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Role of river-derived algae on bioaccumulation in fixed bed reactors; a low-cost safe drinking water plant

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ABSTRACT

River-derived algae, *Phormidium luridum, Gloeothece rupestris* and *Chlorococcum infusionum* a low-cost material were studied for its ability to remove water pollutants for safe drinking water at neutral pH. The algae were grown on naturally occurring gravels in a glass column of nutrient enriched with raw-water media. The effect of flow-rate and temperature on bioaccumulation was investigated. Sorption isotherm followed the Langmuir model with a high Q_0 value (22.7 mg g^{-1}) and the value is in well agreement with the break through capacity (16.8 mg g^{-1}). Different physicochemical and bacteriological parameters were studied to investigate the purity of the effluent. The method effectively permits the quantitative removal (>95%) of both chemical (F^-, AsO_4^{-3}, PO_4^{-3}, Fe^{+3}, As^{+3}) and bio-pollutants (total coli form, Faecal *coli form*, *E. coli*), from raw water.

Keywords: Bioaccumulation; Self maintained bed; river–derived algae; *Phormidium luridum;* Safe Drinking Water Plant; Removal of Pollutants

1. Introduction

In India, more than 80% of total population is suffering from neurological and renal disturbances, as well as from water borne diseases like Typhoid, Cholera, Gastroenteritis, Hepatitis, Dysentery, Diarrhea, due to the presence of both chemicals as well as bio-toxicants. The removal of metalic toxicants from raw water is also becoming more important with the increase in industrial activities [1]. Algae, bacteria, fungi and yeasts have proved to be the potential biosorbents of metals [2]. Bioaccumulation is more advantageous over the conventional treatment methods (Reverse Osmosis, Electrodialysis, Ultrafiltration, Ion-exchange and Chemical Precipitation). It is [3] now one of the most interesting areas in analytical chemistry for the removal of chemical and biopollutants, because it is ecofriendly, simple, selective, rapid, highly efficient and cost effective. Biosorbents can easily be regenerated and it needs no additional nutrients for its growth. Moreover, biosorption works at neutral pH. Many studies have shown that the principal factors influencing bioaccumulation by the non-viable algae include pH [4], presence of alkaloids in biosorbents [5], heavy metal species [6], competing ions [7], and the type of algae and fungi [4,8]. Some marine algae show a high capacity for bioaccumulation and bioconcentration of metals and metalloids, and have therefore been proposed for purification purposes of polluted aquatic media [9]. Cyanobacterium *Phormidium sp*. was shown to have the capabilities of endurance against a high concentration stress of arsenic and of accumulation of arsenic [10]. Cyanobacterium *Gloeocapsa sp.* grown at

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neutral pH, and was studied for removal of Pb^{+2} under pH 4 [11]. Algae showed their pronounced susceptibility towards fluoride in neutral pH [12]. *C. humicola* and *A. fertilissima* biomass pretreated with 50 and 100 mg Ca l^{-1}, respectively, showed a considerable increase in biosorptive capacity for fluoride. However, simultaneous removal of chemical toxicants as well as biochemical pollutants including arsenite, arsenate, phosphate, fluoride, total *coli form*, Faecal *coli*, and *E. coli* from raw water at neutral pH by river-derived algae, *Phormidium luridum, Gloeothece rupestris*, and *Chlorococcum infusionum* growing on natural gravels have not been reported till date. The present work advocates a rapid method for the extraction, preconcentration and removal of microgram level chemical toxicants like arsenite, arsenate, phosphate, fluoride and bio-pollutants like total *coli form* (TC), *E. coli* (EC), MPN/100, Faecal *coli* form (FC), from raw water at neutral pH by river-derived algae, *Phormidium luridum*, *Gloeothece rupestris*, and *Chlorococcum infusionum*, growing on natural gravels.

2. Experimental

2.1. Apparatus and reagents

The pH measurements were carried out with a digital Elico L1-120 pH meter combined with glass electrode. The scanning electronic microscopy (SEM) image for the bacterial cell rupturing on the algae was obtained at 15 KV by S530 HETACHI, Japan. Fourier transform infrared (FTIR) spectra of the exchanger in its F^- loaded and unloaded forms were recorded in the range of 4500–500 cm^{-1} on Shimadzu FTIR spectrophotometer (Model no FTIR – 8400S) using sample : KBr pellets in the weight ratio 1 : 10. The amount of fluoride was estimated by an ion selective electrode, Thermo Scientific (Orion 5 Star Benchtop Multi W/ISE mtr, Singapore). A thermostat was used to carry out the extraction under the controlled temperature. Column experiments were performed in two fixed bed reactors (height 0.25 m, diameter 0.08 m, volume 1.256 l) consecutively connected. Different salt solutions were prepared from $NaAsO_2$, $Fe_2(SO_4)_3$, Na_3AsO_4, NaH_2PO_4 and NaF using raw water. Metal ions were estimated spectrophotometrically. Column experiments were run for a period over 16 months. All of these chemicals and solvents used in this work, unless otherwise stated, were of analytical grade (BDH, Mumbai, India /E Merck, Mumbai, India).

2.2. Column experiments

Preparation of exchanger bed: The columns were packed with naturally occurring gravels and algae were inoculated in the core of the column after continuous flow of river water at neutral pH. Algal growth in the column was clearly evidenced by the gradual change of color of the gravels. The identification of *Phormidium luridum*, *Gloeothece rupestris*, and *Chlorococcum infusionum* (Fig. 1) have been made by comparing various standard monographs [13,14] with our microscopic observation using Olympus BX41 epifluorescence trinocular microscope fitted with digital camera, Nikon coolpix 4500 at a magnification of 45 × 10. The growth of the algae gradually increases with the population ratio of *Phormidium luridum* : *Gloeothece rupestris* : *Chlorococcum infusionum* as 6:20:1. It approaches to a limiting value (12.6 gm) after 21 d. The column set up is shown in Fig. 2.

Preparation of sample solution: Stock solutions of As^{+3} (18.8 mg l^{-1}), Fe^{+3}(26.8 mg l^{-1}), Ca^{+2} (1054.3 mg l^{-1}), Mg^{+2} (402.2 mg l^{-1}), AsO_4^{-3} (44.4 mg l^{-1}), PO_4^{-3} (56.2 mg l^{-1}), fluoride (108.0 mg l^{-1}) were prepared by dissolving appropriate amount of $NaAsO_2$, $Fe_2(SO_4)_3$, Na_3AsO_4, NaH_2PO_4, NaF respectively in dilute acid solution. The solutions containing As^{+3} (1.88 mg l^{-1}), Fe^{+3}(2.68 mg l^{-1}), Ca^{+2} (105.43 mg l^{-1}) and Mg^{+2} (40.22 mg l^{-1}), AsO_4^{-3} (4.44 mg l^{-1}), PO_4^{-3} (5.62 mg l^{-1}), fluoride (10.80 mg l^{-1}) were prepared from the respective stock solutions through appropriate dilution with raw water having TDS 225 mg l^{-1}, hardness 220 mg l^{-1}, alkalinity 201.35 mg l^{-1}, color 6.6 Hazen, turbidity 3.7 NTU, TC 1690 MPN/100, FC 1600 MPN/100, EC 700 MPN/100. The reactor beds

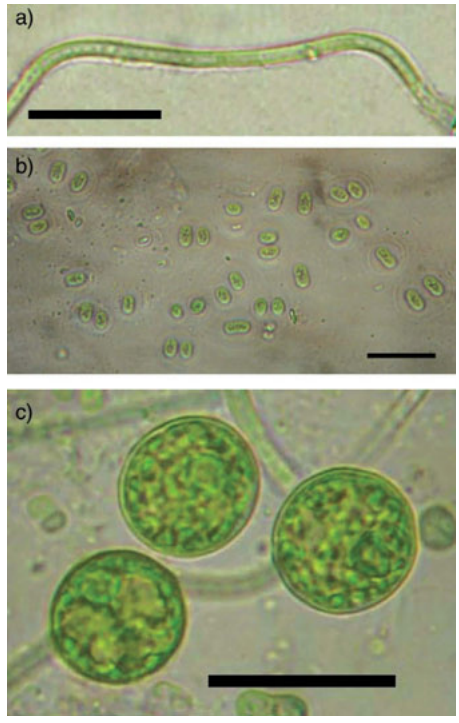

Fig. 1. Micrographs of (a) *Phormidium luridum* (b) *Gloeothece rupestris* and (c) *Chlorococcum infusionum*; Scale bars = 10 µm.

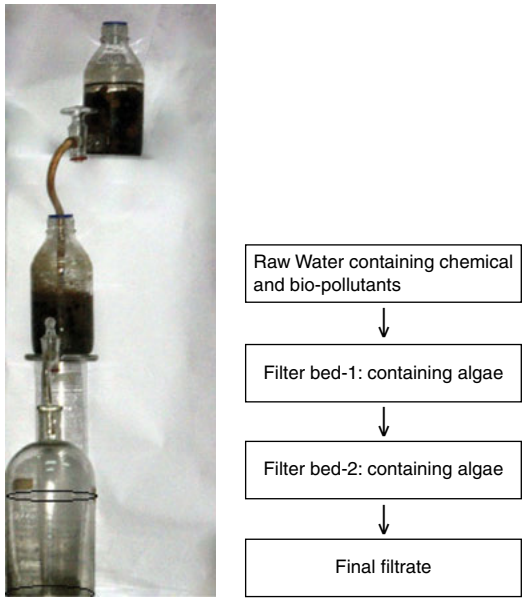

Fig. 2. Experimental prototype of purification plant and experimental set up.

were continuously fed (Flow-rate = 25 ml min^{-1}) with these solutions and the effluents were collected.

2.3. Batch experiment

Adsorption isotherm: A total 2.5 g of the algae was suspended with constant stirring for 30 min in 400 ml As^{+3} solution with different initial concentrations (10–80 µg m l^{-1}) at pH 5.0. The amount of As^{+3} adsorbed per unit mass of the adsorbent (q_e in mg g $^{-1}$) was computed using the following equation:

$$q_e == \left(C_i - C_e\right) V/m \quad (1)$$

here, C_i and C_e are the initial and equilibrium concentrations (mg l^{-1}), m is the mass (g) of the adsorbent, and V is the volume (l) of the solution. The data of the batch experiment are shown in Table 1. Applicability of the isotherm for Langmuir (Eqs. (3) and (4) and Freundlich (Eq. (5)) were examined for goodness-of-fit by using the correlation coefficients, R^2 and the residual root mean square error (RMSE), defined by:

$$\mathrm{RMSE} = \sqrt{[\frac{1}{m-2}\sum_{i=1}^{m}(q_e - q_{e(t)})^2]} \quad (2)$$

where, q_e (average of three determinations) is the experimental value (batch experiment), $q_{e(t)}$ is the estimate from isotherms for corresponding q_e and m is the number of observations in the experimental isotherm. The smaller RMSE value indicates the better curve fitting.

Non linear regression of Langmuir isotherm:

$$q_e = \frac{Q_0 b C_e}{1 + bC_e} \quad (3)$$

The equation can rearrange to:

$$q_e = Q_0 b C_e (1 + bC_e)^{-1} = Q_0 b C_e - Q_0 b^2 C_e^2 \quad (3a)$$

Linear regression of Langmuir isotherm:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b} = \frac{C_e}{Q_0} \quad (4)$$

$$\log q_e = \log K_F + \left(\frac{1}{n}\right) \log C_e \quad (5)$$

here, the adsorption constants K_F and $1/n$ were the indicators of adsorption capacity and adsorption intensity, respectively. C_e is the equilibrium concentration (mg l^{-1}) of As^{+3} and q_e is the amount of As^{+3} adsorbed

Table 1
Data of batch experiment, parameters of Langmuir and Freundlich isotherm and residual root mean square (RMSE) for the As^{+3} sorption experiment at room temperature

Langmuir parameters; R^2 = 0.9916 (0.9989)						Freundlich parameters; R^2 = 0.984			
C_e	q_e	Q_0 (mg g^{-1})	b (l mg^{-1})	$q_{e(t)}$	RMSE	$\frac{1}{n}$	k_F	$q_{e(t)}$	RMSE
2.90	2.0279	14.29 ± 0.014	0.057 ± 0.014	2.0232 (1.8774)	0.21 (0.11)	0.664	1.075	2.1719	0.12
3.40	2.2973	(22.79 ± 0.02)	(0.031 ± 0.02)	2.3154 (2.1711)				2.4172	
4.09	2.6387			2.6966 (2.5636)				2.7317	
4.69	2.9873			3.0089 (2.8933)				3.1347	
5.33	3.3106			3.3240 (3.2353)				3.2818	
6.10	3.6747			3.6805 (3.6285)				3.6526	
6.58	3.8256			3.8912 (3.8458)				3.7660	

Values for non linear regression are shown in the parenthesis.

per unit mass of the adsorbent (mg g $^{-1}$). F-test has been performed on q_e for Langmuir and Freundlich curve and the F-ratio was computed using the following Eq. (6):

$$F = \frac{MS_B}{MS_W} \quad (6)$$

where, MS_B is the between-group mean square value and MS_W is the within-group mean square value.

3. Results and discussion

3.1. Characterization of the bio-sorbent

The exchange capacity is strongly influenced by the type and number of functional groups present on the surface of the adsorbent [15]. The *Phormidium* flocculant is a sulfated heteropolysaccharide to which fatty acids and proteins are bound. The polysaccharide backbone is composed of uronic acids, rhamnose, mannose, and galactose [16]. The FTIR spectrum of the algae showed several intense peaks at around 3425.34–3598.92, 2918.10, 2339.49, 1874.68, 1645.17, 1299.93, 1161.07, 931.55, 725.18 cm^{-1} (Table 2, Fig. 3). The peak at 3425.34–3598.92 cm^{-1} is attributed to –NH_2 & –OH [15,17] and –CH stretching vibrations may be rationalized [15,18] by the absorption peak at 2918.10 cm^{-1}. The absorption peak at 1299.93–2339.49 cm^{-1} could be assigned for –C=O groups [15,17]. Moreover, the presence of –C–O, –C–C and –C–OH may be confirmed by the absorption peak at 1299.93–931.55 cm^{-1} [15,19]. The absorption peak at 725.18–931.55 could be assigned for –P–O, –S–O and aromatic –CH stretching vibrations [15,19]. The shifting of the peaks position assigned for –NH_2 and other anionic groups rationalized the extraction of metal ions (Fig. 2). The binding of F^- occurs at the [–NH_2M]$^{+n}$ moiety [20] due to the electrostatic interaction.

3.2. Break through capacity

At neutral pH, a synthetic mixture containing As^{+3} and F^- was passed through the column containing 21 g (approx.) of algae at a flow-rate of 20.0 ml min^{-1}. It was found that the leakage of As^{+3} and F^- started after passing

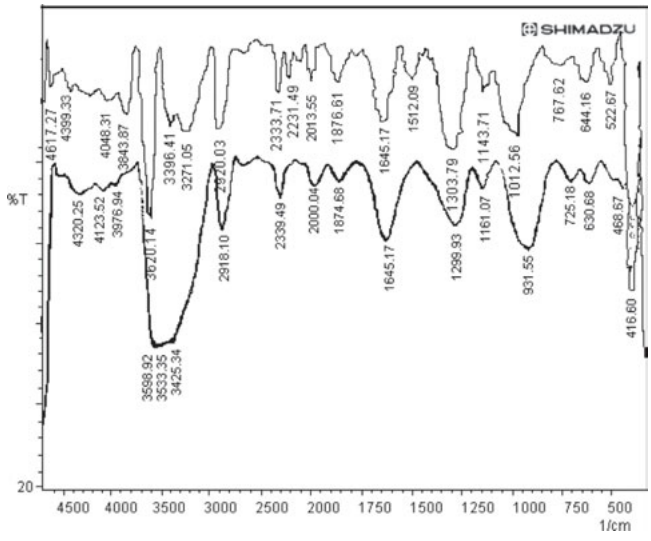

Fig. 3. FTIR spectra of (a) free algae and (b) loaded with chemical and bio-chemical pollutants.

15.85 ml (16.8 mg g^{-1}) and 17.5 ml (18.6 mg g^{-1}) of As^{+3} and F^- respectively (Fig. 4).

3.3. Adsorption isotherm

Langmuir and Freundlich isotherm models were fitted to the experimental data to investigate sorption

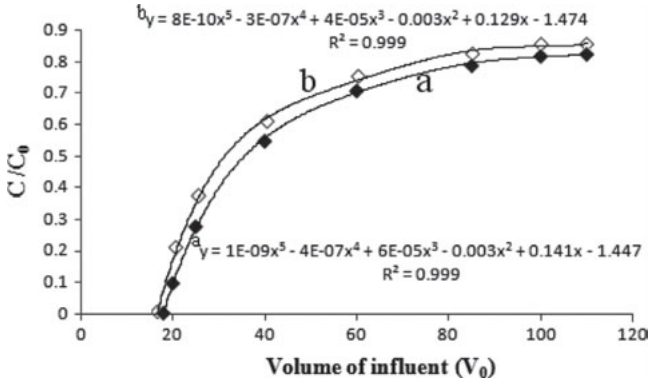

Fig. 4. Break through curves (a) F^- accumulation (b) As^{+3} accumulation.

Table 2
Assignment of IR peaks

Peak position (cm^{-1})	Nature of the peak (intensity)	Peak assignment
725.18–931.55	Broad, medium intensity	–P–O, –S–O and aromatic –CH stretching vibrations [15,19]
931.55–1299.93	Broad, medium intensity	–C–O, –C–C and –C–OH stretching vibrations [15,19]
1299.93–2339.49	Sharp, strong intensity	–C=O stretching vibrations [15,17]
2918.10	Sharp, strong intensity	–CH stretching [15,18]
3425.34–3598.92	Sharp, strong intensity	–NH_2 and –OH stretching vibrations [15,17]

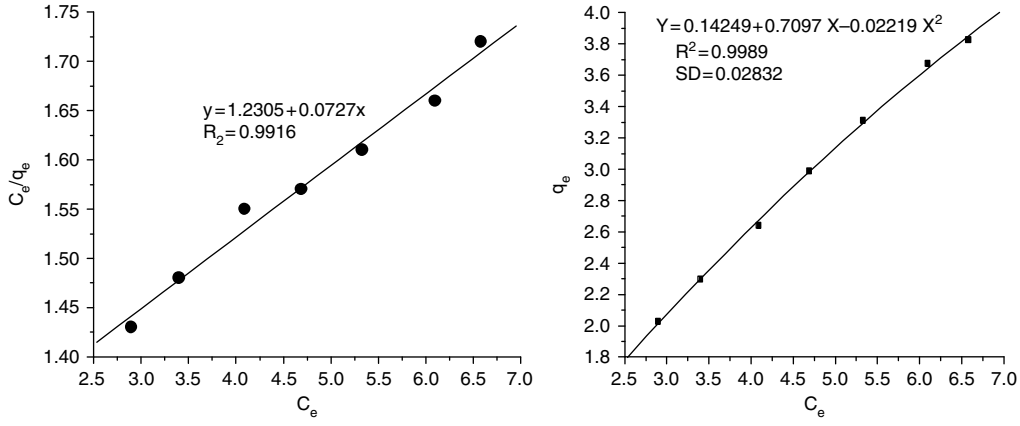

Fig. 5. Plot of C_e. Vs. C_e/q_e (linear regression) and C_e. Vs. q_e (non linear regression).

behavior of the modified exchanger at pH 5.0. An experimental linear plot (y = 0.072X + 1.2305; R^2 = 0.9916; SD= 0.014) of C_e/q_e versus C_e and a non linear regression (y = 0.14249 + 0.7097x – 0.02219x²; R^2 = 0.9989; SD = 0.02832) with very high correlation coefficient closer to unity of q_e versus C_e for the adsorbent (Fig. 5) suggests the validity of the Langmuir adsorption (chemisorption) isotherm (Eqs. (3) and (4)). From the slope and intercept of the linear plot, Q_0 (maximum adsorption) and b (energy parameter of adsorption) were found to be 14.29 ± 0.014 (mg g^{-1}) and 0.057 ± 0.014 (l mg^{-1}) respectively. Comparing Eq. (3a) with the non linear regression, the values of Q_0 (22.7 ± 0.02 mg g^{-1}) and b (0.031 ± 0.02 l mg^{-1}) were obtained from the coefficient of x and x^2 (here, $x = C_e$). The parameters of Langmuir isotherm and Freundlich isotherm are given in Table 1. The observed Q_0 values were in well agreement with the experimental break through capacity value (16.8 mg g^{-1}). The lower value of RMSE (Table 1) of non linear regression of Langmuir isotherm indicates it's goodness-of-fit [21]. However, the observed correlation coefficient is much lower (0.9849) for the experimental plot (Fig. 6) of log q_e versus log C_e ($y = 0.768x - 0.042$; $R^2 = 0.9849$; $SD = 0.01498$) and it denies the Freundlich isotherm models (Eq. (5)). So, it becomes more and more difficult to adsorb the additional adsorbates. The calculated F-ratio (2.49) for the Langmuir isotherm is in agreement with the $F_{Crit.}(6, 14)$ value (= 2.85 at α = 0.05, Table 3). While the F-ratio value (3.73) is considerably higher with respect to the $F_{Crit.}(6, 14)$ value (= 2.85 at α = 0.05) for Freundlich isotherm and it fails to correlate the data points on this isotherm. The S_R^2 values for Langmuir (0.0083 for linear regression and 0.0647 non linear regression respectively) and Freundlich (0.0666) have been calculated from q_e and $q_{e(t)}$. The calculated F value ($\frac{S_{R(Freundlich)}^2}{S_{R(Langmuir)}^2} = 8.02$) is significant with respect to the $F_{Crit.}$ (6, 6) value (= 4.28 at α = 0.05) and also suggests the goodness of Langmuir regression in comparison with that of Freundlich statistically.

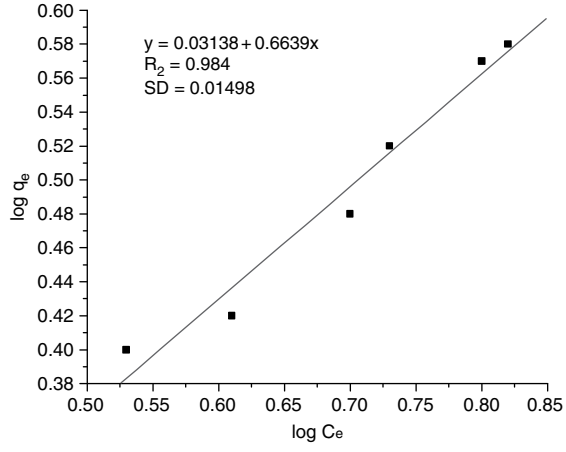

Fig. 6. plot of log C_e Vs. log q_e.

Table 3
F-Test parameters for Langmuir and Freundlich isotherm models

Langmuir isotherm (SD = 1.10 ± 0.50)			Freundlich isotherm (SD = 1.16 ± 0.16)		
MS_B	MS_W	F	MS_B	MS_W	F
5.27	2.12	2.49	0.112	0.03	3.73

The dimensionless separation factor [22] R_L, was calculated from Langmuir constant (b) (Eq. (6)):

$$R_L = \left(1 + bC_i\right)^{-1} \quad (6)$$

where, C_i (10–80 μg m l^{-1}) is the initial solute concentration. Using initial solute concentration ranging between 10 and 80 μg m l^{-1} the values of R_L (0.65–0.19) were calculated. The R_L values lie between 0 and 1, indicating a favorable exchange process.

3.4. Mechanism of extraction

Systematic studies have been made for the removal of metal ions, fluoride, arsenate, phosphate and coli form groups of bacteria, Faecal *coli form*, *E. coli* from several synthetic solutions (Table 4). Poor extraction for both the anions (F^-; AsO_4^{-3}; PO_4^{-3}) and metal ions were observed from their respective synthetic solutions at the neutral pH. However, synergistic extraction for both anions (F^-; AsO_4^{-3}; PO_4^{-3}) and metal ions were observed from their synthetic multicomponent mixtures and it suggests the binding of F^-; AsO_4^{-3}; PO_4^{-3} at the $[-NH_2M]^{+n}$ moiety through the formation of ion pair. Coli form group of bacteria was completely removed from water sample after filtration. The bacterial population was not found at the algae surface also. The SEM images (Figs. 7 and 8) of the filter bed containing algae were taken at different intervals of time after the filtration. The images clearly show that the Coli form group of bacteria (0.6–1.2 μm

Table 4
Removal of cations and anions from synthetic solutions

Composition of solution	Initial concentration	Final concentration	% of removal	RSD
F^-(mg l^{-1})	10.80	4.31	60.10	1.10
AsO_4^{-3}(mg l^{-1})	4.44	2.34	52.70	1.61
PO_4^{-3}(mg l^{-1})	5.62	3.76	66.90	1.97
Mg^{+2}(mg l^{-1})	40.22	29.05	27.80	1.34
Ca^{+2}(mg l^{-1})	105.43	70.34	33.30	1.62
Fe^{+3}(mg l^{-1})	2.68	1.25	53.40	1.44
As^{+3}(mg l^{-1})	1.88	0.62	67.00	1.90
F^-(mg l^{-1})	10.80	0.14	98.70	1.78
Ca^{+2}(mg l^{-1})	105.43	6.09	94.20	1.16
F^-(mg l^{-1})	10.80	0.12	98.90	1.09
Fe^{+3}(mg l^{-1})	2.68	0.02	99.30	1.22
F^-(mg l^{-1})	10.80	0.086	99.20	1.23
As^{+3}(mg l^{-1})	1.88	0.02	98.90	1.26
F^-(mg l^{-1})	10.80	0.066	99.40	1.58
Mg^{+3}(mg l^{-1})	40.22	1.86	95.40	1.19
F^-(mg l^{-1})	10.80	0.059	99.50	1.78
Fe^{+3}(mg l^{-1})	2.68	0.018	99.30	1.43
Ca^{+2}(mg l^{-1})	105.43	4.07	96.10	1.62
F^-(mg l^{-1})	10.80	0.056	99.50	2.06
Mg^{+3}(mg l^{-1})	40.22	0.72	98.20	1.86
Ca^{+2}(mg l^{-1})	105.43	3.67	96.50	1.74
F^-(mg l^{-1})	10.80	0.054	99.50	1.98
As^{+3}(mg l^{-1})	1.88	0.017	99.10	1.32
Mg^{+2}(mg l^{-1})	40.22	1.63	95.90	2.01
Ca^{+2}(mg l^{-1})	105.43	3.57	96.60	2.22
AsO_4^{-3}(mg l^{-1})	4.44	0.35	92.12	1.36
Zn^{+2}(mg l^{-1})	6.56	0.28	95.73	1.45
PO_4^{-3}(mg l^{-1})	5.62	0.44	92.17	1.78
Na^{+}(mg l^{-1})	12.78	0.48	96.24	1.68
TC (MPN/100 ml)	>1600	9	99.40	1.69
FC (MPN/100 ml)	1600	4	99.80	1.11

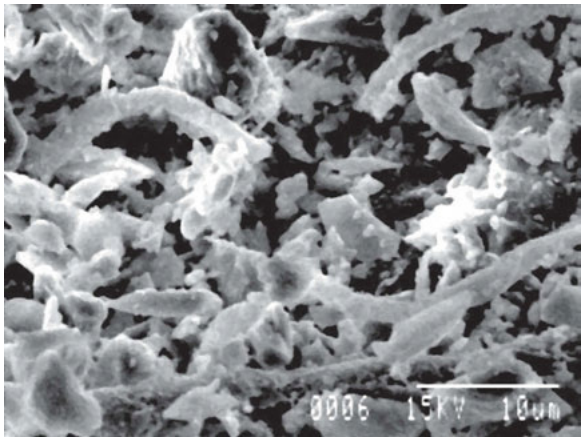

Fig. 7. SEM photograph of *E-Coli* bacteria on the algae surface. Image was taken after 1 h of adsorption.

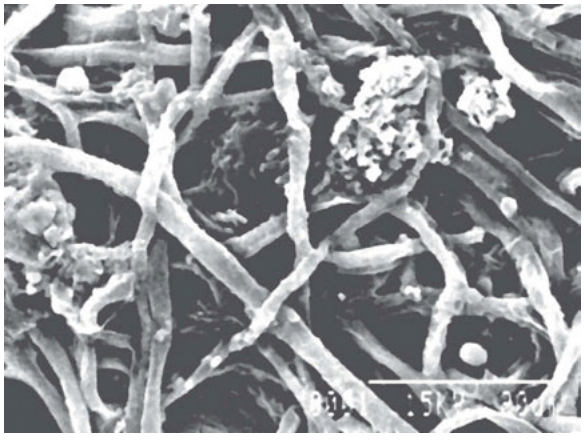

Fig. 8. SEM photograph of ruptured *E.coli* bacteria on algae surface. Image was taken after 2 h of adsorption.

in diameter by 2–3 μm in length) was adsorbed on the algae surface and subsequently it confirms the rupturing of the bacterial cells at the algae surface. Camptothecin [23] is a cytotoxic plant alkaloid isolated from Camptotheca acuminata (family Nyssaceae) which inhibits the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in HeLa cells and induces degradation of DNA [24]. Here, the sorbent algae contain two 2-alkylpyridine alkaloids [25] (Fig. 9). These alkaliods probably bind with the DNA of *E. coli* through hydrogen bonding at the three probable reactive sites, conjugated double bonds, nitrogen and –OR group and prevents DNA religation for the bactirial growth [24] .

3.5. Application to real samples

The effectiveness and the feasibility of the proposed method were judged from several relevant standard water quality parameters of the effluent after filtration (Tables 4 and 5). The effluent quality for both the surface and ground water with respect to standard water quality parameters met the requirements of drinking water. The sorption kinetics is much faster for the effluent containing F^- and bio-pollutants. However, for the effluent containing As^{+3} the sorption kinetics is relatively slower. The proposed method is simple, cost effective,

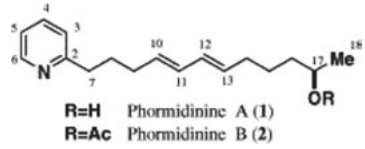

Fig. 9. Chemical structure of 2-alkylpyridine alkaloids.

Table 5
Changes of physico-chemical parameters of treated raw water samples

Sl. No.	Parameters	Initial concentration	Final concentration	% of Removal	RSD
1.	Turbidity (NTU)	27.90	0.21	99.30	1.22
2.	Color (Hazen)	20.56	0.35	98.30	1.49
3.	TDS (ppm)	719.00	120.00	83.30	1.91
4.	pH	8.51	7.20	–	–
5.	Iron (mg l^{-1})	0.58	0.18	67.40	2.01
6.	Alkalinity (mg l^{-1})	269.62	199.74	25.90	1.19
7.	Hardness (mg l^{-1})	488.00	160.00	67.20	1.71
8.	Chloride (mg l^{-1})	566.54	24.63	95.70	1.67
10.	Fluoride (mg l^{-1})	21.00	0.50	88.10	1.98
11.	Calcium (mg l^{-1})	111.90	13.98	87.50	2.16
12.	Magnesium (mg l^{-1})	50.34	8.48	83.10	1.27
13.	Arsenic (mg l^{-1})	0.46	0.01	96.80	1.41
14.	TC, MPN/100 ml	1600	9	99.40	1.88
15.	FC, MPN/100 ml	1600	4	99.80	2.01

rapid, ecofriendly and needs no nutrient other than that present in raw water.

4. Conclusions

The algae (*Phormidium luridum*, *Gloeothece rupestris*, and *Chlorococcum infusionum*) grown on naturally occurring gravels showed a significant uptake of chemicals (metallic toxicants) and bio-pollutants (coli form groups of bacteria, Faecal coli form, *E-Coli*) from raw water. The filtrate satisfactorily met the required standards of drinking water so far as the physico-chemical parameters are concerned. The coli form group of bacteria is removed and remarkably destroyed on algae surface. The algae are readily available in tropical countries like India and these are also cheap. The filter bed works at neutral pH and room temperature. The filter bed needs no treatment or activation because the algae on the bed take up their nutrients from the raw water itself and grow on the bed. Thus the bed is self maintained.

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