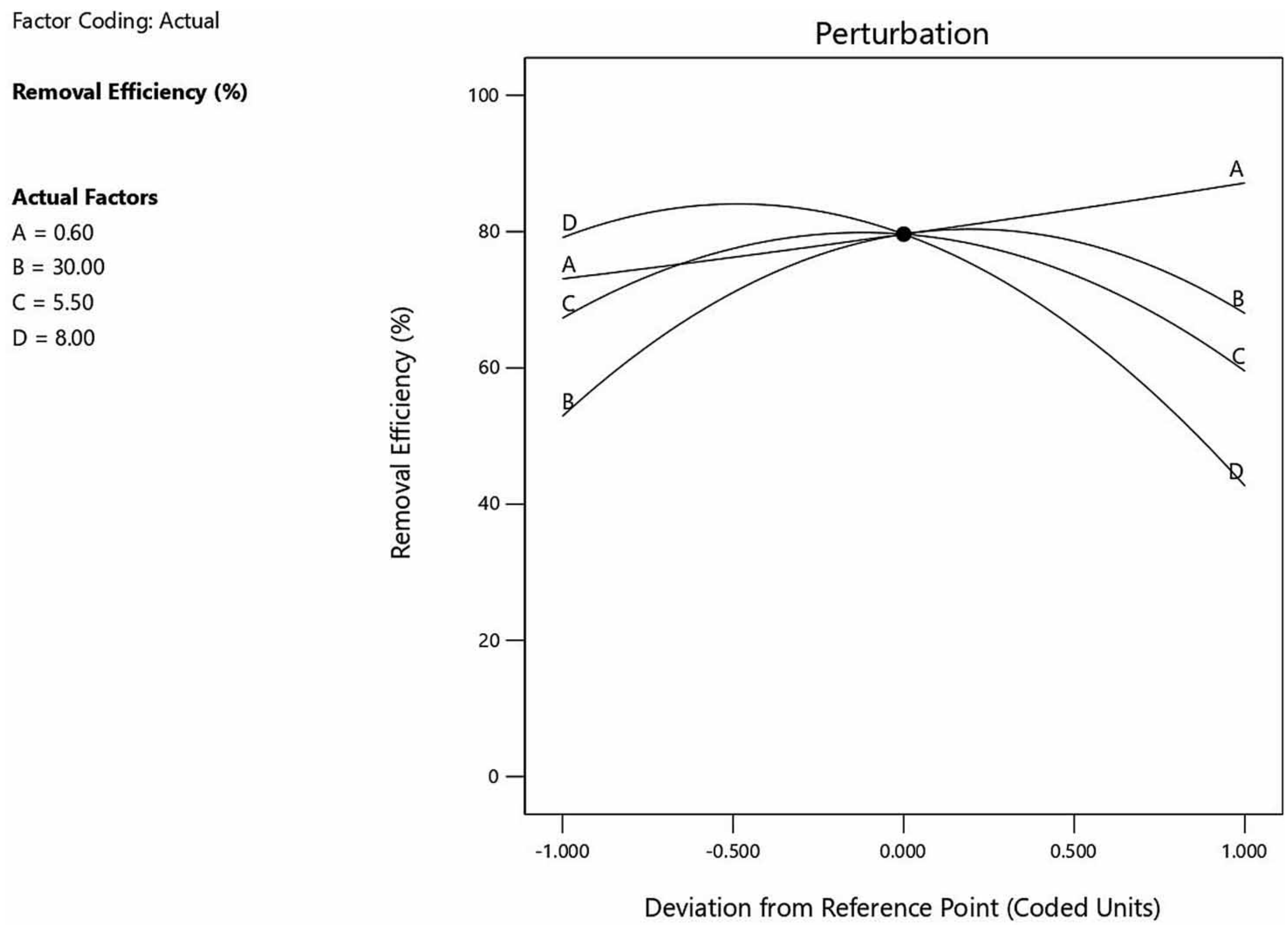

Figure 2 | Perturbation plot showing effect of factors on fluoride removal from water using *Tulsi (O. sanctum)*.

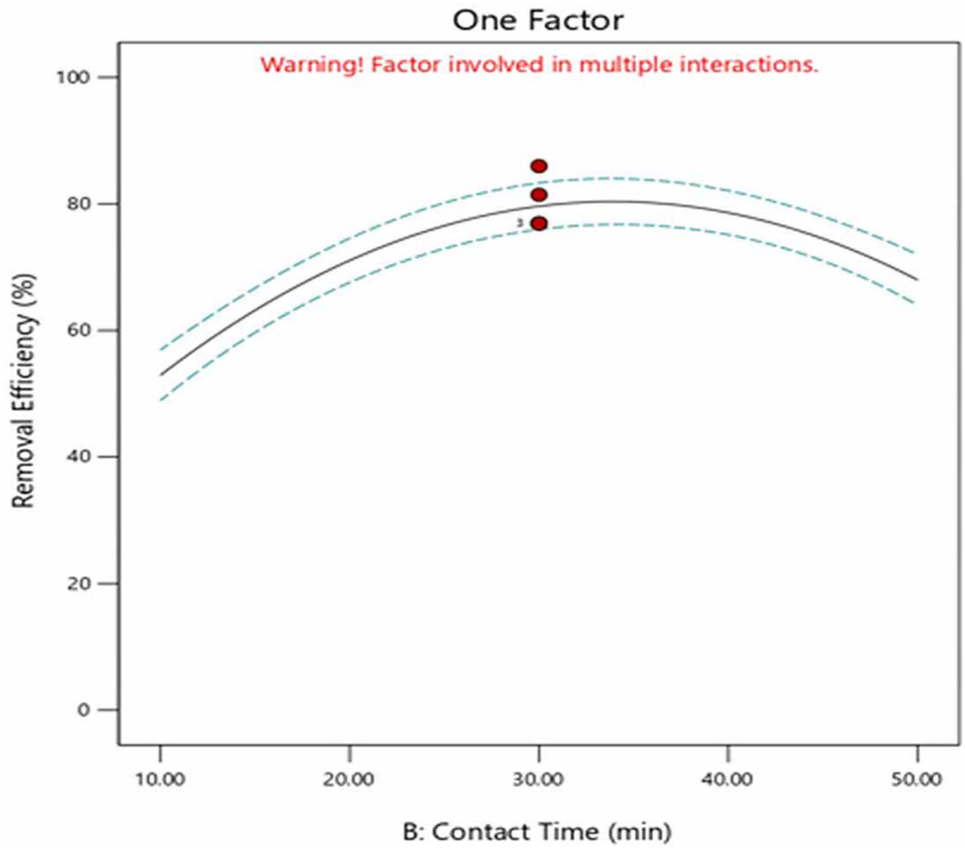

Figure 3 | Illustrates the effect of contact time on fluoride removal efficiency of *Tulsi (O. sanctum)* from water.

Effect of contact time

Contact time that is required to wait after adding adsorbent is also seen as an important factor that has effect on the removal efficiency of fluoride ions from water. Therefore, reduction of fluoride increases with contact time (Malleshaiah & Simha 2015). Similarly, from the result observed, removal efficiency is higher when the contact time waited is higher. As shown in Figure 3, removal efficiency improved from 52.92% at 10 minutes to 80.49% at 32.56 minutes.

Effect of initial concentration

The percentage removal decreases with increasing initial fluoride concentration beyond 5.5 mg/l and changing the initial fluoride ion concentration from 5.5 mg/l to 10 mg/l reduces removal efficiency from 79.76% to 59.52% (Figure 4). This is due to the fact that the adsorbent has a definite capacity and can adsorb only a maximum specific amount acknowledged in prior finding (Yevate & Mane 2017). The result of this study is somewhat lower than the removal efficiency obtained by prior study (Patni & Rambabu 2013), in which removal efficiency was 85.35 with an initial concentration of 2.25 mg/l. This discrepancy may be due to the effect of the interaction of other ions than fluoride ion present in water.

Effect of pH

The level of pH at which the water is treated may play a significant role in defluoridation of *Tulsi*. A study reveals a maximum of removal efficiency (74%) was achieved at a pH of 7 (Amgaokar & Kamble 2012). From this result, Figure 5 illustrates that the maximum removal efficiency was observed at pH of 6.44, which is in line with previous study. At this level of pH, removal efficiency of 84.17%, which is better removal efficiency than the previous study, was achieved. The variation may be that the prior study did not consider the interaction effect of factors. As the pH is beyond this value, the removal efficiency continuously decreased and lowest removal efficiency was observed at a pH of 12, which is 42.68%, suggesting that the plant adsorbed fluoride ions at lower pH. Therefore, this plant adsorbed fluoride ions at lower pH.

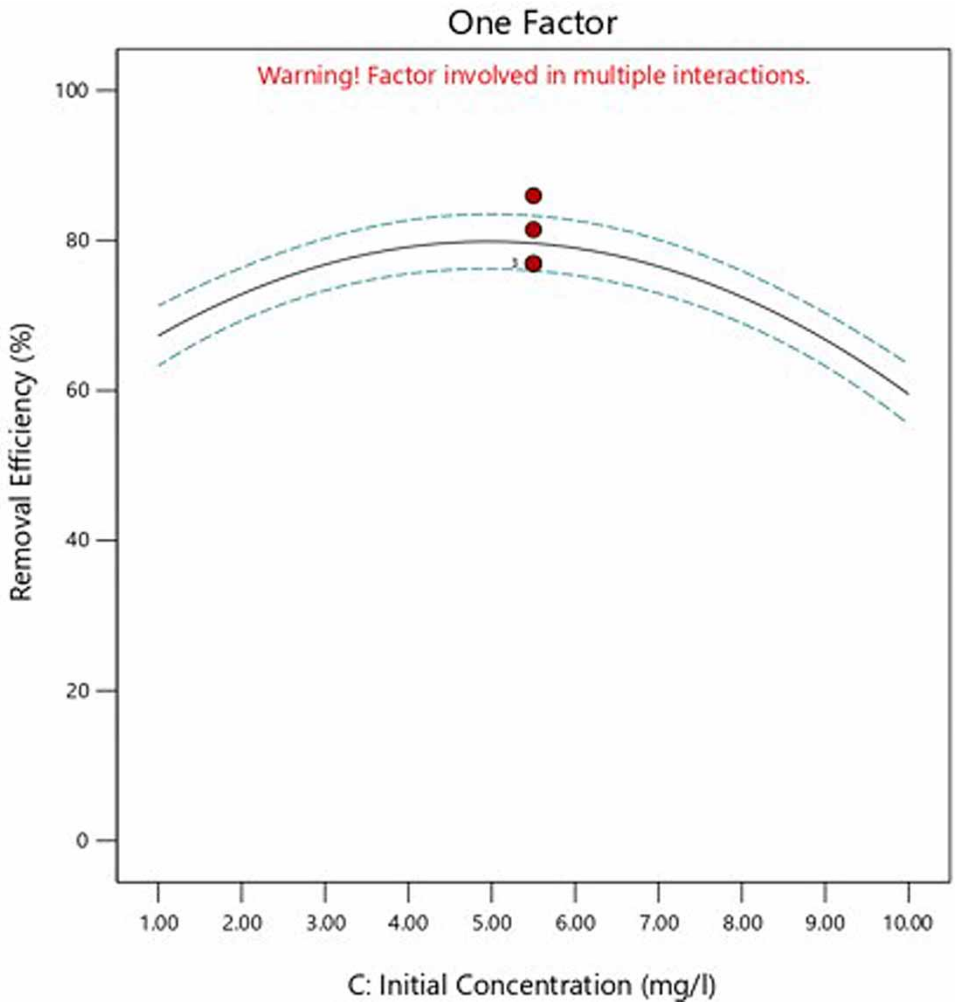

Figure 4 | Illustrates the effect of initial fluoride concentration on fluoride removal efficiency of *O. Sanctum* from water.

Effects of factor interaction

In addition to the main factor effects, interaction of factors also played a significant effect on fluoride removal efficiency of *Tulsi* from water. Interaction effect of initial fluoride concentration and adsorbent dose was shown in a contour plot (Figure 6). Removal percentage increases with decreasing initial fluoride concentration and increasing adsorbent dose. The removal efficiency of 51.51% was achieved at 0.33 gram of adsorbent dose and 9.75 mg/l of initial concentration. As the initial concentration decreased from 9.75 mg/l to 6.17 mg/l and as adsorbent dose increased from 0.33 g to 0.68 g, removal efficiency improved from 51.51% to 80.29% at contact time of 30 minutes and pH 8. In this interaction effect, the maximum removal efficiency 87.28% was achieved at 0.99 g of adsorbent dose, 6.06 mg/l of initial concentration, 30 minutes contact time and pH 8 (Figure 6). This result is higher compared to prior study in which maximum removal efficiency of 23% achieved with adsorbent dose of 0.5 and 1 mg/l initial concentration (Haneef & Kurup 2016). This variation may be due to difference in amount of adsorbent dose and not controlling (making constant) other factors like contact time and pH.

This experimental study reveals that increasing adsorbent dose and decreasing pH value improves the removal efficiency of *Tulsi*. The maximum removal efficiency (88.89%) was achieved at higher dose (0.96 gram) and lower pH value (pH 6.22).

From the graphs (Figure 7) 41.27%, removal efficiency was achieved at 11.4 min, 1.38 mg/l of initial concentration, adsorbent dose 0.6 g and pH 8. Increasing contact time from 11.4 minutes to 34.72 minutes removes the maximum amount (80.77%) with higher initial concentration of 4.79 mg/l.

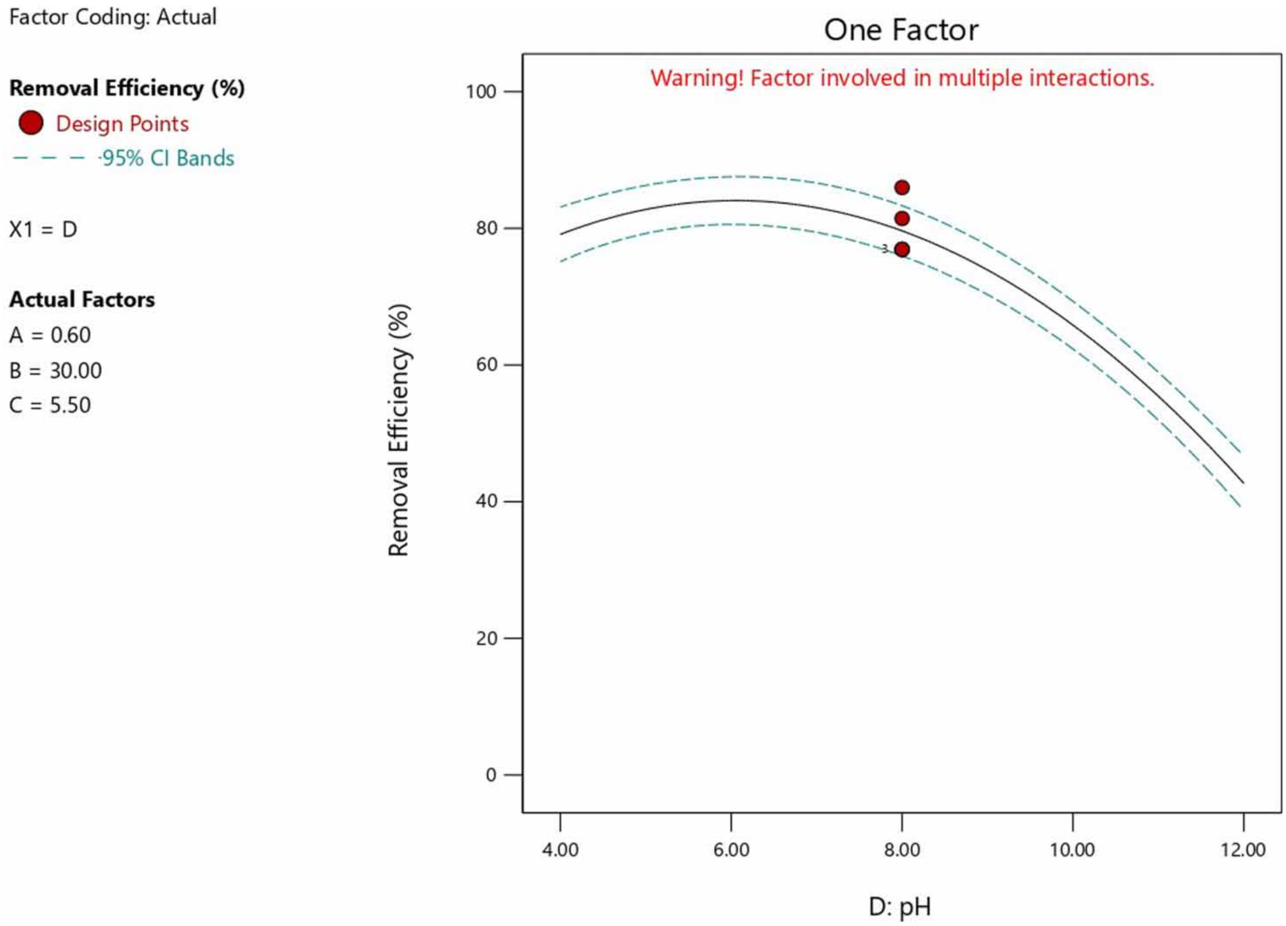

Figure 5 | Illustrates the effect of contact time on fluoride removal efficiency of *Tulsi* (*O. Sanctum)* from water.

This experimental study also shows that to achieve the higher removal efficiency (84.85%) with a higher initial concentration (4.52 mg/l), the pH was decreased from 11.72 (at which 34.2% removal efficiency was achieved with an initial concentration of 1.85 mg/l) to pH 5.91 by keeping contact time and adsorbent dose at 30 min and 0.6 g.

Process optimization

In the Design Expert software's (DOE) numerical optimization, the possible goals are maximizing, minimizing, target, in range and set to an exact value (factors only). Therefore, in the present study, the desired goal for each factor as well as the response function was selected from the menu.

A weight is usually assigned to each goal in order to adjust the shape of the particular desirability function. The goals were then combined to an overall desirability function. Therefore, in this study, the desired goal for each factor as well as for the response function was selected, which was to treat maximum fluoride concentration. Therefore, it was set a goal maximum for initial fluoride concentration, pH in a range, contact time minimum, and adsorbent dose minimum in order to treat maximum initial concentration with minimum adsorbent dose, minimum contact time, and finally pH in range. The DOE software output for the desired goal is presented in Table 2.

Finally, 35 optimum factor combinations were generated by DOE version 12 software and the first option with a combination of 0.2 g, 22.66 min, 6.79 mg/l of initial concentration and pH 5.67 was selected as a best factor combination, at which removal efficiency was 68.4% with a desirability of 0.754.

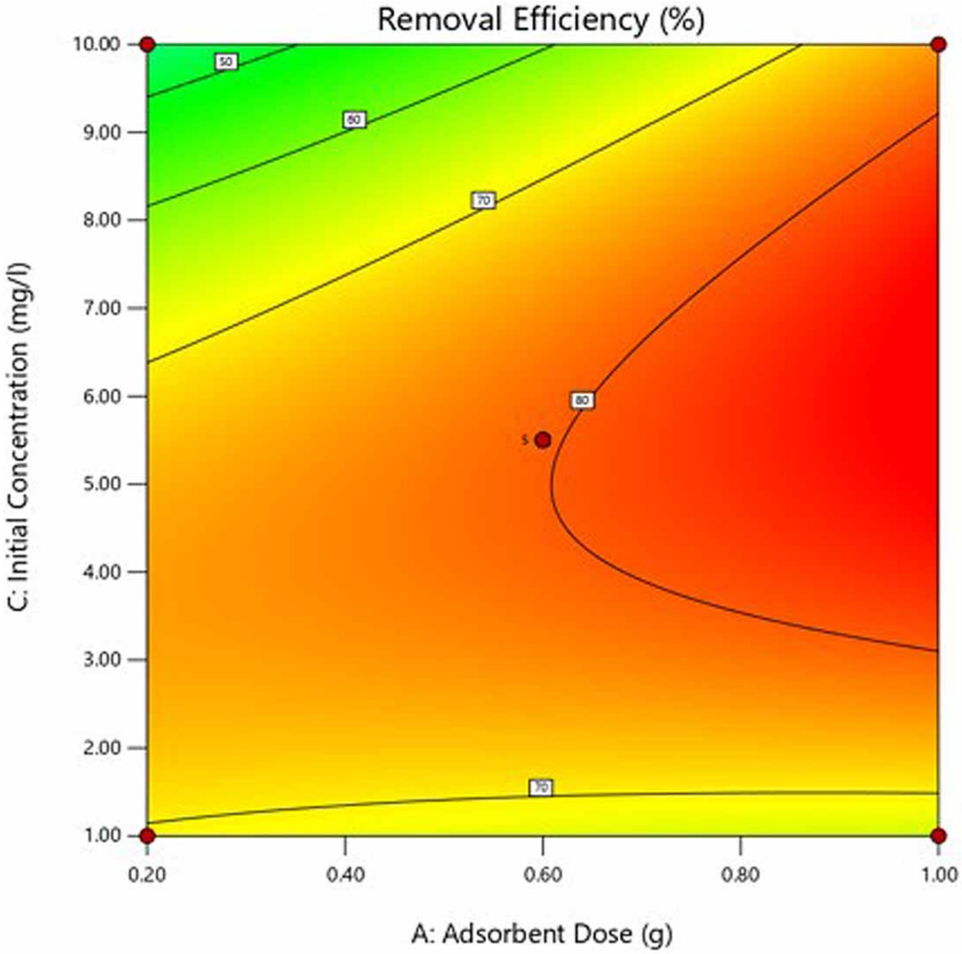

Figure 6 | Contour plot showing the interaction effects of adsorbent dosage and initial fluoride concentration in determining fluoride removal efficiency of *Tulsi (O. sanctum*) from water.

Experiments for validation of models

In order to test the model validation, among different factor combinations, four combinations were purposely selected for confirmatory experiment, as indicated in the Table 3. The experimental values closely agreed with the predicted values of developed models with acceptable percentage errors and the details are given in Table 3.

Application of *Tulsi (O. Sanctum)* for removal of fluoride from natural water

To be more confident and to say, '*Tulsi (O. sanctum)* has defluoridation effect on fluoride water' a batch adsorption experiment was conducted on natural water, sampled from different sources. Water samples were collected from Adama town, where high fluoride concentration in ground water is common, and the second sample was taken from Harar town from a deep well water source.

Onsite testing was conducted immediately after sampling by using the Paline test method to understand the transportation effect on fluoride concentration. In the laboratory, before treating it with the prepared adsorbent, initial concentration and pH was measured and it was found as observed in Table 4.

The adsorption process was conducted on 100 ml of water sample from each sampling source with *Tulsi* dose of 0.2 g. The suspensions were stirred gently using a glass rod stirrer with the same process as the aqueous solution then maintained for a contact time of 22.66 min. Before doing this, 5 drops of TISAB solution were added to control interference of other ions and to adjust pH to 5.67 as obtained from an optimization study of the batch experiment on the initial concentration of 2 mg/l and 1.2 mg/l for both sample sources.

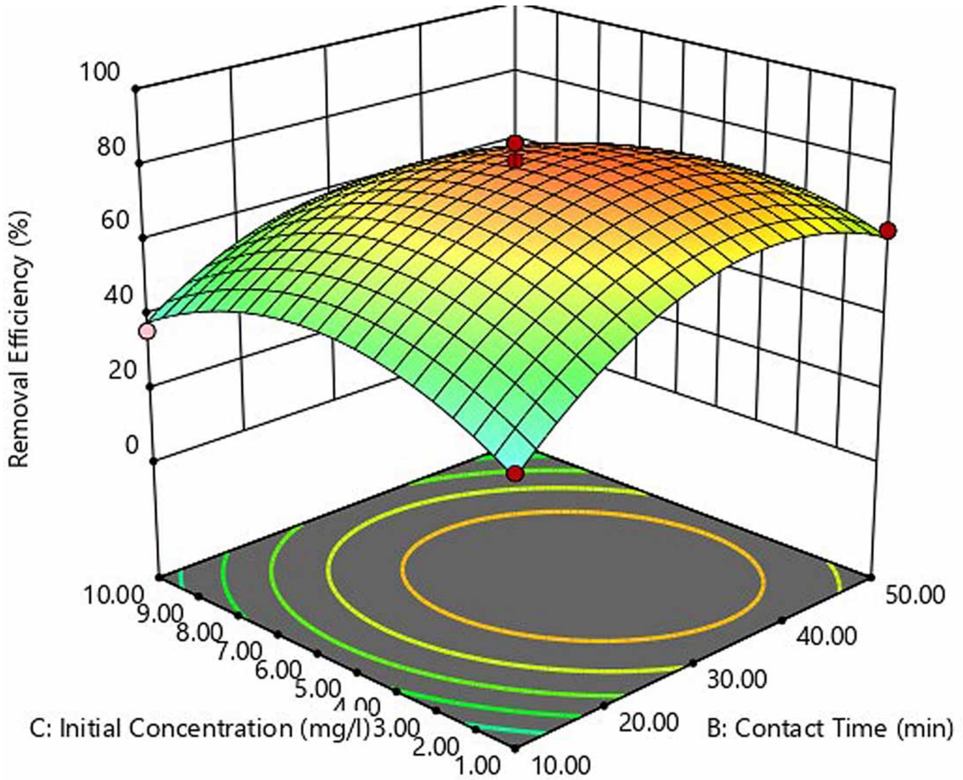

Figure 7 | 3D surface graph showing the interaction effect of initial fluoride concentration and contact time in removing fluoride ion by using *Tulsi (O. sanctum*) from water.

Table 2 | Criteria of setting factors to be optimized to remove fluoride from drinking water by using *Tulsi (O. sanctum)*

Name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
A: Adsorbent dose	Minimize	0.2	1	1	1	3
B: Contact time	Minimize	10	50	1	1	3
C: Initial concentration	Maximize	1	10	1	1	3
D: pH	Is in range	4	12	1	1	1
Removal efficiency	Maximize	18.0995	85.9729	1	1	5

Table 3 | Confirmatory experiment on percent removal for optimized factor combinations to remove fluoride from drinking water by using *Tulsi (O. sanctum)*

	Factor combination				Removal (%)	
No	A	B	C	D	Predicted	Experimental
1	0.2	22.6	6.7	5.6	68.435	66.11
33	0.2	24.28	7.1	5.57	68.26	67.17
34	0.2	20.9	7.1	5.58	64.36	62.54
35	0.2	20.7	6.49	4.9	67.02	65.24

Table 4 | Onsite and after transported analysis result raw water sample collected from two sites

S. No	Sample source	Sample volume	Absorbance before transportation	Absorbance after transportation	F^- conc. before	F^-conc. After transportation	Conc. in 10 ml (mg/l) before transportation	Conc.in 10 ml (mg/l) after transportation	Temp.	pH
1.	Adama Town	3 ml	30	29	0.65	0.6	2.16	2.0	23.5	7.5
2.	Harar	10 ml	44	43	1.29	1.2	1.2	1.2	21.8	7.8

Onsite temperature and pH were 24.8 & 8.14 respectively for Adama sample and 20.89 & 7.8 pH for sample from Harar.

Fluoride removal efficiency of *Tulsi (O. sanctum)* was found to be 74.21% for the sample from Adama and 86.73% for the sample taken from Harar. This result is somewhat higher than aqueous solution efficiency due to the difference in initial concentration of water sample and aqueous solution concentration. Therefore, this result indicates that *Tulsi* is an efficient adsorbent for the removal of fluoride from water.

STRENGTH AND LIMITATION OF THE STUDY

This study was conducted with great care and safety including accurate measurement of chemicals, reagents, and samples as well as calibration of instruments before each working day based on manufacturer's standardized instruction. Since this study is a first work in the context of Ethiopia, it may initiate many researchers for further study with the same or different methodology. However, working only in the same temperature range (at room temperature), using adsorbent powder sieved through only 500 μm, not charactering the sludge and using adsorbent without extraction are the limitation of this study.

CONCLUSION

The efficiency of *Tulsi* on removing fluoride from water determined using a response surface methodology (RSM) based on four variables on Box-Behnken design shows that adsorbent dose of 0.2–1 g, at contact time of 10–30 min, with initial fluoride concentration of 1–10 mg/l at pH range of 4–12. The optimum conditions found from numerically optimized value were: initial fluoride concentration 6.67 mg/l, adsorbent dose of 0.2 g, and contact time 22.66 min. Under these optimum conditions, maximum fluoride removal was obtained as 68.44%. Based on the result, the following recommendation has been forwarded. Further study should be conducted to study the effect of temperature and its interaction effect with other factors on fluoride removal efficiency as the current was conducted at room temperature. It is also recommended to study by using the adsorbent after extraction as well as characterization of the sludge generated during filtration. Moreover, further study on the effect of size of adsorbent for adsorption is needed as the current study was on the plant powder after it was sieved through only 500 μm.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest regarding the publication of this paper.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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